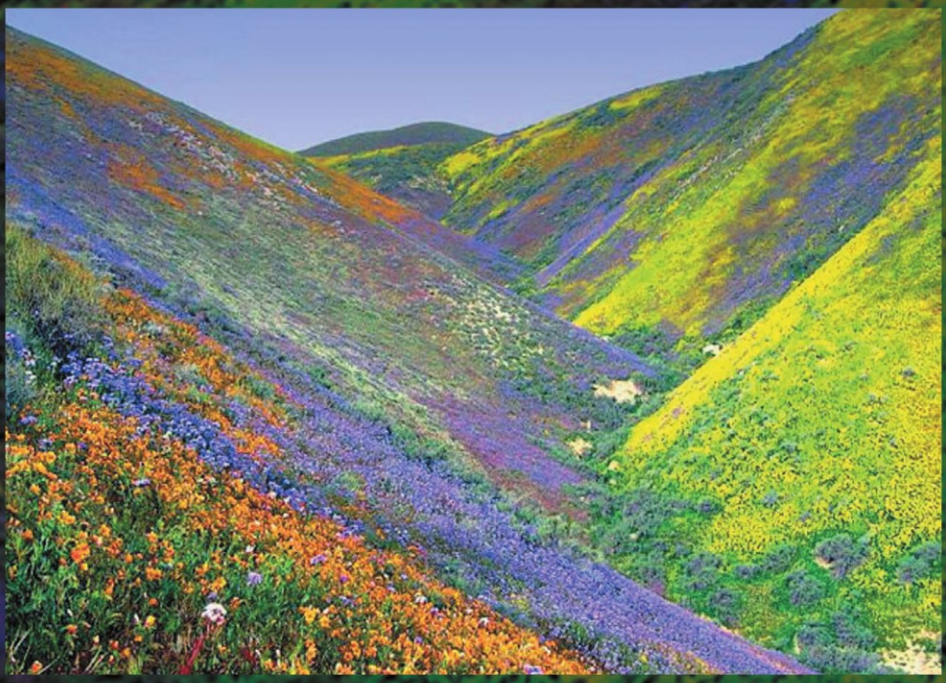
Flower Breeding and Genetics

Issues, Challenges and Opportunities for the 21st Century

Edited by
Neil O. Anderson



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FLOWER BREEDING AND GENETICS

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Issues, Challenges and Opportunities for the 21st Century

Edited by

NEIL O. ANDERSON

University of Minnesota, St. Paul, Minnesota, U.S.A.

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Caption of cover illustration:
Wildflowers of the Carizzo Plains (San Luis Obispo County, California, U.S.A.) burst into bloom after heavy winter rains in Spring, 2005. This photo illustrates the many wild, flowering species across the globe which have yet to be collected, bred, and domesticated as flowering crops.

Photo Credit: Jean Gordon, Jeff Gordon (San Luis Obispo, California, U.S.A.)

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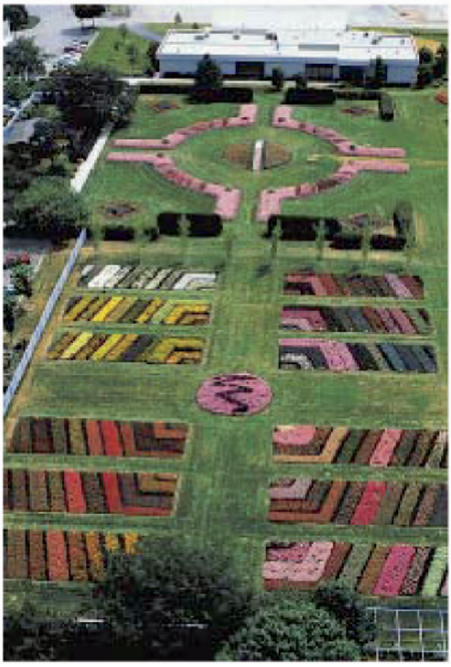

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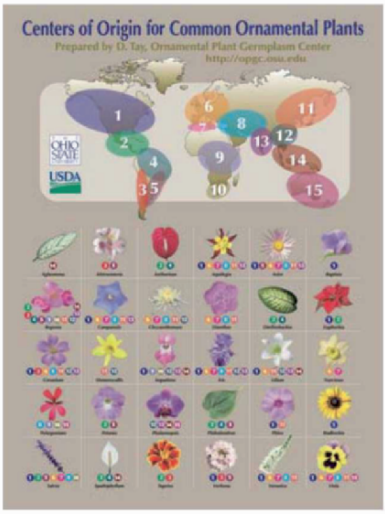

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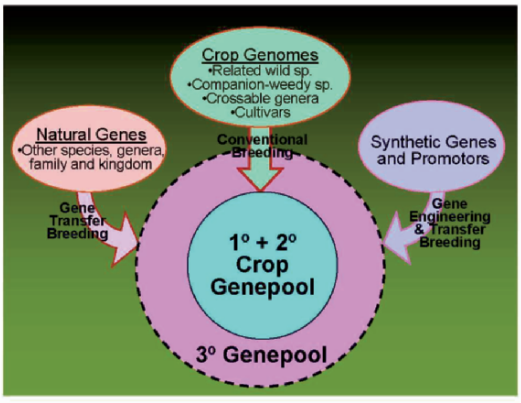

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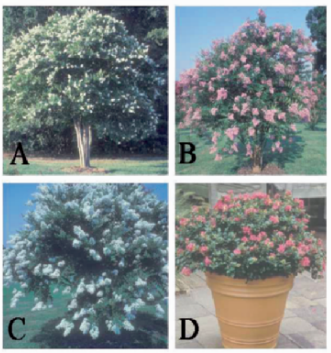

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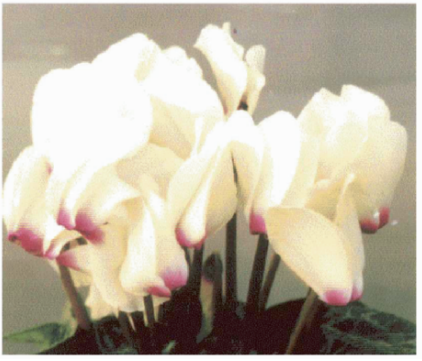

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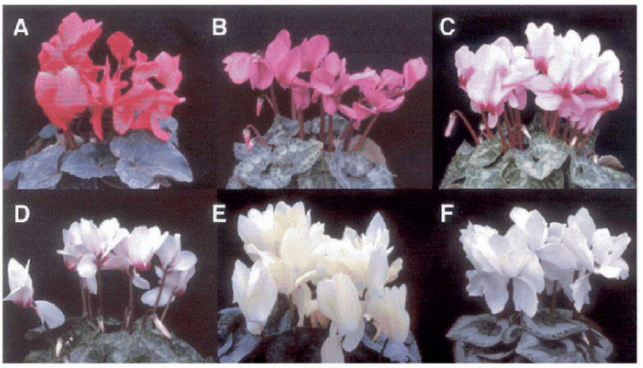

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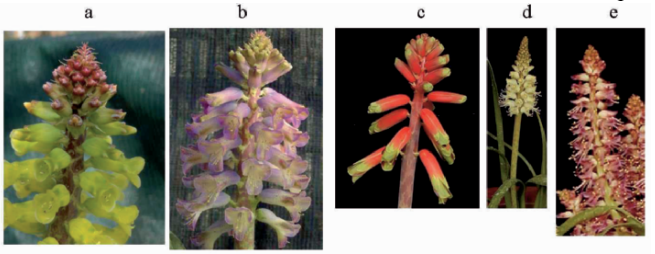

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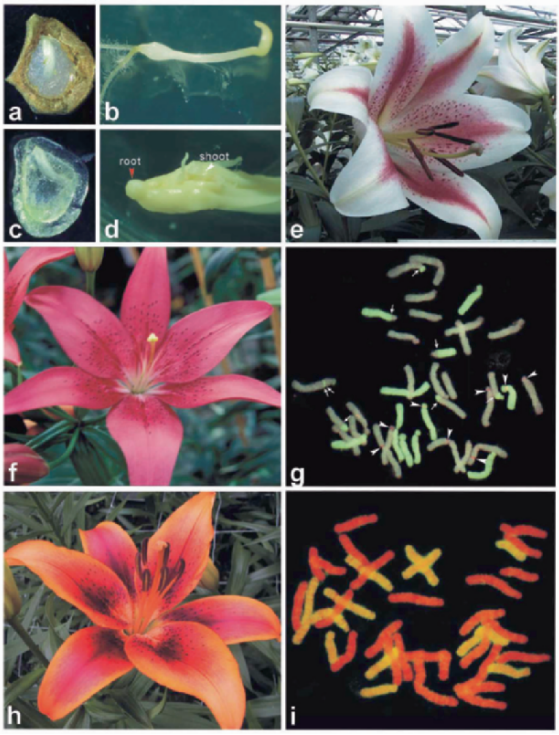

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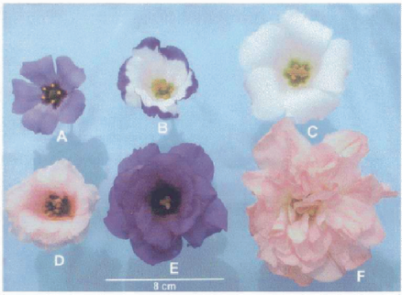

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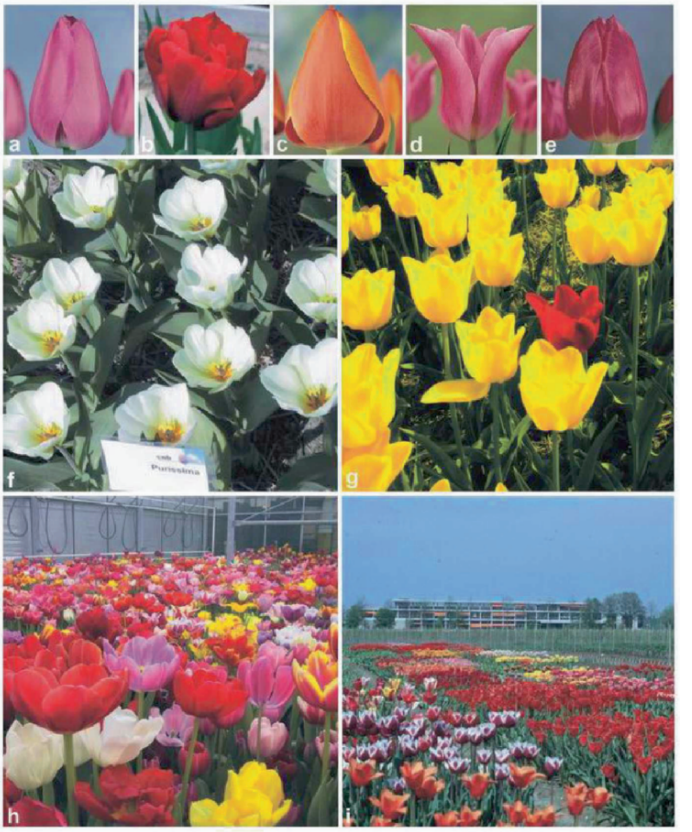

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PART I

FLOWER BREEDING PROGRAM ISSUES

Introduction

FLOWER BREEDING & GENETICS

Issues, challenges, and opportunities for the 21st Century

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Flowers have long been associated with each civilization and culture in the world. Dating as far back as the Neanderthals, flowers were used to decorate graves and celebrate major life events, expressing emotions in ways that words are deficient. Numerous cultures have incorporated flowers into their everyday lives as expressions of beauty and art. The first flower breeder is unknown, but the historic record provides us with a rich accounting of numerous flower selections and cultivars, presumably many of which were either selected as mutations or the result of directed breeding. Many seed and vegetatively-propagated cultivars of flower crops have been preserved as heirlooms, landraces, and are important sources of historic germplasm.

The science and art of flower breeding and genetics is not well documented. Much of the knowledge is transferred from one generation of flower breeders to the next within each public and private sector breeding program. While there are many monographs devoted to specific crops, few, if any, attempt to combine a diverse array of floriculture crops and address important issues for the current and following generations of flower breeders/geneticists. Oddly enough, the early genetics work of the 20th century (e.g. research by East, Mangelsdorf, etc.) was conducted on flowering crops, such as snapdragons and flowering tobacco. Unfortunately, subsequent genetic research often shifted to agronomic (food) crops. Flower breeders, while hard at work researching, collecting wild germplasm, domesticating seed and vegetative crops, and producing thousands of new cultivars for the commercial market, remained predominantly beneath the radar screen. As a result, our current floricultural crops have a paucity of genetic and breeding data in the public record since most of the last

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century's efforts are proprietary in the private breeder companies. A recent publication on Flower Seeds (edited by McDonald and Kwong, CABI publishers, 2004) has helped to change this dilemma. This monograph is dedicated to alleviating this oversight with the goal of preserving much of the genetic and breeding information for future use, as well as spawning the domestication of future floricultural crops.

It was under this pretext that the present monograph was conceptualized by many members of the Ornamental Plant Breeding Working Group, under the auspices of the American Society of Horticultural Scientists. Additional ideas and input arose from discussions with other scientists at the annual meetings of the International Society for Horticultural Science. Undoubtedly, the primary reason no previous monograph has been devoted to such a wide range of topics as are covered in this book is that it is a monumental task. I was eager to assume editorship of this monograph, having directed both private and public sector flower breeding programs, as I felt there was a profound need for such a reference. The call for submissions began in 2001; reviewing and final editing took four years to accomplish due to the scope of topics and crops covered, as well as the busy schedules of each and every contributor.

This book has many significant contributions to the science of flower breeding and genetics. The first six chapters are devoted to topics of wide interest to floriculturists and all plant breeders. Dr. John Erwin provides a thorough examination of the factors affecting flowering in Chapter 1. The essential elements of flowering need adequate characterization and manipulation for any flowering crop to be successfully bred, domesticated, and introduced into the market. Advent of new traits in floriculture crops are explored in many chapters, including the phenomenon of 'annualized perennials' by Drs. Wilkins and Anderson (Chapter 2). The science and art of cultivar trialing in private and sector breeder trials and display gardens is exquisitely delineated by the foremost trial coordinator of our time, Mr. Jim Nau of Ball Horticultural Company (Chapter 3). Marketing of floriculture crops is probably the most advanced of any horticulture or agronomic commodity and can serve as a template for many other crops. Ms. Penny Aguirre provides detailed explanation of all the necessary protection, which can be offered to new flower products in any country of the world (Chapter 4). Never before have we had the opportunity to have a reference devoted to plant germplasm collection, maintenance, and the necessary rules of adherence to international and national treaties governing plant germplasm. Dr. David Tay, the Director of the Ornamental Plant Germplasm Center for the United States Department of Agriculture, provides an exhaustive description of important procedures on this topic (Chapter 5). New issues continually face the global floriculture market, not least of which is crop

invasiveness. Dr. Anderson brings this topic to the attention of our readers to encourage flower breeding programs and all other parties in the distribution channel to research and develop strategies to prevent continued release of invasive flowering crops (Chapter 6).

The sections of crop-specific breeding & genetics are divided into four floriculture commodity groups: bedding plants, flowering potted plants, cut flowers, and herbaceous perennials. Breeding and genetic techniques for a total of 24 floriculture crops are covered in these sections. Insertion of each crop into one of these commodity groups was a challenge, since many crops are in multiple groups. I chose to insert each crop into a commodity grouping, based on its predominant use in the marketplace. Chapter formats for each crop follow a similar layout for ease in reading.

The crops covered in these sections represent a diversity of life history (annual—perennial), age (new—old crops), levels of domestication (relatively recent to those >1,000 years old), ploidy (diploid to complex polyploids), breeding systems (self incompatible to self compatible and all combinations in between), propagation mode (seed, vegetative), etc. Many crops have relatively little genetic information available. We look to future generations of flower breeders/geneticists to expand this area. Other crops require other research topics to be addressed, such as linkage maps, trisomic development, genetic engineering, regeneration/transformation technologies, shortening lengthy life cycles, cytogenetics, manipulation of ploidy levels, interspecific hybridization, removal of reproductive barriers, manipulation of hybrid breakdown, etc. Each author provides an extensive review of the literature, the current state-of-the-art, crop-specific future needs, and ideotypes for continued transformation of each taxa.

I trust that each reader will use the information contained herein to further the progress in flowering crops for future generations. Flower breeding & genetics is an exciting field of research and discovery. Numerous scientists have devoted their lives to this subject. It is one of the most rewarding professions in the world!

Neil O. Anderson, Editor
15 September 2005

Chapter 1

FACTORS AFFECTING FLOWERING IN ORNAMENTAL PLANTS

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Abstract: Flowering is the cornerstone of floricultural crops, regardless of class (bedding plants, herbaceous perennials, cut flowers, flowering potted plants); the only crop exceptions are those grown for their colorful foliage. During flower breeding and crop domestication, both public and private sector flower breeding programs must conduct research to discern the various control mechanisms for flower initiation and development. Important flowering concepts covered in this chapter include autonomous regulation (phase change; species, meristem size, and environmental factor affects), external regulation (photoperiodism, vernalization, devernalization, irradiance and light quality, and their interactions), irradiance induction, stress induction (ehtylene, water), flower development requirements (photoperiodism, temperature, stress), and dormancy.

Key words: Dormancy, facultative irradiance response, floral evocation, floral induction, flower initiation, flower development, heat delay, irradiance indifference, phase change, plant growth regulators, vernalization.

1. INTRODUCTION

Flowering, or the transition from leaf (vegetative phase) to flower (reproductive phase) production by a meristem, can be stimulated by internal or external cues. Internal or autonomous cues include flowering responses that result from factors such as plant age or size. In contrast, external cues include flowering responses that result from environmental stimuli such as day/night length, low temperature, fire, and/or the presence of water. The development of internal cues to control flowering enable plants to regulate flowering when a plant is at an optimal size or age. The

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development of external cues allows for optimal timing of flowering during a year to ensure successful pollination and seed development prior to inclement conditions as well as for synchronized flowering within a population. Such synchrony is essential to for successful cross-pollination of outcrossing species.

Steps in the flowering process, as well as autonomous and external cues that result in flowering and how they can be applied by flower breeders / plant physiologists will be discussed in this chapter. Both basic and applied literature will be reviewed. The chapter will distinguish between factors that result in flowering as a result of induction versus the breaking of dormancy. In addition, detailed information on specific conditions that promote flowering of a number of herbaceous ornamental species will be presented to enable a commercial grower, private or public sector flower breeder, and/or research scientist to induce flowering at any desired time. Much of this specific information is very recently discovered. In addition, data on classification of species into irradiance response groups has recently been introduced (Erwin and Warner, 2002; Mattson, 2002; Mattson and Erwin, 2003 a, b).

2. THE FLOWERING PROCESS

2.1 Terminology

The processes whereby events in a shoot meristem are altered in such a way to produce flowers as opposed to leaves are collectively referred to as '**floral evocation'**. '**Floral induction'** is the actual signal that results in evocation. Formation of flower buds after induction is referred to as '**flower initiation'**. The process after flower initiation until anthesis is referred to as '**flower development'**. '**Anthesis'** refers to the shedding of pollen by the stamen. It should be noted that flower opening (petal unfolding) can occur prior to, during, or after anthesis.

A meristem is '**competent'** to flower when it can respond, in the expected manner, when given an appropriate developmental signal. Such a meristem is referred to as '**determined',** if it follows the same developmental program even after it is removed from a source of environmental or biochemical stimulus. In some cases the '**expression**' of flowering can be delayed until a second developmental signal is received. For instance, some species require a succession of two different photoperiods for successful evocation. Similarly, some species require a cold temperature treatment followed by a specific photoperiod for successful evocation.

Often, floral induction and flower initiation have occurred, but flower development is interrupted. Such a suspension in flower development is, in some cases, referred to as '**dormancy'**. In most cases, a single or series of environmental

cues must occur for dormancy to be broken. Dormancy is common for flowering in spring-flowering woody species and ephemerals.

The complexity of control mechanisms associated with flowering is enormous. It is amazing that flowering can occur at all, given the sensitivity of each step and the possibility for interruption during the flowering process which is significant. If a single factor is promotive, it is possible for flowering to be inhibited if other conditions are not met. Yet, plants have a significant redundancy incorporated into the flowering process to ensure that a number of cues can enable flowering to compensate for environmental fluctuations. The inherent redundancy in the flowering system as well as ways in which several environmental cues can result in the same flower induction is apparent in recent models.

2.2 Autonomous Regulation of Flowering

Organisms pass through a series of developmental phases during growth and maturation. Among animal species, developmental phase changes are ubiquitous throughout the organism. In contrast, among plants such phase changes take place only in the shoot apical meristem; flowering is only possible if a meristem is competent to flower and it receives an inductive signal.

Whether a meristem is competent to flower is dependent on the phase, which the meristem is in. The transition between phases in development is referred to as '**phase change**'. A plant passes through three phases: the juvenile, adult vegetative (competent), and adult reproductive (determined) phases. The critical difference between the juvenile and adult phases is inherent in the ability of that meristem to successfully flower, which is only observed in the adult phases. The critical difference between the adult vegetative and adult reproductive phases are simply whether that meristem has or has not been evoked to flower or is determined.

Whether a plant is competent or determined with respect to flowering can be evaluated using grafting experiments (McDaniel, et al., 1992). If a non-flowering scion is grafted onto an induced rootstock and the scion flowers, then the scion must have been competent to respond to the floral stimulus. In contrast, if the scion does not flower, it is not yet competent. If a scion is grafted onto a juvenile rootstock and the scion flowers regardless, it is likely to be determined. For instance, *Betula verrucosa* J.F. Ehrh. tissues derived from the base of the tree (juvenile) grafted onto a rootstock remain juvenile or vegetative, i.e. the scion was not competent to flower (Longman, 1976). In contrast, plant tissues collected from the top of the flowering tree (mature) were competent to flower after two years.

It must be emphasized that the transition from juvenile to adult phases is a continuous process and not discontinuous. For instance, the ability to flower is a process and is transitional. *Lunaria biennis* L. (Wellensiek, 1958), *Brassica oleracea var. gemmifera* (Stokes and Verkerk, 1951), and *Beta vulgaris* (Wellensiek and Hakkaart, 1955) pass through a clear juvenile to adult phase transition, as

evidenced by the increasing ability of cold temperatures to induce flowering as the plant ages rather than a sudden transition.

Once a plant attains the adult phase, there is an increasing tendency to flower as the plant ages, regardless of inductive conditions. For instance, *Euphorbia pulcherrima* Willd. Ex Klotzsch 'stock', or 'mother', plants will produce numerous successive populations of vegetative cuttings, i.e. can have an extended adult vegetative phase. However, the percentage of poinsettia cuttings that spontaneously produce floral organs (cyathia) increases with each successive generation of cuttings. As the stock plant gets older, cuttings taken from a stock plant are more likely to flower even under non-inductive conditions (Siraj-Ali et al., 1990; Carver and Tayama, 1992). Similarly, the ability of celery (*Apium graveolens var. dulce* Pers.) plants to flower in response to a low temperature treatment increases as a plant grows older. This is evidenced by a shorter time for flower initiation and the number of days to anthesis after completion of a cold treatment (4 wks at 8°C) (Pawar and Thompson, 1950).

The transition from juvenile to adult phases in woody plant and some herbaceous species can be accompanied by changes in morphology, phyllotaxy, thorniness, rooting capacity, and/or leaf retention, i.e. a phenotypic change. For instance, leaf arrangement in *Antirrhinum majus* L. changes from opposite during the juvenile phase to alternate during the adult phase. Leaf morphology changes dramatically in the aquatic plant *Hippuris vulgaris* (common marestail) as the plant transitions from a juvenile to an adult phase (Goliber and Feldman, 1989). *Hedera helix* L. rooting occurs readily when tissue is in the juvenile phase but is nearly 'non-existent' in adult phases (Poethig, 1990).Since there is a temporal order to the phase changes during plant development, there can be a spatial gradient in phases along a shoot. With some species in which the juvenile phase is relatively short, there may simply be a few juvenile structures at the base of the plant. In contrast, on species in which there is a prolonged juvenile period, a significant portion of the plant may be composed of juvenile structures. Meristematic regions then switch to adult phases and, as a result, the adult or reproductive structures are often located on the peripheral portions of a plant. This can be commonly observed in *Fagus sylvatica* L. and *Quercus* spp. where juvenility is associated with leaf retention during the winter and can be observed on the base of the tree.

As many herbaceous or annual species do not exhibit a morphological or phenotypic change after transitioning from juvenile to adult phases, the phase change from juvenile to adult phase can be identified based on another developmental marker: the number of leaves that have unfolded since germination (since leaf number is a direct measure of a plant's developmental age and is temperature dependent). For this reason, identification of competence to flower, based on leaf number, is commonly used as a general phase change indicator in commercial floriculture production (Dole and Wilkins, 1999).

Once the adult phase is achieved, it is relatively stable. For instance, the juvenile or adult phase is maintained through vegetative propagation as well as grafting (Longman, 1976). For instance, *H. helix* cuttings taken from juvenile tissues will remain juvenile after propagation and vise versa (Poethig, 1990). However, in rare cases, non-optimal conditions and/or stress can cause rejuvenation, or reversion back to the juvenile phase. For instance, low irradiance conditions can resulting in reversion from an adult to juvenile phase. However, the incidence of such reversion decreases as the length of time since the transition from juvenile to the adult phase increases. It is also noted that sexual or apomictic reproduction will result in regeneration of the juvenile phase (Hackett, 1980).

A number of factors affect the transition between phases in plants. Some of those factors that impact the transition from the juvenile phase to an adult phase are outlined below. In particular, the impact of species, meristem size and environmental factors are briefly mentioned.

2.2.1 Species

Woody plant species vary considerably in the length of the juvenile phase (Table 1-1). In general, the 'longer-lived' a species is, the longer the juvenile phase. For instance, some nut-trees can have a 25-year juvenile period (Table 1-1), compared to a 4-month juvenile period (14 leaves) for some herbaceous perennial species (Table 1-2), or a 2-week (3-4 leaf) juvenile phase for some annuals (Table 1-2). In some cases, e.g. *Raphanus sativus* L., there is initially no juvenile phase as imbibed seed can be vernalized (Engelen-Eigles and Erwin, 1997)

In contrast to woody plant species, many herbaceous species juvenile phase lengths are identified based on leaf number. However, the developmental time of the juvenile period differs with herbaceous species as with woody plants as shown in Table 2-2 (Cameron et al., 1996).

Table 1-1. Juvenile phase length period differences between various woody plant species (adapted from Clark, 1983; Goh and Arditti, 1985).

Common name	Scientific name	Juvenile length period (days or years)
Rose	*Rosa x hybrida*	20-30 days
Grape	*Vitis spp.*	1 year
Orchids	*Cattleya, Cymbidium, Oncidium*	4-7 years
Apples	*Malus sylvestris*	4-8 years
Orange	*Citrus sinensis*	5-8 years
English ivy	*Hedera helix*	5-10 years
Redwood	*Sequoia sempervirens*	5-15 years
Sycamore maple	*Acer pseudoplatanus*	15-20 years
English oak	*Quercus robur*	25-30 years
European beech	*Fagus sylvatica*	30-40 years

Table 1-2. Juvenile period lengths (leaf number at which plants become competent to flower) for various herbaceous plants (adapted from Sheldron and Weiler, 1982; Whitman, 1995; Yuan, 1995).

Scientific name	Cultivar	Leaf number (nodes) at which plants become competent to flower
Aquilegia x hybrida	McKana's Giant	12
	Fairyland	15
Calceolaria herbeohybrida		5
Callistephus chinensis		4
Coreopsis grandiflora	Sunray	8
Gaillardia x grandiflora	Goblin	16
Heuchera sanguinea	Bressingham	19
Lavandula angustifolia	Munstead	18
Rudbeckia fulgida	Goldsturm	10

2.2.2 Meristem Size

The association between leaf number and phase transition may be related to other morphological characteristics such as meristem size. There is an association between meristem size and the transition out of the juvenile phase. A plant may progress from juvenile to adult phases after the meristem increases beyond a minimum diameter meristem size which increases as leaf number increases. For instance, *Dendranthema x grandiflora* Tzvelv. (=*Chrysanthemum* x *morifolium* Ramat.) flower primordia are not initiated until after a minimum apex size has been attained (Cockshull, 1985).

Similarly, Singer and McDaniel (1986) showed that tobacco plants produced 37 leaves from terminal buds prior to flowering. After numerous grafting experiments across tobacco cultivars, Singer and McDaniel (1986) concluded that the number of nodes a meristem produces prior to flowering was a function of the strength of the floral stimulus and the competence of the meristem to respond to that flowering signal. Pomologists have also noted that the passage of a tree out of the juvenile period may be associated with the attainment of a specific size (Visser and De Vries, 1970). However, more recent work has shown that phase change in *Hedera helix* is independent of meristem size (Hackett and Srinivasani, 1983). However, this assumption of an association between meristem size and phase transition excludes any involvement of meristematic size or carbohydrate status; the latter impacts leaf number below the flower or juvenile period phase length.

2.2.3 Environmental Conditions

Environmental conditions that retard growth can delay the transition from juvenile to adult phases. For instance, as noted previously, exposure to low irradiance conditions can delay the transition from the juvenile to adult phase or cause reversion from the adult back to the juvenile phase. This association has led

researchers to question the role of carbohydrate availability in the transition from juvenile to adult phase. Such assumptions are borne out by increasing irradiance, which can decrease the length of the juvenile phase developmentally in numerous herbaceous annuals. This is evidenced by a decrease in the leaf number below the first flower (see Irradiance section below). For example, the juvenile period length can be decreased as irradiance increases in *Pelargonium* x *hortorum* L. H. Bailey (Armitage and Tsujita, 1979). Similarly, numerous species juvenile period length can be reduced by addition of supplemental lighting (Mattson and Erwin, 2003a, b; Warner and Erwin, 2001b) or by providing conditions that promote growth (Poethig, 1990).

2.3 Physiological Basis for Transition from Juvenile to Adult Phase

The physiological basis for the transition from juvenile to the adult phases is not well understood. Application of plant growth regulators can hasten or delay the transition from the juvenile to the adult phases or cause a reversion from the adult to the juvenile phase. For instance, application of gibberellins or gibberellic acids (GAs) to *Cupressus arizonica* Greene caused male cone formation (an indicator of the transition to adult phase) when plants were only two months old (Pharis and King, 1985). Similarly, other treatments that result in accumulation of endogenous GAs in conifers can reduce the juvenile phase length.

In contrast to conifers, application of GAs to *Hedera helix* can cause a reversion from an adult to the juvenile phase (Hackett and Srinvasani, 1985). Application of Amo 1618 (a GA-synthesis inhibitor) was not able to cause a phase change from juvenile to adult in *H. helix* (Frydman and Wareing, 1974). However, ancymidol (a GA-synthesis inhibitor) prevented the spontaneous reversion to the juvenile state in low light in *H. helix* (Rogler and Hackett, 1975). Similar inhibition of transition from juvenile to adult phases following GA application has been noted on *Citrus* (Cooper and Peynado, 1958), deciduous fruit trees (Luckwill, 1970), and *Ipomoea caerula* L. (Njoku, 1958).

3. EXTERNAL REGULATION OF FLOWERING

There are four primary external environmental cues that affect flowering in plants: photoperiod, temperature, irradiance, and stress (fire, water levels). In addition, lack of stress or supra-optimal levels of nutrients or saturating water levels can also impact progression towards flowering. Of these environmental queues, photoperiod, temperature, and the presence of water, in particular, allow plants to synchronize flowering with the seasons. Fire can be an indicator of seasonal

transition but is less precise. In general, horticultural manipulation of flowering involves control of photoperiod, temperature, and/or irradiance.

When photoperiod (the duration of light/darkness) impacts flowering, a plant is said to be '**photoperiodic'**. Photoperiodism is primarily associated with longer-lived species that survive for at least one growing season. Development of such species can often depend on photoperiod to synchronize flowering to ensure that flowering occurs at a specific time of year for cross pollination, successful seed set, and seed maturation.

In contrast to photoperiodism, prolonged exposure to temperatures between 0 and 16°C can also synchronize flowering. For example, flowering in biennial and perennial species will often have a cool temperature requirement (2-4°C) for successful flower induction (often 6-12 weeks). Such low temperature induction of flowering is referred to as '**vernalization'**. Presumably, a vernalization requirement ensures a plant will not flower until after winter has occurred in temperate climates.

Further, some species exhibit a dual requirement for successful flowering, i.e. they may require a prescribed sequence of inductive conditions such as short days-long days (SD-LD) or LD-SD. In some cases, e.g. many perennial species in temperate climates, a plant may have a vernalization requirement followed by a LD requirement. Daylength and vernalization can act synergistically as in the case of *Lilium longiflorum* or *Anethum graveolens* L. 'Florida' (Pressman and Negbi, 1980).

A number of other environmental factors such as irradiance, light quality, and/or water stress can impact flowering and/or interact with vernalization and/or photoperiodic induction. These factors appear to be more associated with conditions, which are optimal for flowering under inductive conditions (growth), rather than the inductive process itself. For instance, low irradiance can decrease flowering of the day-neutral *Rosa* x *hybrida* L. by increasing the occurrence of 'blind' shoots, i.e. aborted flowers (Nell and Rasmussen, 1979). In contrast, high irradiance can substitute for a photoperiod requirement entirely in *Hibiscus moscheutos* L. (Warner and Erwin, 2003). Additionally, high temperatures can inhibit flowering induced by photoperiod or vernalization. The following sections elaborate on the primary environmental cues for flowering and the physiological bases for each response.

3.1 Photoperiodism

Photoperiodism refers to the ability of an organism to detect daylength (or more correctly night length). Photoperiodic events can be observed in animals and plants. In animals, hibernation, egg laying, and migration are all controlled by photoperiod. In plants, photoperiodism can regulate growth processes as different as flowering, tuber formation, the onset of dormancy, and rhizome formation.

Wightman Garner and Henry Allard initially discovered photoperiodic control of flowering at the U.S. Department of Agriculture laboratories in Beltsville, Maryland

in the 1920s. Garner and Allard (1920) noted that a mutant cultivar of tobacco ('Maryland Mammoth') did not flower in the field during the summer but flowered in the greenhouse during the winter. They subsequently covered outdoor field grown plants during the summer with black cloth to artificially shorten the daylength; this treatment resulted in flowering plants. Garner and Allard (1920) concluded that day length controlled when plants flower.

Photoperiodism was not applied in the commercial floriculture industry until the 1930s-1950s when Gus Poesch noted that street lights outside of a chrysanthemum facility inhibited flowering (Poesch, pers. comm.). The recognition of the importance of daylength in that chrysanthemum facility resulted in the application of photoperiod manipulation in greenhouse production. This resulted in a year-round flowering chrysanthemum industry, as well as extended flowering seasons for many other potted plants and cut flowers (Poesch, 1931). The application of photoperiod to bedding plant production is in its infancy and is a primary focus of the author's research program at the University of Minnesota.

3.1.1 Photoperiodic Response Groups

Photoperiodic flowering responses can be divided into several different response groups: short-day plants, long-day plants, day-neutral plants, intermediate-day plants and ambiphotoperiodic plants (Thomas and Vince-Prue, 1997). The specific characteristics of each group are outlined below:

Short-Day Plants – those species in which the night length (duration) must be greater than some critical length. Generally species from lower latitudes (i.e. the flowering of coffee, cotton, and rice) or species which flower in the late summer, such as chrysanthemum (Cockshull, 1984).

Long-Day Plants – those species in which night length must be shorter than some critical length. Generally species from high latitudes such as temperate grasses (Deitzer, 1984).

Day-Neutral Plants – flower induction is not affected by night length. Usually species with a wide latitudinal distribution, such as potato and tomato (Halevy, 1984)

Intermediate-Day Plants – those species that require a night length between 12 and 14 hours.

Ambiphotoperiodic-Day Plants – those species in which flower induction occurs under long or short nights but not intermediate nights

3.1.2 Subdivision Within Response Groups

Within short- and long-day plant groups, there are plants that have a facultative (quantitative) or obligate (qualitative) responses. Species that exhibit a 'facultative' or quantitative response will flower under any photoperiod. However, in such taxa flowering is hastened under the prescribed photoperiod. In contrast, species that exhibit an obligate or qualitative response will not flower unless they receive the prescribed photoperiod. Examples of common herbaceous annual species and their corresponding photoperiodic classifications are shown in Table 1-3.

Species within genera can vary widely in their photoperiodic requirement. For instance, Warner and Erwin (2003b) showed that all photoperiodic response groups were evident in a group of *Hibiscus* spp. studied. In addition, early work by Cumming (1969) showed that *Chenopodium rubrum* L. strains collected from different latitudes possessed a wide range of photoperiodic requirements for flowering.

Table 1-3. Photoperiodic and irradiance classifications of herbaceous annual floriculture crops (flowers, herbs, vegetables), based on mean leaf number below the first open flower (Armitage, 1996; Dole and Wilkins, 1999, Erwin and Warner, 2002; Motum and Goodwin, 1987a, b; Nordwig, 1999; Mattson and Erwin 2003a, b; Seeley, 1985; Zanin and Erwin, 2003). Photoperiod classifications: FSDP (facultative short-day plant); FLDP (facultative long-day plant); OSDP (obligate short-day plant) OLDP (obligate long-day plant); DNP (day neutral plant). Irradiance classifications: 'FI' (facultative irradiance response: supplemental irradiance hastened induction developmentally); 'II' (irradiance indifferent response; increasing irradiance did not hasten flowering developmentally) (Erwin and Warner, 2002; Mattson and Erwin, 2003a, b). A question mark identifies an uncertain photoperiodic classification.

Floriculture crop (Scientific name and cultivar)	Photoperiod	Irradiance
Ageratum houstonianum L. 'Blue Danube'	FLDP	II
Alcea rosea	LDP?	
Amaranthus hybridus L. 'Pygmy Torch'	DNP	II
Ammi majus L.	OLDP	II
Anethum graveolens L. 'Mammoth'	OLDP	II
Anigozanthos flavidus	FLDP	
A. manglesii	FSDP	
A. pulcherrimus Hook.	DNP	
A. rufus Labill.	DNP	
Anisodontea x *hypomandarum* K. Presl.	FLDP	
Antirrhinum majus L.	FLDP	FI
Asclepias curassavica L.	DNP	FI
A. tuberosa L.	OLDP	
Asperula arvensis L. 'Blue Mist'	OLDP	II
Begonia x *hiemalis* Fotsch	O/FSDP	
B. tuberhybrida	OLDP	
B. semperflorens	DNP	FI
Bougainvillea spp.	FSDP	FI
Calceolaria herbeohybrida	FLDP	

Floriculture crop (Scientific name and cultivar)	Photoperiod	Irradiance
Calendula officinalis 'Calypso Orange'	FLDP	II
Callistephus chinensis L.	FLDP	
Catananche caerulea L. Per. 'Blue'	OLDP	FI
Carpanthea pomeridiana L. 'Golden Carpet'	DNP	II
Celosia plumosa L. 'Flamingo Feather Purple'	OSDP	II
Centaurea cyanus L. 'Blue Boy'	OLDP	II
Centranthus macrosiphon Boiss.	DNP	FI
Cleome hassleriana Chodat 'Queen Pink'	FLDP	II
C. h. Chodat 'Queen Rose'	DNP	FI
Clerodendrum thomsoniae	DNP	
C. x *speciosum*	DNP	
Cobaea scandens Cav.	DNP	II
Convolvulus tricolor L. 'Blue Enchantment'	DNP	FI
Cosmos bipinnatus Cav. Ann. 'Diablo'	FSDP	II
C. b. Cav. Ann. 'Sensation White'	FSDP	FI
Cosmos sulphureus Cav.	OSDP	
Collinsia heterophylla Buist	FLDP	II
Crossandra infundibuliformis L.	DNP	
Cucumis sativus H.	DNP	
Cyclamen persicum Mill.	DNP	FI
Dendranthema x *grandiflora* Tzvelv.	FSDP	
Dianthus barbatus L.	DNP	
D. chinensis L. 'Ideal Cherry Picotee'	FLDP	II
Dimorphotheca sinuata DC. 'Mixed Colors'	DNP	II
Dolichos lablab L.	OSDP	II
Eschscholtzia californica Cham. 'Sundew'	FLDP	II
Euphorbia pulcherrima Willd. Ex Klotzsch.	OSDP	
Exacum affine Balf. F.	DNP	
Fuchsia x hybrida	OLDP	
F. x *hybrida* 'Gartenmeister'	DNP	
Gazania rigens L. 'Daybreak Red Stripe'	OLDP	FI
Gomphrena globosa L. 'Bicolor Rose'	FSDP	II
Gypsophila spp.	LDP?	
Hatoria gaertneri Reg.	OSDP	
Helianthus annuus L. 'Vanilla Ice'	FLDP	II
Helipterum roseum Hook.	OLDP	II
Hibiscus cisplatinus St.-Hil.	DNP	
H. laevis	OLDP	
H. moscheutos L.	OLDP	FI
H. radiatus Cav.	OSDP	
H. rosa-sinensis L.	DNP	
H. trionum L.	FLDP	
Impatiens balsamina L.	DNP	
I. hawkeri Bull.	DNP	
I. wallerana Hook.f.	DNP	
Ipomoea x multifida Shinn. 'Scarlet'	FSDP	II
Ipomopsis rubra Wherry 'Hummingbird Mix'	OLDP	II
Kalanchoe blossfeldiana Poelln.	OSDP	
Lathyrus odoratus L. 'Royal White'	OLDP	FI

Floriculture crop (Scientific name and cultivar)	Photoperiod	Irradiance
Lavatera trimestris L. 'Silver Cup'	OLDP	FI
Legousia speculum-veneris Chaix	OLDP	II
Leonotis menthaefolia R. Br.	DNP	
Leptosiphon x *hybrida*	OLDP	II
Lilium spp.	FLDP	
Limnanthes douglasii R. Br.	OLDP	FI
Limonium sinuatum Mill. 'Fortress Deep Rose'	FLDP	II
L. s. Mill. 'Heavenly Blue'	FLDP	II
Linaria maroccana Hook. f.	FLDP	FI
Linum perenne L.	OLDP	FI
Lobelia erinus L. 'Crystal Palace'	OLDP	II
Lobularia maritima (L.) Desv.	DNP	
Lycopersicon esculentum Mill.	DNP	
Matthiola longipetala Venten.'Starlight Scentsation'	DNP	II
Mimulus x *hybridus* L. 'Magic'	OLDP	II
Mina lobata Cerv.	OSDP	II
Mirabilis jalapa L.	OLDP	II
Nemophila maculata Benth. 'Pennie Black'	DNP	FI
N. menziesii Hook. & Arn.	DNP	II
Nicotiana alata Link & Otto 'Domino White'	DNP	FI
Nigella damascena L. 'Miss Jekyll'	OLDP	II
Oenothera pallida Lindl. 'Wedding Bells'	OLDP	II
Origanum vulgare L.	DNP	FI
Oxypetalum caerulea D. Don 'Blue Star'	DNP	FI
Pelargonium x *domesticum* L.H. Bail.	FLDP	
P. x *hortorum* L.H. Bail.	DNP	FI
P. peltatum L.	DNP	
Perilla frutescens (L.) Britt.	SDP?	
Petunia x *hybrida*	FLDP	
P. x *hybrida* 'Purple Wave'	OLDP	
Phacelia campanularia A. Gray.	DNP	II
P. tanacetifolia Benth.	FLDP	II
Pharbitis nil (L.) Choisy	FSDP	
Polemonium viscosum Nutt.	OLDP	II
Primula malacoides Franch	OSDP	
Primula obconica Hance	DNP	
Primula x *polyantha*	DNP	FI
Rhododendron spp.	OSDP	
Rosa x *hybrida*	DNP	FI
Saintpaulia ionantha Wendl.	DNP	FI
Salpiglossus sinuata Ruiz & Pav.	LDP?	
Salvia farinacea Benth. 'Strata'	FLDP	FI
S. splendens F. Sellow 'Vista Red'	FLDP	II
Sanvitalia procumbens Lam.	FSDP	II
Scabiosa caucasia Bieb.	LDP?	
Schlumbergera truncata Haw.	OSDP	II
Silene armeria L. 'Elektra'	OLDP	FI
Sinningia speciosa Lodd.	DNP	
Solenostemon scutellarioides (L.) Codd. (=*Coleus*)	SDP?	

Floriculture crop (Scientific name and cultivar)	Photoperiod	Irradiance
Solidago L. *spp.*	SDP	
Streptocarpus x *hybridus* Voss.	DNP	FI
S. nobilis Clarke	FSDP	
Tagetes erecta L.	FSDP	
T. patula L.	DNP	
T. tenuifolia Cav.	FSDP	
Thunbergia alata Bojer.	DNP	II
Tithonia rotundifolia Mill. 'Fiesta Del Sol'	FLDP	II
T. r. Mill. 'Sundance'	FSDP	FI
Verbascum phoeniceum L.	DNP	II
Verbena x *hybrida*	LDP?	
Viguiera multiflora S.F. Blake	FLDP	II
Viola tricolor L.	F/O LDP	II
V. x *wittrockiana* Gams.	FLDP	FI
Zea mays H.	DNP	
Zinnia angustifolia Kurth.	DNP	
Z. elegans Jacq. 'Exquisite Pink'	FSDP	II
Z. e. Jacq. 'Peter Pan Scarlet'	FSDP	II

3.1.3 Photoperiodism in Floriculture Crops

Many herbaceous species grown for spring crop production in temperate climates are photoperiodic. Shillo (1976) showed that flowering of *Limonium sinuatum* Mill. was delayed six weeks and leaf number below the first flower was increased when plants were grown under ambient light (10-12 hr photoperiod) vs. ambient light plus a 4 hr night interruption with incandescent lights. *Limonium sinuatum* was found to be a facultative long-day plant (Table 1-3). *Eschscholtzia californica* Cham. only flowered when grown under 9 hr ambient light plus a 4 hr night interruption (3-4 µmol m^{-2} s^{-1} from incandescent lamps). Thus, Lyons and Booze-Daniels (1986) classified *E. californica* as an obligate long-day plant. Keatinge et al. (1998) noted that *Dolichos lablab* L. flowered after 69 and 172 d when grown under short- (11.5 hr) and long- (14.5 hr) day photoperiods, respectively (leaf number below the first flower not reported); *D. lablab* was classified as a facultative short-day plant. Armitage and Garner (1999) showed *Catananche caerulea* L. Per. was an obligate long-day plant, flowering after 164 d when grown under long days, but did not flower when grown under short days. Recent screening experiments have resulted in the characterization of >50 commercial floriculture or herb species into photoperiodic response groups (Table 1-3; Erwin and Warner, 2002, Mattson and Erwin, 2003a, b).

Flowering response to photoperiod can vary with cultivar. For example, Ross and Murfet (1985) describe three flowering classes of *Lathyrus odoratus* L.: a day-neutral (winter) flowering group, and long-day groups exhibiting a facultative (spring) or obligate (summer) long-day response. Such variation in photoperiodic responses among cultivars is also obvious in *Salvia splendens* and *Petunia* x *hybrida*

cultivars (Erwin, unpublished data; Table 1-3). In particular, the introduction of new germplasm (often wild in origin) can often increase the photoperiod requirement of nearly day-neutral existing cultivars. Perhaps the most obvious recent example is the increase in long-day requirement within *P.* x *hybrida* with the introduction of the 'Wave' and 'Fantasy' series of petunias in recent years (Erwin, unpublished data; Erwin et al., 1997).

3.1.4 Length of Inductive Conditions Required for Flower Induction

The length of time for complete flower induction varies with plant age, temperature, and irradiance. Flowering tendency increases as a plant ages, regardless of whether it is in inductive conditions or not. Evidence of this is seen when cuttings are harvested from poinsettias (*Euphorbia pulcherrima*). Cuttings have a greater and greater proportion that exhibit 'splitting', a condition where buds initiate but do not develop fully (Siraj-Ali et al., 1990; Carver and Tayama, 1992) as the stock or mother plant age increases. Similarly, there is an inverse relationship between plant age and the number of short days required for complete flower induction with *Pharbitis nil* Chois.

In addition to the natural tendency of a plant to eventually flower, the number of inductive cycles required for complete induction will often vary with the age of a plant. For instance, the time for complete induction in *Lolium temulentum* (darnel ryegrass) decreases from 4 LD cycles on plants with 2-3 leaves to 1 LD cycle on plants with 6-7 leaves (Taiz and Zeiger, 1998). Similarly, *Petunia* x *hybrida* 'Purple Wave' required 24 LD to flower when 10 days old and 18 LD to flower when 24 days old (Mattson, 2002). These observations suggest that the effect of photoperiod may be to accelerate a process that is already occurring at a slow pace even under non-inductive conditions.

Although there is no exact number of inductive cycles, an estimate of time to completely induce a plant to flower is important for commercial production. For instance, an understanding of the time required to induce a floriculture crop to flower can identify how long a plant must stay under conditions with supplemental lighting or short days with a blackout curtain before it can be moved to non-inductive conditions. To this end, there are several general observations and estimates. Many species appear to require approximately 21 days for complete induction when grown at 16-20°C. For example, complete flower induction in *Begonia* x *hiemalis* requires 2-3 weeks (Karlsson and Heins, 1992). In some cases, flower induction and initiation can be achieved in 14 days, however, flower development may not be successful. Similarly, flowering of *P.* x *hybrida* 'Purple Wave' occurs when plants are exposed to 20 LD at 20°C and natural daylight conditions (45° N lat., St. Paul, Minnesota, U.S.A.) (Mattson, 2002).

The length of time required for complete flower induction can vary with temperature and irradiance. In general, the time required for complete induction and

initiation increases as temperature decreases and as irradiance decreases. (Zrebiec and Tayama, 1990; Mattson, 2002).

3.1.5 Interaction of Photoperiodic Induction with Temperature

Photoperiod can interact with temperature to affect flowering. It is common for some plant species to be photoperiodic at higher temperatures but exhibit a day-neutral response at lower temperatures. For instance, *Begonia* x *hiemalis* Fotsch is an obligate short-day plant at temperatures greater than 24°C (Sandved, 1969). In contrast *B.* x *hiemalis* is a facultative SDP at temperatures >24°C but a DNP at temperatures lower than 24°C. Similarly, *Calceolaria herbeohybrida* Cav. Stout (a facultative LDP) flower induction will occur under SD conditions (Poesch, 1931). The critical daylength of *Campanula isophylla* Moretti. is 14 hours with temperatures of 15-21°C, however, it increases to 15 hours when temperatures exceed 21°C (Heide, 1965). In most cases, this is due to an increase in the critical photoperiod required for flower induction (Heide and Runger, 1985).

It is also common with many SDP to exhibit delayed flowering under inductive SD conditions when night temperature exceeds 22°C. This phenomenon is commonly referred to as 'heat delay' in the commercial floriculture industry. High night temperature inhibition of flowering has been studied extensively with *E. pulcherrima* and *Dendranthema x grandiflora* (SDP). In addition to these species, *Gomphrena globosa* L. exhibits a heat delay when night temperature is 25°C (Warner et al., 1997). In general, there is evidence that as night temperature increases above 22°C that the length of an inductive long night for successful flowering increases. Therefore, heat delay can be overcome in some cases by providing a longer night to SDP grown with night temperatures higher than 22°C.

3.2 The Physiological Basis for Photoperiodism

Plants perceive daylength by 'measuring' the night length. Short-day plants are determined by the length of the night, regardless of the daylength. Similarly, LDP also measure night length and only flower when the length of the night is less than some critical night length regardless of the length of the photoperiod.

The importance of the night length in determination of photoperiodic responses with respect to flowering was demonstrated by early experiments by Hamner and Bonner (1938) and Hamner (1940) where flowering of *Xanthium strumarium* and *Glycine max* (L.) Merrill only occurred when the night length exceeded 8.5 and 10 hours, respectively. Night break lighting of as little as a few minutes in *X. strumarium* or *Pharbitis nil* (both SDP) prevented flowering even when the total night length was sufficient to promote flowering. Similarly, night break lighting can result in stimulation of flowering in LDP even when night length is inhibitory to such flowering. In general, the length of time of a night break required to inhibit

flowering in SDP is considerably less than the time required to promote flowering in LDP.

The point at which a night break occurs greatly impacts the effectiveness of that break in either promoting or inhibiting flowering. In general, SDP and LDP are most sensitive to receiving a night interruption eight hours after the onset of darkness (Vince-Prue, 1975; Salisbury, 1963; Papenfuss and Salisbury, 1967). Harder and Bode (1943) showed that inhibition of *Kalanchoe blossfeldiana* flowering by light was most effective when the night interruption occurred between 5-7 hours after the onset of darkness. In contrast, Salisbury and Bonner (1956) showed that *X. strumarium* was most sensitive to night interruption lighting eight hours after the onset of darkness. This observation is the basis for the commercial practice of interrupting the night from 10 pm to 2 am (2200-0200 HR) with low intensity lighting. Such an interruption is long enough to achieve a desired promotive or inhibitory effect and is delivered at the critical time of the night.

The observation of night interruption lighting led to the development of an action spectra for photoperiodism. Different wavelengths of light varied in their ability to inhibit flowering of the SDP *X. strumarium* (Hendricks and Borthwick, 1954). Red light (640-660 nm) was shown to be most effective in inhibiting flowering. Perhaps most significant was the observation that the night-break action spectra for inhibition of flowering in SDP (*Glycine* and *Xanthium*) was similar to that for stimulation of flowering in LDP (*Hordeum vulgare* and *H. niger*) indicating a similar pigment as a photoreceptor (Borthwick et al., 1948).

Interestingly, species can differ in the 'mixture' of light that is optimal for stimulation or inhibition of flowering. Whereas many SDP are most sensitive to red light (660 nm), stimulation of flowering in some LDP by a night interruption is increased when there is a mix of both red and far red light. It is for this reason that induction of flowering in the LDP *Fuchsia* x *hybrida* requires a light source that contains both red and far red light (incandescent lamps and non-fluorescent lamps).

The observation that light delivered in the middle of the night inhibited flowering of tobacco (a SDP), led to the discovery of phytochrome. Borthwick et al. (1952) showed that red light was the primary color of light which inhibited flowering when delivered as a night break. In contrast, a subsequent exposure to far red light restores the flowering response and suggested a photoconversion process (Downs, 1956). Action spectra of the inhibition of flowering and an action spectra for reversal of inhibition identified a peak at 660nm and 720-740nm, respectively (Saji et al., 1983). There is also evidence for the involvement of a blue light receptor although these data are not as clear as with phytochrome involvement (Millar et al., 1995; Hicks et al., 1996; Zagotta et al., 1996).

Subsequent to this work was the significant finding that the red light inhibition of flowering was reversible by far red light (720 nm). Hendricks et al. (1956) demonstrated that red/far red light effects were reversible. This reversibility was

likely a result of two forms of a pigment: a red and far red light absorbing form. The pigment was named phytochrome (Butler et al., 1959).

The variation in sensitivity of plants during the dark period to a night interruption lighting is not well understood. Bunning (1948) suggested that plants exhibit diurnal cycles between a photophile and skotophile phase. The photophile phase was light sensitive and the skotophile phase was not. Carr (1952) subsequently showed that the effectiveness of night interruption lighting in inhibiting flowering followed a periodic 24 hour rhythm when the night was extended. Important to such a time keeping mechanism is the observation that this rhythmicity is mostly temperature independent (Bunning, 1963; Takimoto and Hamner, 1964).

It is important to note that the effectiveness of night interruption lighting in inhibiting flowering of SDP is affected by temperature. The effectiveness of a red light night interruption decreases as temperature increases from 18.5 to 25°C (Takimoto and Hamner, 1964). In addition, the length of night required to stimulate flowering in SDP is altered by temperature. The degree to which temperature affects this required night length for flower induction in SDP is species specific (Salisbury and Ross, 1969). In addition, those plants that are sensitive to high temperatures in the night are most sensitive 8 hours after the onset of darkness.

3.3 Photoperiodic Stimulus

The photoperiodic stimulus is perceived in the leaves. When a single leaf of *X. strumarium* is exposed to short days and the rest of the plant is grown under long days, the plant will flower (Hamner and Bonner, 1938). Similarly, in a classic study by Zeevaart (1969), a single excised leaf of *Perilla crispa* (L.) Britt. exposed to short-day conditions was capable of repeatedly causing plants grown under long days to flower after being grafted to those plants. Interestingly, stimulation of flowering occurred even with a day-neutral plant when a single leaf of *Glycine max* 'Agate' was grafted onto the short-day *G. max* 'Biloxi'. Flowering was induced in 'Biloxi' even when grown under long days (Heinze et al., 1942). Also, leaves that were induced indirectly via grafting experiments can themselves also be donors and result in flowering of non-induced plants (Lona, 1946; Zeevaart and Lang, 1962; Wellensiek, 1966). Studies by Handro (1977) demonstrated that only *Streptocarpus nobilis* L. tissues derived from leaf tissue were capable of flowering *in vitro* under inductive conditions.

The degree to which an induced leaf can induce a non-induced donor plant to flower can vary with species and the degree to which the leaf has been induced. *Xanthium strumarium* leaves will remain induced for a few days and are competent in inducing plants to which they are grafted (Carr, 1959). Imamura (1953) showed that a *Pharbitis nil* leaf also was also induced for a few days following a single inductive night but could remain in an induced state for many days when the leaves

received numerous SD compared to one. Similarly, *P. crispa* leaves could be inductive for a few days or many months depending on the number of inductive short-days received prior to removal from the donor plant (Zeevaart, 1957).

Grafting between species in a different photoperiodic class showed the stimulus to be similar across species. For instance, grafting LDP *Hyoscyamus niger* L. to SDP *Nicotiana tabacum* L. caused the SDP to flower (Melchers and Lang, 1941). Although more difficult, pairing a flowering SDP to a LDP has induced flowering in the LDP (Lang and Melchers, 1943; Zeevaart, 1957). In addition, flowering of parasites such as dodder can be stimulated when it is parasitizing flowering plants from any photoperiodic class (Frattianne, 1965). Together, the grafting experiments suggest the existence of a mobile flowering promoter, which is produced by induced leaves. The term used to describe this promoter is 'florigen'. Although considerable effort has been spent trying to identify the nature of florigen, it remains unknown.

In contrast to the theory that photoperiodism is associated with a floral promoter, there is evidence that photoperiod can affect the synthesis of a floral inhibitor. Removal of all leaves of the LDP *Helleborus niger* resulted in flowering suggesting that the leaves produced an inhibitor and photoperiod (LD) acted to depress the synthesis of that inhibitor during LD (Lang and Melchers, 1943). Guttridge (1959) was able to show a similar induction of flowering following leaf removal on the SDP strawberry (*Fragaria*). Leaves from a photoperiodic tobacco (non-inductive conditions) are capable of inhibiting flowering of a day-neutral tobacco cultivar (Lang et al., 1977).

Wellensiek (1959) identified an association between the day length and degree of inhibition in SDP. Supportive of the flowering inhibitor concept is the observation that reduced irradiance during the day (Krumweide, 1960) or low temperatures (Wellensiek, 1959; Ogawa, 1960; de Zeeuw, 1957) can promote flowering under non-inductive photoperiods. However, this contradicts more recent work when higher irradiance levels overcome a LD requirement in *H. moscheutos* when grown under SD conditions (Warner and Erwin, 2003b).

Regardless of whether there is a chemical promoter/inhibitor, this substance is believed to be transferred in the phloem. Imamura and Takimoto (1955) estimated the timing of the flowering stimulus by developing a two-branched *Pharbitis nil* system and identifying the node at which flowering was induced. Subsequent experiments showed translocation of the compound from the leaf to a receptive bud is comparable or somewhat slower than that for the translocation of sugars (2-4 mm h^{-1}) (Evans and Wardlaw, 1966; King et al., 1968).

Of all the known plant growth regulators, there is evidence that a promoter/inhibitor of flowering is associated with gibberellins. A 100 ppm application of GA_3 to a facultative SDP *Cosmos* was able to substitute for SD when plants were grown under an 18 hour photoperiod (Wittwer and Bukovac, 1959). *Lolium temulentum* plants are committed to flower after exposure to a single long

day (plants will not flower under SD). Excised terminal meristems exposed to a single long day will flower in tissue culture in the presence of GAs. Short-day-exposed meristems in tissue culture will not flower in the presence of GAs. Therefore, long days are required for determination of *Lolium temulentum* L., and GAs are required for the expression of the determined state (McDaniels and Harnett, 1996).

4. VERNALIZATION

Vernalization is the low temperature induction of flowering in an imbibed seed or a growing plant (Chouard, 1960; Taiz and Zeiger, 1998). As with photoperiodism, plants must advance through the juvenile stage into an adult stage before they have the capacity to perceive a vernalization treatment. The length of time required for this varies. Winter annuals have the capacity to perceive a vernalization treatment as an imbibed seed. *Raphanus sativus* similarly has the capacity to perceive a vernalization treatment as an imbibed seed.

Plants can be vernalized with a wide range of temperatures. In general, vernalization can occur at temperatures ranging from -6 to 14°C (Chouard, 1960). There is an optimal temperature range from 6-10°C for most species (Lang, 1959; Chouard, 1960). Still other studies show that plants can be vernalized at temperatures between 5 and 17°C. However, the length of time that plants must be exposed to cool temperatures increases as the temperature deviates from the common optimum of 4°C.

The time required for complete vernalization varies with species as well. For instance, *Lunaria biennis* L. requires nine weeks (Wellensiek, 1958); *Apium graveolens* L. (celery) and *Secale cereale* L. (winter rye) require six weeks (Ramin and Atherton, 1991; Friend, 1965), respectively. *Alstroemeria* L. hybrids commonly require six weeks at 5°C (Healy and Wilkins, 1981, 1982). In contrast, vernalization time can be as short as 6-8 days for *R. sativus* 'Chinese Jumbo Radish Scarlet' (Englen-Eigles and Erwin, 1997; Erwin et al., 2002). Some species exhibit sensitivity to vernalization immediately after seed is imbibed; a subsequent period of temperature insensitivity is followed by a period of increasing sensitivity to low temperature induction (Napp-Zinn, 1969).

The length of time required for vernalization is related to whether a species has an obligate or facultative vernalization requirement. Plants that require a short vernalization period (<30 days) such as *Brassica campestris pekinensis* L. (Suge, 1984) and *Arabidopsis thaliana* L. (Bagnall, 1993; Martinez-Zapater and Somerville, 1990) often have a facultative vernalization requirement and photoperiod is often the primary flower induction stimulus. In contrast, plants that require a long vernalization period often have an obligate vernalization requirement.

A noted exception to this is *R. sativus* "Chinese Jumbo Radish Scarlet' (Englen-Eigles and Erwin, 1997).

4.1 Perception of Vernalization

The site of perception of vernalization is the shoot tip. Studies conducted where different portions of the plant were cooled relative to the rest of the plant indicate that the shoot tip is solely capable of perception (Curtis and Chang, 1930; Metzger, 1988, 1996). Similarly, Gregory and de Ropp (1938) showed that excised winter rye embryos, or even a fragment thereof, can still be effectively vernalized in tissue culture. However, Wellensiek (1961, 1962) reported that young expanding leaves of *Lolium biennis* were capable of being vernalized. The commonality between there two tissues is the presence of active cell division. Therefore, active cell division is required for vernalization as isolated cells in tissue culture (originating from different locations on a mother plant) are capable of perceiving vernalization as well (Metzger et al., 1992).

Once tissue has been effectively vernalized, all growth that develops from that tissue is vernalized (Schwabe, 1954). Regrowth of temperature perennials following flowering results in a 'reversion' to a non-vernalized state in those buds (Schwabe, 1954). Unlike photoperiodism, the vernalization stimulus is not graft transmissible.

4.2 Devernalization

The period between the completion of a vernalization treatment and flower initiation can be divided into two phases. Phase I is a period immediately after vernalization when flower induction can be reversed/eliminated by exposure to warm temperatures, low irradiance, and/or SD conditions. The reversal of vernalization by environmental conditions is referred to as 'devernalization' (Lang, 1965). Phase II is that period after Phase I when flower induction is stable and cannot be reversed.

Devernalization refers to the reversal of the vernalization process (Purvis and Gregory, 1952). This results from an interaction between the degree of vernalization and environmental conditions immediately after the vernalization treatment, i.e. during Phase I (Table 1-4). The most common agent of devernalization is high temperature (25-30°C). However, devernalization by short day conditions and/or low irradiance during or after vernalization has also been demonstrated in some species (Thomas and Vince-Prue, 1997). Devernalization treatments are most effective immediately after vernalization, if a plant is not completely vernalized (Table 1-1), when light intensity during and/or after vernalization is low, and/or when plants are exposed to short-day conditions (Thomas and Vince-Prue, 1997). Interestingly, exposure of *Beta vulgaris* to SD caused devernalization at any stage of development, even when stem elongation had initiated. *Oenothera biennis* L. and

Cieranthus allionii L. can also be devernalized by transfer to short day conditions (Wellensiek, 1965). In fact, nearly all cold-requiring plants can hypothetically, can be devernalized. For instance, Petkus winter rye (Purvis and Gregory, 1952), *C. allionii* (Barendse, 1964), carrot (Hiller and Kelly, 1979), cauliflower (Fujime and Hirose, 1980), kohlrabi (Wiebe et al., 1992), celery (Boojiu and Meurs, 1993), *Lilium longiflorum* Thunb. (Miller and Kiplinger, 1966) and cineraria (Yeh et al., 1997) can all be devernalized. Devernalization can be a commercial problem in Easter lily, cineraria and Regal geranium production (Erwin, unpublished data). The first five days after the vernalization appear to be the most critical period, i.e. non-optimal temperatures or irradiance after this period have little impact on induction (Erwin, unpublished data).

Table 1-4. Progressive stabilization of vernalization with increasing duration of exposure to cold (2-8 weeks) in winter rye 'Petkus' (adapted from Thomas and Vince-Prue, 1984).

Cold treatment	2 wks.	3 wks.	4 wks.	5 wks.	6 wks.	8 wks.
% plants remaining vernalized >2 days at 25C	0	42	44	75	84	97

4.3 Photoperiod Interactions

Photoperiod interacts with vernalization to affect flower induction (Yui and Yoshikawa, 1991; Thomas and Vince-Prue, 1997; Yeh et al., 1997). In some cases, as with winter cereals, short day conditions substitute for vernalization entirely. For instance, *Coreopsis grandiflora* Hogg ex Sweet. Per. is a SDP-LDP plant; however, the short day requirement can be substituted with a vernalization treatment (Ketellapper and Barbaro, 1966). In contrast, long day conditions are additive with vernalization in hastening flower initiation of some species, including *L. longiflorum* (Wilkins, et al., 1968). In addition, thermoinduction affects photoperiodic requirements after vernalization (Thomas and Vince-Prue, 1997). Most cold requiring plants require long-day conditions after cooling. The number of long days required/the critical daylength required for flowering after vernalization decreases as the length of the vernalization treatment increases (Lang, 1965).

4.3.1 Irradiance & Light Quality Interactions

Irradiance during vernalization is critical with some species. For instance, *Apium graveolens* does not perceive vernalizing temperatures unless plants are exposed to light during the vernalization process (Ramin and Atherton, 1994). Similarly, *Dianthus caryophyllus* L. and *Ajuga reptans* L. flowering was hastened as the irradiance during vernalization increased (Elzroth and Link, 1970).

Recent research demonstrates that a low red:far red light ratio is antagonistic to vernalization of *R. sativus* (Engelen-Eigles, 1996) and *Lilium longiflorum* (Erwin and Engelen-Eigles, unpublished data). In contrast, a high red:far red ratio appears to hasten vernalization. For instance, *L. longiflorum* plants were completely induced by a vernalization treatment in 4 weeks when bulbs were exposed to light with a high red:far red ratio but were not completely induced until after 8 weeks when bulbs were exposed to light with a low red:far red ratio.

4.4 Stage of Sensitivity to Vernalization

The point at which a plant becomes sensitive to a vernalization treatment or the length of the juvenile period varies with species. Some species can be vernalized as imbibed seed. For instance, *Beta vulgaris*, *Oenothera* (Thomas and Vince-Prue, 1997) and *Raphanus sativus* (Englen-Eigles and Erwin, 1997) can be vernalized as imbibed seed. In contrast, most plants must reach a specific stage of development before the meristem is capable of responding to vernalizing temperatures. *Helleborus niger* must grow for ten days and *L. biennis* must grow for seven weeks before plants will respond to a vernalization treatment (Thomas and Vince-Prue, 1997). *Daucus carota* 'Chantenay Red Cored' required 8-12 leaves before plants were capable of responding to vernalizing temperatures (Atherton et al., 1990). Similarly, many herbaceous perennial species have a minimum leaf requirement (often >10) before plants can successfully induce flowering in response to a vernalization treatment (Lopes and Weiler, 1977; Sheldron and Weiler, 1982a, b; Iverson and Weiler, 1994; Armitage et al., 1996; Whitman et al., 1996). In addition to variation among species, variation in sensitivity among cultivars of a given species in leaf number required to achieve a mature state and in the required length of the vernalization treatment to induce flowering also exists (Yui and Yoshikawa, 1991; Wurr et al., 1994, Englen-Eigles and Erwin, 1997).

4.5 Stability of the Vernalization Process

The vernalization process, once saturated, can be very stable (Table 4) (Yeh et al., 1997). For instance, once *Hyoscyamus* is vernalized (requires both vernalization and long-days for flowering), the vernalized state can persist for several months under short day conditions: flowering occurs when plants are returned to long day conditions (Thomas and Vince-Prue, 1997). *Lilium longiflorum* bulbs exposed to vernalizing temperatures retained that information even when several warm temperature periods occurred prior to the completion of a vernalization treatment (Erwin and Engelen-Eigles, 1997). No loss of the vernalization stimulus was evident on henbane after 190 days. In fact, there was no measurable loss in degree of vernalization until 300 days had passed (Thomas and Vince-Prue, 1997). Similarly, some cereal seeds can be moistened, vernalized, and then re-dried and

maintained for months without loss of the vernalization status (Purvis and Gregory, 1952).

4.6 Physiological Basis for Vernalization

Early work on the physiology of vernalization focused on the identification of a specific cold-induced, gibberellin responsible for flowering (Lang, 1965). Gibberellins are known to interact with vernalization to affect flowering of a number of species (Metzger and Dusbabek, 1991). Application of GAs to certain biennial species, including wild-type *Arabidopsis thaliana* grown under unfavorable conditions for flowering, promotes flower formation and bolting (Lang, 1957; Zeevaart, 1983). Conversely, application of 2-chloroethyltrimethylammonium chloride (CCC – Cycocel), a GA synthesis inhibitor, delayed flowering in several wild-type *A. thaliana* lines and suggested that the presence of GA is essential for direct flower induction (Napp-Zinn, 1985).

Although exogenous applications of specific gibberellins can overcome a cold-requirement for flowering of some species, these applications are successful on some species and not others (Ketellapper and Barbaro, 1966). It has been proposed that the vernalization process is associated with a progressive demethylation of DNA. Demethylation of DNA is believed to remove a block in gene expression that ultimately leads to floral initiation. Evidence for this theory is based on effects of exogenous 'demethylators' on flower induction and changes in the degree of demethylation of DNA during cooling. For example, application of 5-azacytidine, a demethylator, promoted flowering of non-vernalized cold requiring *Thlaspi* and *A. thaliana* (Metzger, 1996). In addition, vernalizing temperatures result in demethylation of plant DNA (Burn et al., 1993). Transformed *A. thaliana* that have reduced levels of DNA methylation because of the presence of a methyltransferase (METI) antisense gene flowered earlier than untransformed control plants (Finnegan et al., 1998). However, a 70% reduction of methylation occurred in antisense plants where the promotion of flowering was comparable to that of a vernalization treatment that resulted in only a 15% decrease in DNA methylation. These data suggest that other methyltransferases may be affected by vernalization, since METI only affected one.

5. IRRADIANCE INDUCTION OF FLOWERING

In general, increased irradiance reduces the length of the juvenile period with many plant species. In contrast, low irradiance can extend the length of the juvenile period with some species. Irradiance reduction of the juvenile period is the basis for the common practice of lighting seed geraniums for earlier flower induction where

the general 'rule-of-thumb' is that every day those seedlings are lighted with supplemental light results in a single day decrease in time to flower.

In addition to photoperiod, irradiance can affect earliness of flowering of many species (Armitage and Tsujita, 1979; Armitage et al., 1981; Dole and Wilkins, 1999; Erwin et al., 1997; Zhang et al., 1996). For instance, *Achillea millefolium* L. 'Summer Pastels' grown under a 16 hr photoperiod in growth chambers flowered after 57, 45, and 37 d when grown under 100, 200, or 300 µmol m^{-2} s^{-1}, respectively (plant temperature and leaf number below the first flower not reported) (Zhang et al., 1996). An increase in mean dry weight gain per day (MDWG) from 0.32 to 1.02 g d^{-1} occurred as irradiance increased from 100 to 300 µmol m^{-2} s^{-1} in *A. millefolium* (Zhang et al., 1996). Armitage and Tsujita (1979) grew four *Pelargonium* x *hortorum* Bailey cultivars under natural daylight conditions (Feb-Mar 1977, Guelph, Ontario, Canada) plus supplemental HPS and LPS lighting (27 or 54 µmol m^{-2} s^{-1}). Irradiance from high pressure sodium (HPS) lamps (27 µmol m^{-2} s^{-1}) hastened flowering for some cultivars but not others, suggesting that *P.* x *hortorum* cultivars flowering responses to supplemental HPS lighting varied. *Asclepias curassavica* L. node number below the inflorescence decreased from 21 to 16 nodes when plants were grown under continuous lighting versus night interruption lighting (Nordwig, 1999). However, it is often unclear whether irradiance effects are a result of a hastening of flowering strictly through infrared heating from supplemental lighting or from a reduction in juvenile period length as determined through leaf number data.

More recent studies on irradiance effects on flowering have sought to distinguish between thermal hastening of flowering versus developmental hastening of flowering. Erwin et al. (1997) showed supplemental lighting hastened flowering developmentally in *Viola x wittrockiana* and *P. x hybrida*. For example, *P. x hybrida* 'Fantasy Pink Morn' flowered in as little as 28 d after germination when grown at constant 24 ºC under 8-9 hr ambient daylight conditions (St. Paul, Minn) plus continuous 100 µmol m^{-2} s^{-1} HPS lighting. Similarly, Adams et al. (1999) showed *P.* x *hybrida* flowering was hastened by long days, but that decreased daily light integrals (DLI) lengthened the time to flowering. Pearson et al. (1993) also noted that increasing DLI shortened the time to anthesis for *Dendranthema x grandiflora*. Further, the reduction in time to flower was related to DLI rather than to maximum light intensity. Erwin and Warner (2002) reported that 11 species flowered earlier developmentally, when plants were grown with ambient light (10-14 hr photoperiod, Sep 1999-May 2000, St. Paul, Minn.) plus 25-50 µmol m^{-2} s^{-1} HPS lighting for up to 18 hrs daily. Hedley (1974) showed that *A. majus* 'Orchid Rocket' flowering was hastened developmentally (leaf number below the flower decreased from 80 to 34 leaves) under long-day conditions as irradiance increased from 115 to 500 *u*mol m^{-2} s^{-1}.

We have 'coined' the term '**facultative irradiance response'** to describe a developmental hastening of flowering by addition of supplemental lighting. Species

that exhibit a facultative irradiance response (FI) will show a decrease in leaf number below the first flower as irradiance increases (Erwin and Warner, 2002). The increased irradiance is capable of reducing the length of the juvenile period. In contrast, we refer to plants that do not have hastened flowering, developmentally, in response to increased irradiance as '**irradiance indifferent'** (Erwin and Warner, 2002). With these species, increased irradiance does not reduce leaf number below the first flower, i.e. juvenile period length is not reduced developmentally with additional lighting.

Some species exhibiting a facultative irradiance response include: *Convolvulus*, *Dianthus*, *Gazania*, *Lavatera*, *Limnanthes*, *Linaria*, *Nemophila*, *Nicotiana*, oregano, *Silene*, snapdragon, petunia, pansy, seed geranium. Some species with a neutral irradiance response include: *Ageratum*, *Celosia*, *Cleome*, *Cosmos*, *Gomphrena*, *Statice*, *Lobelia*, *Mimulus*, *Nigella*, *Salvia*, *Tithonia*, *Zinnia*.

The majority of species studied here did not flower earlier developmentally with increasing irradiance (Table 1-3; Warner and Erwin, 2002; Mattson and Erwin, 2003a, b). Erickson et al. (1980) found a linear relationship between DLI and the DTF for *P.* x *hortorum* until a threshold level between 6.89 and 9.01 µmol m^{-2} d^{-1} was reached (leaf number not reported). Fausey et al. (2001) noted that 100% flowering of *Digitalis purpurea* L. 'Foxy' was only reached with DLI greater than 11 µmol m^{-2} d^{-1}. Supplemental irradiance (at 30, 60, and 90 µmol m^{-2} s^{-1}) on *Gerbera jamesonii* H. Bolus hastened flowering by up to 23 d in the winter, but only up to 11 days during the spring (leaf number below the first flower not reported) (Gagnon and Dansereau, 1990). Thus, the impact of supplemental irradiance on flowering can be dependent on ambient light conditions. In our experiment, average DLI's (Table 1-2) under all lighting treatments were greater than the threshold levels found by Erickson et al. (1980) and Fausey et al. (2001). It is likely that the light saturation point for impact on flower induction was reached under the lowest irradiance treatments for many of the species during the relatively high natural light conditions of spring in Minnesota. The five species identified as having a FI response represent plants that likely have a higher threshold of light than other species.

The hastening of flowering with exposure to supplemental irradiance is likely due to two components: increased plant temperature from infrared and long-wave radiation from the HPS lamps and additional light available for photosynthesis. More research is needed to determine if FI responses seen on some species are a result of increased photosynthesis or a photomorphogenetic high irradiance response (Beggs et al., 1980). In our experiment, ADT at plant level was 1-2°C greater under the highest lighting treatments (data not shown). Faust and Heins (1998) reported that supplemental irradiance increased shoot-tip temperatures of *Catharanthus roseus* L. by 1.2, 1.5, and 1.7 ºC with HPS light additions of 50, 75, and 100 µmol m^{-2} s^{-1}, respectively. Pietsch et al. (1995) concluded that increased development rates of *Catharanthus roseus* under supplemental light were a result of increased

shoot-tip temperatures. Graper and Healy (1991) conducted an experiment to separate the components of HPS lighting into the effects of thermal radiation vs. photosynthetic photon flux (PPF) using a circulating water bath. They concluded that light plays a greater role in development of *Petunia* x *hybrida* seedlings via greater photosynthesis than the increase in plant temperature associated with supplemental HPS lighting. Leaf number below the first flower was not reported.

Clearly, controlled environment chamber experiments where lighting conditions can be precisely controlled are necessary to more accurately evaluate the effect of irradiance on herbaceous ornamental plant species flower induction. Determining the threshold levels of irradiance required for optimal growth and impact on flower induction of herbaceous ornamental plant species would be useful information for growers deciding whether to purchase supplemental lighting.

6. STRESS INDUCTION OF FLOWERING

Short-term plant stress will cause a shortening of the juvenile phase in some species. Perhaps the best example of this phenomenon is the induction of early flowering in celosia after a short-term water stress (Erwin, unpublished data). Similarly, it appears as though short-term heat stress can cause earlier flowering on newer cultivars of garden mums. High or low temperatures can also affect a plant's photoperiodic response. For instance, at temperatures below 17°C, cineraria is a day-neutral plant. At temperatures above 17°C, cineraria is a short-day plant. In contrast, when temperatures exceed 23°C, flowering of many species can be delayed. Some species are sensitive to high night temperatures (*Euphorbia pulcherrima*, *Gomphrena globosa*, *Tagetes erecta*), some species are sensitive to high day temperatures (*Fuchsia* x *hybrida*, *Antirrhinum majus*), and some species are sensitive to high day or night temperatures (*Impatiens hawkeri*, *Dendranthema x grandiflora*, *Schlumbergera truncata*, many *Pelargonium* spp.) (Erwin and Warner, 1999a).

6.1 Ethylene

Exposure to ethylene is capable of inducing flowering in some plant species. Most notably, ethylene exposure induces flowering in Bromeliads. Clark and Kerns (1942) showed that application of auxin to pineapple could stimulate early flowering. Subsequent research by Burg and Burg (1966) demonstrated that the induction of flowering was associated with auxin induced stimulation of ethylene. Commercially, pineapple is now stimulated to flower by applying ethephon which releases ethylene on plants. Application of ethephon also stimulates early flowering in mango (Chacko et al., 1976).

7. FLOWER DEVELOPMENT

Once flower induction and initiation have occurred, successful flower development must follow for flowering. This can require specific environmental conditions. For instance, some species require a specific photoperiod (which can be different than that for flower induction) for successful flower development. Additionally, species may have a minimum irradiance required for flower development, as was noted with *Rosa spp*. previously (Nell and Rasmussen, 1979). Temperature also interacts with flowering as described below. In contrast, other species may have no environmental requirements for successful flower development other than a lack of stress.

7.1 Photoperiod Requirement

Flower development can have a photoperiodic requirement. For instance, *Dendranthema x grandiflora* flower development has a shorter critical photoperiod than for flower induction. *Dendranthema x grandiflora* (SDP), therefore, has evolved in such a way as to naturally induce flowers as daylength starts to shorten after June 21st and then has successive flower development as the daylength continues to shorten into the fall of the year and the vernal equinox. Therefore, if a grower induced *D. x grandiflora* in the spring and then exposed plants to a photoperiod greater than the critical photoperiod for flower development, development would cease and a 'crown bud' would develop (Dole and Wilkins, 1999). Similarly, if a LDP is induced very early in spring and placed back under short-day conditions after induction in a lighted environment too early, flower bud development can be arrested.

7.2 Temperature Requirements

There is an optimal temperature for flower development that is likely species, photoperiod, and irradiance dependent. Whether temperature effects are due to impacts on the latter stages of flower initiation or whether the effect is on actual flower bud abortion is not clear. Likely, depending on the timing of non-optimal temperature conditions, both conditions are occurring. Additionally, whether non-optimal temperature conditions constitute a 'stress' is a matter of debate. Here, non-optimal temperature conditions are separated from temperature stress as discussed below.

High temperature inhibition of flowering has been noted for some time. For instance, the node number at which the first flower cluster appears in *L. esculentum* increases dramatically at 27°C versus 10-16°C (Calvert, 1957). In general, many plant species have temperature optima for flowering that are between 18 and 22°C. As temperature increases or decreases from this optimum, flower number decreases.

There is evidence that anecdotally suggests that these temperature optima are based on differences in flower bud abortion during development. Such a conclusion is based on the observation that temperature significantly affects flower number on mature day neutral plants where flower induction is continuous. For example, *Pelargonium spp*. flower number per inflorescence decreases as average daily temperature increases. Flower number per inflorescence on *P. peltatum* 'Nicole' decreased from 9 to 3.8 flowers per inflorescence as average daily temperature increased from 12 to 29°C (Erwin, 1999). Similarly, *P.* x *domesticum* flower number per inflorescence decreases as average daily temperature decreases and flowering is inhibited altogether at average daily temperatures above 17°C (Erwin and Englen, 1992). *Pelargonium* x *hortorum* 'Veronica' flower number decreased from 52 to 15 flowers per inflorescence as average daily temperature increased from 12 to 29°C (Erwin and Heins, 1992). *Fuchsia x hybrida* L. 'Dollar Princess' flower number per node increased from 2.3 to 6 flowers as average daily temperature increased from 12 to 15°C then decreased to 2.3 flowers per node as average daily temperature further increased to 25°C (Erwin and Kovanda, 1990).

Flower development of different species is sensitive to temperature and can vary diurnally. *Schlumbergera truncata* and *Euphorbia pulcherrima* flower development is most sensitive to night temperature. For instance, flower number was greatest on *S. truncata* 'Madisto' when night temperature was 20°C (Erwin et al., 1990). In contrast, *Antirrhinum majus* flower development appears to be most sensitive to day temperature. *Impatiens hawkeri*, *Dendranthema x grandiflora*, and *Fuchsia x hybrida* flower development is most sensitive to day and night temperature. *Pelargonium spp*. flower development appears to be most sensitive to average daily temperature (Erwin and Warner, 1999a).

7.3 Impact of Stress

High temperatures can decrease flowering. Whether such temperature exposures constitute a 'stress' is a matter for debate. However, temperature exposure that results in an extended period of reduced growth should be considered a stress.

Prolonged exposure to high temperatures reduces flowering of numerous flower crops. Exposure of *Antirrhinum majus*, *Calendula officinalis*, *Impatiens wallerana*, *Mimulus* x *hybridus*, and *Torenia fournieri* to high temperature reduced flowering of all species compared to cooler temperatures across a variety of irradiance treatments (Warner and Erwin, 2001b). The basis for reduced flower number is likely due, in part, to reduced photosynthesis. Exposure of *Impatiens hawkeri* and *Viola* x *wittrockiana* to high temperatures (35°C) for as short as two hours reduced photosynthetic rates for at least three days for some cultivars compared to 'un-stressed' plants (Warner and Erwin, 2002). Photosynthetic recovery varied between *I. hawkeri* and *V.* x *wittrockiana,* with the latter recovering more quickly.

Such variation in high temperature tolerance can be seen across cultivars within a species as well. For instance, prolonged exposure of *V*. x *wittrockiana* to high temperatures (30°C) compared to a 'non-stress' environment (20°C) reduced flowering of all cultivars studied (Warner and Erwin, 2003a). However, there was cultivar variation with flowering being reduced 23% for 'Crystal Bowl Purple' and 79% for 'Majestic Giants Red and Yellow'. Similarly, the effect of high temperature exposure (32/28°C day/night) affected flower number per cluster on *Lycopersicon esculentum* cultivars differently (Warner and Erwin, 2003a). Lastly, Strope (1999) showed *I. hawkeri* cultivars varied in their ability to continue to flower under high temperature conditions (30°C).

In addition to reducing flower number, yield in some crops can be reduced by high temperature induced floral sterility resulting in reduced seed set, fruit size, and yield. Such high temperature reduction in yield is a common problem in commercial horticulture production of peppers as well as many crops worldwide.

High temperature sensitivity of flowering may be due to reduced overall carbon assimilation under high temperatures (Ranney and Peet, 1994), increased respiration (Berry and Bjorkman, 1980), or to a reduced ability of floral organs to recruit photoassimilates compared to vegetative tissues (Dinar and Rudich, 1985). Photosynthesis is a metabolic process that is susceptible to high temperature stress (Larcher, 1995). For example, photosynthetic rates of five birch taxa declined sharply when temperature exceeded 30°C (Ranney and Peet, 1994). This may be in part due to a reduction in chloroplast number as chloroplast biogenesis is reduced at temperatures greater than 32°C in barley (Smillie et al., 1978). Respiration rates continue to increase at temperatures above the optimum temperature for photosynthesis resulting in a net carbon loss (Berry and Bjorkman, 1980). Lastly, reduced concentrations of plant growth hormones in reproductive tissues following high temperature exposure likely reduce the ability of those tissues to attract assimilates (Kuo and Tsai, 1984).

The author's laboratory is currently evaluating the physiological basis for high temperature inhibition of flower development. Promising data are emerging showing variation in *Arabidopsis thaliana* heat sensitivity, which may provide a basis for gaining new insight into this significant floriculture crop issue.

8. DORMANCY

Dormancy will only be discussed briefly since it is not directly related to flower evocation, only to continued flower development in a limited number of floriculture crops. Most notably, some bulb crops and woody plant species can initiate flowers, but then have flower development stopped at a specific stage until specific environmental conditions are met (Dole and Wilkins, 1999).

Bulb crops can vary considerably when flowers are initiated during the year and during the life cycle of the species. Hartsema (1961) has classified species into the following five response groups:

1. Flowers initiated during the spring or early summer of the year preceding that in which they reach anthesis and before the bulbs are 'lifted' (*Narcissus*, *Galanthus*, *Leucojum*).
2. Flowers initiated following the previous growing period, so that the bulbs have initiated flowers by replanting time in the autumn (*Tulipa*, *Hyacinthus*, *Iris reticulata*).
3. Flowers initiated after replanting, at the low temperatures of winter or early spring (bulbous *Iris*, but not *I. reticulata*).
4. Flowers initiated more than a year before anthesis (*Nerine*).
5. Flower initiation alternates with leaf formation through the whole growing period (*Hippeastrum*, *Zephyranthes*).

The physiological basis for flowering in each of these groupings can differ significantly. For instance, there is evidence that group 3 has a vernalization requirement, whereas groups 1 and 2 require dormancy. Dormancy requirement is defined as flower development, which can be arrested until the appropriate environmental, or time constraints are fulfilled. For example, spring ephemerals, i.e. *Tulipa* x *hybrida* or *Narcissus pseudonarcissus* are not photoperiodic, as the length of a growth cycle is relatively short. Such plants will only successfully flower after these requirements are met. These plants are said to be '**dormant'**. Temperature control of dormancy is common in temperate perennial species and water control of dormancy is common in locations where water can be limiting, i.e. a desert region. For instance, water stress can induce dormancy in *Achimenes* hybrids (Zimmer and Junker, 1985).

In general, flower bud dormancy can be broken on many woody plant species by exposing tissue to conditions similar to vernalization. Six to 12 weeks of temperatures between 2-6°C is often optimal. In some cases, GA_3 application can overcome dormancy and enhance shoot emergence from tubers (Lurie et al., 1992). Application of GA_3 to overcome flower bud dormancy is common commercially in the florists Azalea (Dole and Wilkins, 1999).

9. CONCLUSIONS

At this point, one must take some latitude and emphasize the need for traditional physiological research on floriculture crops during crop domestication and breeding. It seems that a complete understanding of what factors result in flowering in floriculture crops are essential to the success of any breeding or physiology project related to that crop. It is amazing how much research has been conducted on crops

in which we have little or no understanding of the flowering mechanisms in that crop! How valid are the conclusions?

Our recent work on photoperiodism in annual crops was started by questions from Terry Smith (Smith Garden, Bellingham, Washington) when he simply wanted to know how to flower petunias from February 1 to March 1. What grew out of that question was a realization flowering of many of the commercially significant bedding plant crops is in its infancy. There is clearly a need to renew fundamental research on the flowering physiology of flower crops to meet the needs for scheduling, timing and marketing that are upon the floriculture industry. A fundamental understanding of flowering physiology of floriculture crops will provide the foundation from which we can use classic flower breeding and molecular biology to improve our crops and enhance our understanding of photoperiod and temperature effects.

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Chapter 2

CREATION OF NEW FLORAL PRODUCTS

Annualization of Perennials--Horticultural and Commercial Significance

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Abstract: The recent surge in popularity of new floral products has been due to a variety of factors. Annualization of perennials is one such factor and has primarily arisen due to the advent of 'annualized' perennials which can be grown from seed or cuttings and flowered the first year. These products offer a distinct advantage to growers who can produce flowering herbaceous perennials quicker thereby creating less expensive cultivars for the gardening public. Since numerous taxa are herbaceous perennials yet to be domesticated and commercialized there are numerous opportunities for flower breeders to create new products. The challenges of breeding, producing, distributing, retailing, and growing reliable perennials throughout the distribution channel are discussed. Traits such as life history, the five factors of plant growth, dormancy, juvenility, flowering (flower bud initiation, development), are provided with numerous examples of crops which require one or many factors to be manipulated to achieve flowering. Marketing and horticultural significance of herbaceous perennials are presented as example methods whereby continued interest in such products can be maintained or increased to continue the vibrant floriculture industry.

Key words: annualized perennials, factors of plant growth, herbaceous perennials, marketing, sales, trends

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1. THE PERENNIAL MARKET

Winter hardy, herbaceous perennials offer many benefits to the gardening public over annual bedding plants. With the advent of computer technology, instantaneous communication and purchasing power on the worldwide web through *e*-Commerce, people's busy schedules leave less time for gardening. Important consequences of these technological innovations have led to consumer demands of more efficient gardening with less time inputs. A noticeable advantage of perennials over annuals is that they only have to be planted once--rather than annually--provided they are winter hardy. In addition, flower breeders have focused on creating low-maintenance perennial products, i.e. self-pinching garden chrysanthemums (*Dendranthema x grandiflora*) in the My Favorite™ series (Anderson, 2002), day neutral plants that do not require deadheading (*Gaura lindheimeri*) (Anderson and Peters, 2002), reflowering cultivars in taxa that normally flower once in a season (*Hemerocallis* 'Stella d'Oro', *Lilium x formolongi, Iris sibirica* 'Butter & Sugar') (Bluebird Nursery, 2002), season extenders flowering beyond the normal time-period for the species (*Geranium sp.* 'Appleblossom', 'Rozanne') (Blooms of Bressingham, 2001), and heat and/or drought tolerant species (*Aster novae-angliae, A. novi-belgii, A. tataricus, Epimedium grandiflorum, E. xperralchium, E. x rubrum, Eryngium agavifolium, Gaura lindheimeri, Yucca filamentosa, Zauschneria californica*) (Armitage, 2000).

A tremendous wealth of perennial taxa is available for spectacular performance in any garden condition and climate throughout the globe (Jelitto and Schacht, 1950-51, 1995). The common "perennial border" in landscape design has been expanded in recent decades by a wide variety of commercialized new crops marketed under novel uses (Still, 1994). Major use categories of perennials include: natives; ornamental grasses; fragrant foliage; fragrant flowers; plants not favored for browsing by deer, rabbits, or squirrels; plants that attract butterflies; plants that attract hummingbirds; sun/shade tolerance; vines or sprawling habit; aggressive plants; plants for wet and boggy places; cut flowers; evergreen (winter interest); drought tolerance; heat tolerance; ground covers; colorful foliage/fruit (Armitage, 2000; Bluebird Nursery, 2002). These categories and monographs have increased consumer popularity with perennials (Armitage, 2000; Still, 1994).

Likewise, emphasis has been placed on propagator (stock plant producers, cutting and rooting stations, plug producers, distributors) and grower (pre-finishers, finishers) traits that have allowed perennials to be grown similar to annual bedding plants. For instance, early flowering (less than 16 weeks from seed to flower for bedding plants), short stature, minimal nutritional inputs, pest/disease resistance, and "annualized" perennials. One of the newer traits of interest to propagators and growers is annualized perennials (Runkle, et al., 2001b; Simbo, 2001). The concept of obtaining flowering seedlings in less than one year made perennials more attractive to growers. This has spurred flower breeders to create annualized

perennials in genera with extensive juvenility periods, as well as obligate cold treatments.

As more and more breeder/producer companies have added perennials to their product offerings, there is a critical need for increased research on herbaceous perennials. The perenniality of these crops, unlike that of annuals, has meant that many additional production, growing, and marketing factors need to be resolved in the commercialization process. In this chapter, we will focus on the major issues that must be resolved prior to the release of a successful new herbaceous perennial product.

2. LIFE HISTORY

A perennial has been defined a plant which lives from season to season, opposed to annuals or biennials, which live for only one or two years, respectively (Bernier, et al., 1981; Chouard, 1960). Hardy herbaceous perennials exclude woody plants. The former non-woody species have a crown and roots, which live over the winter while their tops most frequently die down. There are a few herbaceous perennials which are evergreen (e.g. wintergreen, *Gaultheria procumbens;* heartleaf bergenia, *Bergenia cordifolia*), but these are the exception to the following general characteristics of herbaceous perennials. Thus, in most instances, the root systems and crowns are the surviving portion in an herbaceous perennial. During the winter these plants, biennials, woody and herbaceous perennials, receive a cold treatment. When the environment becomes appropriate (temperature and light duration) their dormant terminal, axillary or adventitious buds (apical meristems) become active. Their respective shoots (still vegetative or previously reproductive) elongate in the spring. The meristem, if vegetative, becomes reproductive and flowers. A biennial plant dies in the second year, whereas a perennial forms adventitious buds and recycles (Bernier, et al., 1981; Chouard, 1960).

While this chapter focuses on herbaceous perennials, similar physiological responses are present in woody perennial species. Regardless, we must now define the state of "inactive growth" during the winter. The broad term used is "dormant" or "dormancy". However, this state is more complex. The term "rest" is used when plants are unable or not capable of responding to favorable growing environmental conditions. Certain internal physiological and biochemical changes must occur and are thought to be occurring during the cold (moist) period or horticultural (commercial) treatments. Quiescence is a term used to delineate that the above "requirement" has occurred, but the correct environmental conditions are not present. Once the environment is correct, growth and flowering occurs (Rees, 1985; Rietveld, et al., 2000). These environmental conditions are temperature and light (Runkle, et al., 1998). Both will be discussed in later sections.

Another factor is juvenility or lack of maturity. If a plant is placed under the optimal or correct environmental conditions for growth, but flowering does not occur, the plant is "juvenile". Therefore, there are a minimum number of leaves and or available carbohydrates before floral initiation can occur. Simply, this is frequently thought of as plant size, a critical leaf number, or apical meristem size. Plant growth is a complex process requiring the proper temperature at various stages of development, as well as, light (quantity, duration, and quality), water, proper levels of ionic nutrients and lastly, carbon dioxide. All of these interact with juvenility. We will mainly discuss temperature and light (duration and intensity) as they interact with the various herbaceous species along with plant maturity (juvenility) or its capacity to flower (Bernier, et al., 1981; Ramen and Atherton, 1991; Runkle, et al., 2001a).

Flowering is frequently controlled by specific cold treatments for floral induction, initiation and early development. Warmer temperatures are required for stem elongation, later stages of flower development, and anthesis. The term most commonly used to describe the cold/moist treatment is vernalization. However, the senior author prefers the simplistic term cold treatment. Why? Frequently the temperature range for flowering is quite large. As an example with *Lilium longiflorum* that range is from 1^{o} to 20^{o}C (Dole and Wilkins, 1999). Secondly, vernalization was first used as an absolute requirement for floral induction with a seed-propagated biennial species, i.e. wheat. With the numerous horticulture herbaceous species within a genus as well as cultivars within a species, floral initials may or may not be present at the beginning of the cold period. Thus, for these basic reasons we will use the term cold treatment. The response of a cold treatment may be indeed required for floral induction or for further differentiation, development, as well as, internode elongation. Some example genera with a cold requirement are *Campanula, Coreopsis, Heuchera, Monarda, Saxifraga,* and *Veronica* (Clough, et al., 2000; Runkle, et al., 2001b).

3. DORMANCY

Low temperatures are commonly used to break dormancy. These low temperature treatments vary in length. For instance, in *Tulipa* a 16-week treatment at 9^{o}C is an average, whereas with *L. longiflorum*, six weeks at 4^{o}C is commonly used (Dole and Wilkins, 1999). These treatments are the most efficient temperature level and duration for commercial expediency. During treatments the primordia may continue to develop slowly. Cold treatments remove growth inhibitors. For example, in *L. longiflorum*, the inner scales that have not experienced a cold treatment contain the inhibitors. The outer scales have endured a cold treatment from the previous winter, such that those inhibitors have been removed. Afterwards, at warmer temperatures, stem elongation occurs and, in time, flowers reach anthesis (Reese,

1983). Seeds of many temperate zone perennials also require a low temperature treatment to germinate and commence the vegetative phase. *Lunaria, Digitalis*, and *Althaea* are additional examples (Metzger, 1996; Wallensiek, 1958).

Cold temperature treatments thus can be used as an absolute (obligate) requirement for floral meristems to form and eventually reach senescence as with *Leucantha* (Runkle, et al., 2000a). Or, these cold treatments are used to merely hasten the process (facultative) response. To further complex the issue, long-day photoperiods can substitute for facultative cold temperature treatments and visa versa. *Anemone, Campanula, Coreopsis, Gaillardia* and *Veronica* are examples (Clough, et al., 2000; Runkle, et al., 2001b)

Regardless, most herbaceous perennials must pass through a juvenile vegetative developmental phase. They must have reached a minimal size, leaf number and/or age. If they are not "mature", they will neither respond to the cold temperature treatment nor to the respective photoperiod required for floral induction, differentiation and development (Wang, et al., 1995).

Cold temperature treatments are progressive and the impact becomes increasingly stable as the length of treatment increases. Frequently, if the respective species are returned to or placed into high temperature, the cold temperature response can be "erased" after brief treatments are given or partially reversed if not given for longer durations. Example crops include *Aquilegia, Astilbe,* and *Gaillardia* (Runkle, et al., 2001b).

In some species gibberellic acid (GA_3) can substitute or partially substitute for the cold requirement for induction of flowering. In other species GA_3 can substitute for the appropriate long day light treatment. This is not true for all species. The complexity of the GA_3 treatment actually resulting is the formation of a reproductive meristem – or – resulting in internode elongation. Regardless, GA_3 can substitute LD as for cold, but not for both. Short day (SD) plants are not responsive to GA_3. Commercially these respective GA_3 treatments are not commonly used for academic interest (Lang, 1965; Hazebroek and Metzger, 1990).

4. JUVENILITY

Juvenility exits in many plants in that they will not flower in response to stimuli (cold and/a photoperiod) until they reach a certain stage (leaf number or age). This response is valid for both long day, short day, day neutral plants, as well as when plants have a cold treatment response. We define juvenility as the early vegetative developmental phase during which the plant is totally non-responsive to any environmental conditions that at some time in the future will promote floral induction and development. There may be, indeed, a period of increasing sensitivity to the respective floral stimulus(-i). Early on the response may be "nothing", then a long-term stimuli will result in a response. Later, as the plant "matures" a relatively

brief treatment time is required for the response to be observed. True this juvenile period will vary between genera within a species and between cultivars within a species (Bernier, et al., 1981; Rameu, et al., 1991).

The actual age is perhaps not an accurate method to determine if plants are mature and capable of responding to the flowing stimulus(-i). Age does not take into consideration the growth rate from seed germination, as influenced by photosynthetic exposure, appropriate temperature for rapid vegetative growth, as well as adequate nutrition and $C0_2$. Leaf number in herbaceous perennial plants is a more frequent measure or method used to determine when the juvenile phase has ended and the meristems are capable of becoming reproductive. This method is not always infallible, as plants grow under poor nutrition or low light levels, as an example, may influence the capability of the meristem to become reproductive. These factors may indeed impact carbohydrate levels. Regardless, leaf number is the most commonly used method (Thompson, et al., 1991). *Gaillardia*, for example, should have developed up to 16 leaves prior to the cold treatment (Yang, et al., 2000).

Horticulturally, the mere presence of a flower is not acceptable if the flower number(s) is not commercially appropriate for sale or public acceptance. Consumers prefer purchase of perennials in flower, with multiple flower buds present for future flowering potential. Physiologically or scientifically the phase shift is of great interest. Thus, in the annualization of perennials, plants must acquire a greater vegetative mass so that, when placed under the respective treatment(s), which is species dependent, the flowering plant will be of an acceptable economic value (Runkle, et al., 2001b).

Other considerations as to the phase shift from juvenile to mature, we frequently consider the increasing meristem size as a prerequisite to flowering capacity. Regardless, once a plant has the capacity to become reproductive this phase is quite stable.

5. FIVE FACTORS OF PLANT GROWTH

Horticulturally one may shorten the time span from seedling juvenility to the capacity to become reproductive and yet produce an acceptable, commercially valuable flowering plant. Consequently, all plant growth factors are optimized [temperature, light duration, intensity and quality, water, maturation, and CO_2] in order to minimize the time for the juvenility phase.

Some techniques used are high intensity discharge (HID) photosynthetic lamps, optimal temperature, constant optimal nutrient, water, and CO_2. Thus, with these strategies the goal is to produce a potted, flowering herbaceous perennial in a limited time period (usually less than 10 months) and not using naturally occurring out-of-door cold temperatures. Further, the aim is to have a perennial plant in flower during

the spring marketing season versus the normal flowering period, which may be normally in the summer or autumn. Traditionally these plants were sold as vegetative plants or sold during their natural flowering season. Sales potential are greater if plants are in flower and particularly during the spring plant buying period. Again, the plants in flower or must be "customer attractive" or acceptable. The flowering requirements for these various species are published by Greenhouse Grower Magazine & Michigan State University (2000) and Runkle, et al. (2000b, 2001a, b).

Runkle, et al. (2001b) have proposed the following:

i) germinate the seed in a plug tray, grow these seedlings to a specific size (node number) according to the species or cultivar.

ii) Expose this plant (now in the mature vegetative phase) to a cold treatment if required (qualitative) /or if cold enhances (quantitative) flowering (Tables 2-1, 2-2).

iii) Expose this plant to long day (night interruption) for a specific duration either to enhance (quantitative) flower development or as a direct qualitative (obligate) requirement (Tables 2-1, 2-2). True, a few perennials are short day plants (chrysanthemum, aster). Note: if production is during the time span of poor light intensity (winter) and of short duration, high intensity photosynthetic lamps may be used.

iv) Plants were grown at 14^{o}C plus temperature during the photoperiodic treatments.

As with many of the examples used to describe vernalization (cold treatment), long days and their interactions are frequently species- and cultivar-dependent; the response may vary between them.

Table 2-1. Herbaceous perennials which will flower without a cold treatment (Cameron, et al., 2000).

Category	Genus	Species	Cultivar or Series	Notes
Seed	*Aquilegia*	*x hybrida*	Songbird series	Only this series does not require cold.
	Asclepias	*tuberosa*		If not first exposed to short days
	Campanula	*carpatica*	Blue Clips	
	Coreopsis	*grandiflora*	Early Sunrise	
	Hibiscus	*x hybrida*	Disco Belle Mixed	Non-dormant plants will receive chilling injury.
	Primula	*veris*	Pacific Giants	
Cuttings (vegetative)	*Coreopsis*	*verticillata*	'Moonbeam'	
	Perovskia	*atriplicifolia*		
Cold hastens or improves				

Category	Genus	Species	Cultivar or Series	Notes
flowering				
Seed	*Armeria*	*x hybrida*	Dwarf Ornament Mix	
	Armeria	*latifolia*		
	Asclepias	*tuberosa*		If plants are dormant, following exposure to short days
	Delphinium	*elatum*	Blue Mirror	
	Dianthus	*deltoides*	Zing Rose	
	Echinacea	*purpurea*	Bravado	
	Gypsophila	*paniculata*	Double Snowflake	
	Lobelia	*x speciosa*	Compliment Scarlet	
	Oenothera	*missouriensis*		
	Physostegia	*virginiana*	Alba	
	Platycodon	*grandiflorus*	Sentimental Blue	
	Rudbeckia	*fulgida*	'Goldsturm'	Immature plants will flower much slower
	Veronica	*spicata*	Blue	
Cuttings	*Scabiosa*	*caucasica*	Butterfly Blue	

Table 2-2. Cold treatments (vernalization) and greenhouse forcing requirements for herbaceous perennials (Cameron, et al., 2001).

Category	Genus	Species	Cultivar or Notes
Provide long days for optimal flowering (4 hr. night interruption)	*Achillea*		Most, but not all, cultivars
	Anemone	*hupehensis*	
	Asclepias	*tuberosa*	
	Astilbe	*chinensis pumila*	
	Campanula	*carpatica*	Blue Clips, White Clips
	Coreopsis	*grandiflora*	Early Sunrise, Baby, Sun, Sunray
	Coreopsis	*verticillata*	Moonbeam
	Echinacea	*purpurea*	Magnus, Bravado
	Gaillardia	*x grandiflora*	Goblin, Baby Cole
	Gaura	*lindheimeri*	Whirling Butterflies, Siskiyou Pink
	Hibiscus	*moscheutos*	Disco Belle hybrids
	Hosta	*x hybrida*	All cultivars (for growth & flowering)
	Lavandula	*angustifolia*	Hidcote, Munstead
	Leucanthemum	*x superbum*	Snow Cap
	Lobelia	*x speciosa*	Compliment Scarlet
	Oenothera	*fruticosa*	Youngii-Lapsley
	Pennisetum	*setaceum*	
	Penstemon	*digitalis*	Husker Red

Category	Genus	Species	Cultivar or Notes
	Rudbeckia	*fulgida*	Goldsturm
	Sedum		Autumn Joy
Day neutral (once adequate cold has been provided, begin forcing and flowering)	*Aquilegia*	*x hybrida*	All types
	Armeria		All types
	Astilbe	*chinensis*	Superba
	Campanula		Birch Hybrid
	Delphinium		All types
	Hemerocallis		Stella D'Oro
	Heuchera	*x hybrida*	All types
	x Heucherella	*x hybrida*	All types
	Perovskia	*atriplicifolia*	
	Salvia		
	Scabiosa	*caucasica*	
	Stokesia	*laevis*	
	Tiarella	*wherryi*	
	Veronica		All that have been tested to date
No cold treatment required (some may tolerate cold storage)	*Asclepias*	*tuberosa*	
	Campanula	*carpatica*	Blue Clips, White Clips
	Coreopsis	*grandiflora*	Early Sunrise
	Coreopsis	*verticillata*	Moonbeam
	Hibiscus	*moscheutos*	Disco Belle Hybrids; provide long days to seedlings to prevent dormancy
	Pennisetum	*setaceum*	
	Sedum		Autumn Joy
Cold treatment (4-6 wks.)	*Achillea*		Moonshine
	Aquilegia	*flabellata*	Cameo
	Campanula		Birch Hybrid
	Delphinium		Blue Mirror, Belladonna
	Geranium	*dalmaticum*	
	Leucanthemum	*x superbum*	Snow Cap
	Lobelia	*x speciosa*	Compliment Scarlet
	Penstemon	*digitalis*	Husker Red
	Perovskia	*atriplicifolia*	
Cold treatment (>6 wks.)	*Anemone*	*hupehensis*	
	Armeria	*x hybrida*	Dwarf Ornament Mix
	Astilbe	*chinensis pumila*	

Category	Genus	Species	Cultivar or Notes
	Astilbe	*chinensis*	Superba
	Coreopsis	*grandiflora*	Baby Sun, Sunray
	Gaillardia	*x grandiflora*	Goblin, Baby Cole
	Gypsophila	*paniculata*	Double Snowflake
	Hemerocallis		Stella De Oro
	Heuchera	*sanguinea*	Bressingham Hybrids
	Lavandula	*angustifolia*	Hidcote, Munstead
	Oenothera	*fruticosa*	Youngii-Lapsley
	Rudbeckia	*fulgida*	Goldsturm
	Salvia	*nemorosa*	May Night
	Salvia	*x superba*	Blue Queen
	Saxifraga	*umbrosa*	Aureo-punctata, London Pride
	Scabiosa	*caucasica*	Butterfly Blue
	Stokesia	*laevis*	Klaus Jellito
	Tiarella	*wherryi*	
	Veronica	*longifolia*	Sunny Border Blue, Red Fox

6. CURRENT RESEARCH ON ANNUALIZING PERENNIALS

Certainly, the research center for production of perennials in flower for spring sales has been at Michigan State University (Runkle, et al., 2001b). They have published over 200 forcing schedules for various genera, species and cultivars. Within a genus, as an example, with *Hosta* and cultivar dependent, the weeks of cold for 100% of the plants to break dormancy and for the vegetative shoots to emerge varies from zero to six weeks. The required photoperiod for continual growth and eventual flowering is $\geq$ fourteen hours or normal days with temperatures for forcing all cultivar responses were similar. However, the days for shoots to emerge (dormancy breaking) varied between cultivars from four to fifteen weeks and for weeks to flower varied from nine to fifteen weeks.

In contrast with some seven species of *Achillea* and five cultivars of one of these species, only one species required a cold treatment for emergence or flowering. All required long days [16-hour or with a night interruption of 4 hours (2200 to 0200 HRS)] except for the cold requiring species. The balance of the various growth responses to the recommended photoperiod and forcing temperatures (64^{o} - 68^{o}F or 17.78-20^{o}C) were similar.

7. EXAMPLES OF PLANT GROWTH CYCLES

Regardless of the genera, species or cultivar a producer will germinate the respective seed under the required environmental conditions. For efficiency these seeds are germinated in plug trays for automation of seeding and future potting procedures. Plants are allowed to increase in size until maturity a size (leaf number) or age has been (as discussed) achieved. This phase or stage should be under the most optimal environmental conditions [high (intensity and duration), temperature, nutrition, CO_2 and moisture] possible. This stage also may be thought of as the vegetative plant.

After the vegetative production phase has passed and maturity is reached, the appropriate environmental condition for the floral induction is given. As with the two examples gives, *Hosta* has an absolute cold requirement and there is no response to photoperiod. The response to light would be for quality and rapidity of leaf and flower (which is not of value for this genus) development. On the other hand, *Achillea* essentially has no cold requirement only LD treatment. Regardless, after these treatments are given for the reproductive phase, the plant responses and develops to an acceptable commercial saleable plants.

8. MARKETING ASPECTS

Perennial sales have dramatically increased in the past two decades. It is difficult to obtain reliable sales data to establish the trends and values within each country. For example, in the United States, the U.S. Department of Agriculture's census is not consistent from year-to-year (United States. Department of Agriculture. National Agricultural Statistical Service, 1998, 2000). The 1998 U.S.D.A. census reported US$627,236 in sales (wholesale, farm-gate value) for all 50 states. In 2000, the data was only collected from 36 states at a value of US$426,435. Under nursery crops in the section 'Other Environmentals', sales of US$32,9430,000 were reported for 'other herbaceous plants' which included hostas, peonies, daylilies, irises, etc. Herbaceous perennials may also have been reported in other categories, e.g. groundcovers or vines (Marvin Miller, 2002, personal communication). In 1988, some US$32,943,000 in sales were reported for "other ornamentals", exclusive of annuals from the 36 states routinely surveyed. Regardless, it is realistic to state that interest in the production and sales of perennials is dramatically increasing (Marvin Miller, 2002, personal communication).

Public awareness of herbaceous perennials has grown due to publicity and marketing by the Perennial Plant Association **(**www.perennialplant.org**).** The Perennial Plant Association is a professional trade association serving the U.S. and Canada, with the primary goal of improving the industry, providing education to enhance production, the promotion of, and use of herbaceous perennials. Each year

the association chooses a 'Perennial Plant of the Year' which is already in production with established performance across the continent. The marketing arm of the association promotes this to growers and consumers alike.

Additional interest in herbaceous perennials arises from northern temperature gardeners who are anxious to purchase reliably winter-hardy cultivars. To partially fulfill this need, the University of Minnesota has established winter hardiness test sites across the state of Minnesota (U.S.A.) at 45°N latitude and northward, constituting U.S.D.A. Winter Hardiness Zones 3-4. A brand of herbaceous perennials has been established, 'Minnesota Tough & Terrific™' (www.florifacts.umn.edu), to demarcate those which have survived the three-year test with outstanding performance (for disease and/or pest susceptibility, tolerance, or resistance; cultural responses to cutback, growback; plant morphology, i.e. height x width measurements; stem strength; flowering—100%, duration; reseeding or invasiveness--tendency to form asexual and/or sexual propagules, their dissemination, and competition with the parental source) during each growing season. The trials conducted in Minnesota are the first systematic, scientific, public trialing system to evaluate winter hardiness and garden performance of herbaceous perennials. The trials are unique by testing replicated samples of seed and/or vegetatively propagated products for a duration of three years (three growing seasons and three winters). A national test system anywhere in the world for herbaceous perennial plants does not currently exist. Individual breeder/producer/distributor companies may have organized public and/or private trialing of herbaceous perennials. For instance, Blooms of Bressingham Perennials™ N.A. (Lapeer, Michigan, U.S.A.) conducts performance evaluations and hardiness tests for each year's new releases. This provides a gauge of performance/hardiness across various regions, although none are as rigorous as the Minnesota U.S.D.A. Z3-4 test sites.

The public has become aware of the repeated flowering capacity of herbaceous perennials and the establishment of varied dates of flower, colors, and textures. This is a natural progression as the public learns more about gardening and moving away from annuals which must be replanted every spring. The emphasis of plant breeding technology on spring flowering annualized perennials has an added marketing/sales incentive. Thus, a *Sedum spectabile* or a *Dendranthema x grandiflora* may be sold in May in flower even though it is not normally in flower until September. Regardless, during the summer this allows for further growth and establishment of the plant during the summer, a quieter establishment and display of the respective species. Hence, if not flowered "out of season" the gardener would be forced to visit a garden center on a biweekly or monthly basis. This is not reality, as the average gardening public visits and purchases plants during a very limited time span in the spring.

9. HORTICULTURAL & COMMERCIAL SIGNIFICANCE

The annualization of flowering perennials sold in the spring has been mainly responsible for the increase in demand of new herbaceous perennials. An added feature of annualized perennials is their 'dual-use' nature wherein they can be grown as flowering potted plants and then planted out-of-doors for continued enjoyment in subsequent years. At this time, the expansion of perennials sold in the spring in flower has ceased to be as dramatic as in the past. The market of perennials is now being shared with vegetatively propagated flowering or tropical foliage plants, which are sold as annuals.

An important component of continued market appeal, which creates reliable demand for new products, is the influence of the 'gardening gurus'. Each country has their own well-known and not-so-well-known gardening gurus who promote floricultural crops such as annualized herbaceous perennials. Their attunement to fashion and design trends, e.g. Madison Avenue in the U.S., the British Royal Family in the U.K., etc., promote instantaneous sales of plants to their gardening audiences and followers. For instance, Martha Stewart, a self-proclaimed gardening guru in the U.S. (http://www.marthastewart.com/) has a website devoted to her own line of flowering potted plants (many of which are herbaceous perennials) and cut flowers (MarthasFlowers, www.marthasflowers.com). Martha Stewart was influential in creating a demand for unusual plant materials and emphasizing colors and cultivars, which were 'scruffy' or out-of-the-ordinary. These differed from the uniform annual bedding plant and annualized herbaceous perennial offerings, which the flower breeding companies were producing. Alan Titchmarsh has educated and inspired gardeners in the U.K. and beyond with his characters such as 'Gordon the Garden Gnome' (http://www.alantitchmarsh.com/).

Other countries have similar television, radio, blogs, and news media 'stars' that promote gardening and products. The Garden Gurus (Perth, Western Australia; http://www.thegardengurus.com/), Trevor Cochrane and Neville Passmore, introduced their television show the year Perth established a water restriction on the city. Trevor Cochrane and Neville Passmore rose to the occasion and promoted water-wise gardening practices for the Perth residents. This led to the establishment of the Swan River Trust in The garden Gurus' River Friendly and Fertilise Wise public education campaigns. Perhaps the first gardening gurus to establish a blog on the web were Connie Groop, Gretchen Mayer, and Kelda Pharris, gardeners at the American News in Aberdeen, South Dakota, U.S.A. (http://blogs.aberdeennews.com/garden_gurus/2005/07/welcome_to_gard.html).

The interest in herbaceous perennials generated by new and old crops bred by public / private sector breeding programs and brought to the market by the breeder/producer companies remains unabated. Given the extraordinary number of plant taxa awaiting domestication and the promotional ability of the gardening gurus

worldwide, there will continue to be an exciting array of new annualized perennials for the gardening public.

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Chapter 3

CULTIVAR TESTING

America's trial gardens

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Abstract: Trial gardens, both public and private, provide an essential service to the flower breeding industry. In such trials, new cultivars and plant products are evaluated in replicated plantings (in field, hanging basket, or container trials) over years and locations, along with market standards (comparisons) to ensure superior performance, stability, and marketability. Most trials are grown as comparison or row trials with varying numbers of plant material (replications). Display gardens provide a landscaped alternative to classic row trials. The cultural factors, evaluation and rating systems (quantitative/qualitative, objective/subjective) are presented and discussed relative to product class and trialing objective(s). Trial gardens and cultivar testing are essential prior to introducing new or improved products into the floriculture market.

Key words: Combination containers, display gardens, hanging baskets, row trials, trial gardens, trial rating systems

"To thoroughly know seeds and the relative value of their products, both of his own and his competitor's stock, to learn the comparative merits of newly introduced or proposed novelties, carefully conducted trial grounds are to the progressive seedsman, and his assistants, the open book of nature."
--W. Atlee Burpee, August 14, 1893.

1. INTRODUCTION AND HISTORY

In his presentation at the 1893 World's Columbian Exposition in Chicago, U.S.A., W. Atlee Burpee promoted the importance of trialing one plant with another (Burpee, 1894). This fundamental practice of comparison had only recently been adopted, even though the number of horticultural companies opening their doors for business had been increasing since the close of America's Civil War. During the

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1880s and 1890s, many horticultural companies, mostly in the eastern U.S., were establishing production sites for harvesting seed. In addition, the state of California was just starting to devote land to flower and vegetable seed production and the eastern U.S. companies wanted to compare these products with their European counterparts.

Well into the 1900s, most of the seed sold in the United States was produced in Europe (Burpee, 1894, 1895). There was a national sentiment that European-grown seeds were superior to those grown in America. Breeding out unwanted characteristics, as well as a greater attention to production, seed harvesting, and the selection of improved strains were seen as the key benefits of European firms. As the number of American and Canadian seed firms grew, their cultivars began to compete with some of the European introductions. As companies introduced a myriad of new varieties propagated by seed, they realized the need to evaluate their strains with competitive lines, and so the basis of trialing plants was established in the United States in the late 1800's.

A single seed company could not actually produce all the cultivars they offered in their catalogs during the 1800s. While they purchased a vast portion of seed from Europe, they also bought and marketed varieties produced by other seed companies in the US and Canada. The larger seed firms used trial gardens to evaluate whether their selections were the best available, as well as confirm or deny the extravagant claims on plant performance blazoned across the pages in rival catalogs.

The premise of any trial garden is based on comparison (http://www.all-americaselections.org/). Breeder/producer seed or vegetative companies compare their selections with what is available from others around the world in the same class (termed 'market standards'). W. Atlee Burpee once wrote "...the wide awake" seed professional "…will not be content merely to test the strains of seeds which" s/he "…is selling, but will also want to know how they compare with seed of the same varieties sold by other growers. This is the most important use of the trial grounds" (Burpee, 1894).

2. TODAY'S TRIAL GARDENS

There are many trial gardens throughout the world operated by commercial greenhouse growers, retailers, public universities and colleges, botanical gardens and arboreta, independent horticultural organizations, amateur societies, merit award organizations (e.g. All America Selections, http://www.all-americaselections.org/; Fleuroselect, http://www.fleuroselect.com/), and horticultural seed and plant (breeder, producer, distributor) companies [both in private/secure trials and those for wholesale customers, e.g. the California Pack Trials (Greenhouse Grower Magazine, 2005) and the European Pack Trials (Beytes, 2005)]. Some of these test sites evaluate only flowering plants, while others grow and test a combination of flowers,

vegetables, ornamental grasses, herbs, and more. Cultivars are reviewed and evaluated for use in the home garden, commercial indoor and outdoor landscape, mixed containers, and hanging baskets. The following highlights the major types of trialing in the horticulture industry.

2.1 Comparison or Row Trials

Many seed producer companies conduct row trials, which are traditionally the primary basis for trialing annual plant material (Figure 3-1). Most of these row trial gardens are flat, open stretches of ground with minimal obstruction around them to avoid shading sun-loving plants (Allard, 1960). Conversely, shade-loving plants are often grown in a shade house constructed of wood or metal, and covered by a shade cloth stretched over the top. The degree of shade delivered by the cloth is available in a number of percentages. Shading needs vary from southern to northern latitudes, but a range of 35% to 55% shade is commonly used, depending on the crop (http://www.americannettings.com/commercial_products/shadecloth/shadecloth.htm)

Logistics within any row trial depend on the plant being evaluated, but plant population and replication are key for most crops (Anderson, 2001). The number of plants that are used to determine a trial result is significant in making a correct assumption based on performance during the growing season. Some trial gardens use n=12 plants to verify color and performance although more replications increase accuracy. At the Ball Seed Company trials, n=18 to 24 plants are preferred to obtain more accurate data of plant (cultivar) performance (http://www.ballseed.com/). This is especially true when evaluating a brand new plant that has never evaluated before.

The spacing used in row trials is also important. In the Ball Seed Company Trial Gardens in West Chicago, Illinois (Figure 3-1), plants of a single cultivar are spaced at 72 cm (1 ft.) intervals with 3-4 rows across and six plants / row, for a total of n=18 to 24 plants per population – all spaced on center (72 cm apart) from all directions. There is a 144 cm (2 ft.) spacing between two adjacent cultivars. Larger or smaller-sized crops at maturity may be planted at different spacing, depending on the trial site. Regardless of the crop being trialed, proper spacing is required to determine which selections fill in the area fastest, the cultivars with the most 'flower power' (color), are the most attractive, as well demonstrating which market segment they amenable to (hanging baskets, containers, gardens, etc.).

Figure 3-1. Example comparison or row trials planted in a color-coordinated grid system at Ball Seed Company, West Chicago, Illinois, U.S.A.

While managing a trial garden involves keeping it orderly, clean, disease-free and nutrient-rich, a row trial is meaningful to the trained professional in charge of the garden. Without the observations of people trained to look for new opportunities in plants, a trial garden is simply 'pretty'. It is vital to determine what is already available on the market (using market comparisons) and what the market can bear (specific product classes or other niches) (Anderson, 2001). To recommend that a plant that performs well but has been superseded by others lessens the impact as well as the credibility of a trial location and of the person collecting the data.

Consider an F_1 hybrid purple petunia (*Petunia x hybrida*), of which there are many on the bedding plant market worldwide. Mundane you might think, as 'purple' is 'purple'. But imagine that a selection had a dark center rather than a yellow or clear one, or the outer petals were flat rather than frilled, or the blooms were slightly smaller but held up better in the wind, hail, and rain, or the plants spread across the ground rather than mounded. Petunia Wave® Purple offered all of these advantages. In 1993, a purple-flowering petunia was entered in the All-America Selections trials

by Kirin Brewery of Japan, and was the first trailing petunia from seed (http://www.all-americaselections.org/). The plants grew only 18-36 cm (3-6 in.) tall but could spread from 0.6-1.3 meters, each dotted with 12-15 cm (2-2.5 in.) burgundy-purple flowers. This selection was something entirely different than any other seed petunia ever seen before in the history of the industry. Not since the introduction of double-flowering petunias in the 1920s or the advent of F_1 hybrid 'Comanche' in 1953 (Rice, 1999) had there been such a major improvement in petunias. In their trial gardens throughout North America, each of the All-America Selections (AAS) judges voted to honor petunia Wave® Purple with an AAS medal.

It is well-known that gardeners are always looking for something new and different, e.g. new colors in flowers that didn't exist before (white marigolds); seedless fruit (triploid watermelon); small, flavorful tomatoes that look like oblong cherries and are eaten like clustered grapes (grape tomatoes) or new habits within existing crops (petunias Wave® Purple or the more recent Fantasy series with a diminutive habit). Truly innovative plants that add design, texture or color add an intrigue that gardeners want to try at home.

The planning of trials begins in the fall of the year and continues throughout the winter and spring. Seeds are sown from autumn to May depending on the plant and its crop time (Anderson, 2001; Dole and Wilkins, 1999). In southern latitudes, for instance, Park Seed Company in South Carolina will sow seed of plants with a long crop time in November or December (http://www.parkseed.com/). In more northern latitudes, other trial sites may delay sowing until late December or January before germinating the same cultivar. Numerous references are available to assist with crop production (timing and scheduling) for any latitude (Amos, 2003; Armitage, 1997, 2001; Armitage and Kaczperski, 1992; Banner and Klopmeyer, 1995; Beytes, 2003; Darke, 1999; Dole and Wilkins, 1999; Hamrick, 2003; Holcomb, 1994; MacKenzie, 1997; Nau, 1996, 1999; Ouellet, 2001; Schmid, 2002; Styer and Koranski, 1997; White, 1993).

Most annuals are planted and sold in 'cell packs' or 24 cm dia. (4 in.) pots. However, 18 plants per standard flat (66x132 cm or 11 x 22 in.) are excellent for larger plants like seed geraniums (*Pelargonium x hortorum*) and other robust items. Finally, perennials are best planted from 24 cm dia. (4 in.), 0.95 liter (1 quart), or 3.78 liter (1 gallon) containers. Obviously the larger the plant is when it is planted into the garden means it will reach its potential earlier for quicker evaluation. However, it is also important to realize what container size the market can or will accept. If the input costs of the plant, soil medium and container are too high then the plant may not have the sales to warrant its promotion.

In the southern U.S., trial gardens from South Carolina to Texas will commence planting warm season annuals and perennials in April and May, while more northern sites will delay until late May and June. For instance, planting at the West Chicago, Illinois trials is started the last two weeks of May and completed by June 12. Cool season plants, like dicentra (*Dicentra spectabilis*), viola and pansy (*Viola cornuta,*

V. x wittrockiana), English daisy (*Bellis perennis*), or snapdragon (*Antirrhinum majus*) can be planted earlier. Earlier planting is facilitated by a 'hardening off' treatment in a cold frame or cool house when they are grown at 10°C or 50°F (nights). Before planting the ground beds, conduct a soil/pH test and, if necessary, apply a pre-plant fertilizer to the beds. Each cultivar is labeled with its name, source, accession or sowing number, or bar code to assist in the evaluation process.

2.2 Display Gardens

While row trials highlight an age-old method of evaluating annuals from seed (Burpee, 1894), the greatest contribution of display gardens is to demonstrate how to use the plants in the home garden and/or landscape setting. While row trials are created on a grid system, display gardens are based on landscape design. The purpose remains the same, i.e. trialing, however the plants may be mixed together in large borders and beds, or grouped together in one large bed. In addition, combining flower and foliage colors, textural features and other design elements helps those who view the trials to choose new varieties and potential combination plants.

Display beds are also useful for landscape professionals who design commercial landscapes and golf courses, as these environments provide extreme conditions for annual and perennial plants. Corporate installations often lack large trees, preferring more intermediate or dwarf selections, and the heat from surrounding concrete or asphalt parking lots and parkways may not dissipate until after midnight in the middle of summer. Annuals and perennials chosen for these extremes must be durable since they are often used in mass for striking color and appeal and are exposed to the full sun all day. Some excellent display gardens include the Missouri Botanic Garden (http://www.mobot.org/), the New York Botanic Garden (http://www.nybg.org/), and the Chicago Botanic garden (http://www.chicago-botanic.org/).

2.3 Hanging Baskets and Combination Containers

Hanging baskets have long been a key facet for bedding plant sales in the North American horticultural market. Likewise, their popularity is expanding in the world market (Figure 3-2). Twelve and 14 in. baskets (72 cm and 84 cm, respectively) are commonly grown for evaluation for the home garden market, while 20 and 24-in. (120 cm and 144 cm, respectively) baskets (Figure 3-2) are produced for comparison by the commercial or professional landscape market. Smaller hanging baskets in the trial gardens often feature one cultivar while the larger baskets have a combination of plants.

Figure 3-2. An example large-sized hanging basket at the Ball Seed Company trials, featuring mixed plantings of flowering and foliage annuals and perennials. This basket contains *Plectranthus* 'Nicoletta', *Lantana camara* 'Samantha', *Perilla* 'Magilla', *Petunia x hybrida* 'Suncatcher Pink', and *Sanvitalia procumbens.*

Mixed combination planters are also important (Figure 3-2). Fourteen-inch (84 cm) and larger containers are planted with a wide range of plants: annuals, perennials, ornamental grasses, herbs, vegetables and even small decorative trees and shrubs in the largest planters. The most traditional of these combinations is vinca vine (*Vinca major* 'Variegata'), red geraniums (*Pelargonium x hortorum*), and spikes (*Cordyline indivisa*). In the past ten years, scaevola (*Scaevola aemula*), diascia (*Diascia barberae*), nemesia (*Nemesia x hybrida*), osteospermum (*Osteospermum ecklonis*), dichondra (*Dichondra argentea, D. repens*), bacopa (*Sutera cordata*) and a myriad of other never-before-heard-of crops have entered the market and are now key products to include in combination planters.

Typically two planting/growing strategies can be used, depending on space availability in the production greenhouses: 'plant and grow' (grow-together) or 'grow and plant' (put-together) (Gaston, et al., 2002). Both methods have distinct advantages, resulting in differing end products (Gaston, et al., 2002). For instance, the plant and grow method promotes a more 'natural' appearance as the plants have time to grow and intertwine together. This natural garden effect commands higher price points at the wholesale and retail levels (Gaston, et al., 2002). Conversely, the grow and plant method has the advantage of allowing the greenhouse grower to produce each crop in their optimal environment, rather than compromising cultural conditions (Gaston, et al., 2002). Regardless of which method is used, the principles and elements of design are followed when planting the baskets or containers to ensure proper size, color, and flowering/foliage combinations at maturity (Gaston, et al., 2002). Care must be exercised to ensure that the final containers are not too large in size. While larger containers would be beneficial for evaluation, they may be too large and weigh too much for home gardeners to purchase at retail.

To underscore the importance of the container trialing system, the trial gardens of most distributor companies had 15 to 25 combination planters during any one season in past decades (1975-1995) (Nau, unpublished data). However, by 1997, due to the dramatic increase in 'new' vegetative and seed crops, there were over 100 and the number topped 500 in 2004 at the Ball Seed Co. trials (Nau, unpublished data). Most consumer's homes are now commonly graced with a combination pot or hanging basket rather than planted beds of flowers or vegetables, since these "all-in-one" mini-gardens are easier to maintain (Gaston, et al., 2002).

3. EVALUATION AND RATING

Plants are bred for stability in performance; flower breeders seek to balance genetic x environmental interactions such that a new cultivar performs similarly across a wide range of growing conditions (Allard, 1960). Such releases must perform as they were bred, selected, and produced. It is the trial garden that can equate them, rate, and "make or break" new cultivars. In most trial gardens, the evaluation data is collected weekly, biweekly, or monthly during the peak growing season (Anderson, 2001). In many commercial trials, plants are evaluated on the "Three Times" rule. Judgments are not made on the outcome of one trial, but plants are looked at in three different trials and three different times. At least one of these trials is based on the field or garden performance of the plant. Plants are also tested in greenhouses with similar production methods as the commercial greenhouse grower uses to verify results (Anderson, 2001). If the first trial hasn't captured interest on the visual features of the plant, a trial may be repeated two to three years later. On the other hand, if the plant piques interest during its first trialing year, the remaining two trials are often conducted within the next 12 months to verify the first

results, detail the cultural information, and obtain the photography needed for marketing.

3.1 The Trial Rating System

Every trial garden has its own methods and standards for recording the data gathered from evaluation of each variety (Nau, unpublished data). Criteria may be selected based on a number of aspects (Anderson, 2001). Data for flowering plants taken at universities and public trialing locations may include objective, quantitative data such as flower color (using Royal Horticultural Society color charts; Royal Horticultural Society, 1995), plant habit, flower size, flower type, plant height and spread (cf. see www.florifacts.umn.edu/). Visual evaluation of the plant is also performed, but is a subjective rating based on the individual's tastes, acceptance, degree of knowledge of the market, and other biases. In many cases, this reflects market acceptance and the personal training/bias of a trial manager.

While over 20 different criteria may be evaluated, it becomes evident with just the visual impact if a "winner" is present. However, there has to be starting point. Information on height, habit, spread, flower color and flower size are all important, but additional notes are required. These include:

- The number of days to first flower, 50% bloom and full color in a flat or a given number of pots. This information is recorded, the trial replicated for verification, and then compared to market comparison (if they exist) (Anderson, 2001). This information allows professional greenhouse growers to schedule plants more easily.
- Seasonality. It is assumed a plant will grow and flower from the time that it is planted until a killing frost (in the north) or other environmental factors which limit or cease growth (e.g. heat, drought). Some plants, including calendula (*Calendula officinalis*) and annual coreopsis (*Coreopsis grandiflora*) perform best from May to July in the north, but often 'die out' by the end of August. Other crops like diascia (*Diascia barberae*) and lobelia (*Lobelia erinus*) will flower as long as the night temperatures are cool to warm, but may stop flowering when night temperatures and relative humidity levels increase.
- Regional performance. Seed and vegetative companies often test their introductions at their own secure trialing sites, as well as at a number of independent locations to verify that a cultivar can be used in various climatic conditions throughout the intended market region. In some cases, a N. American company will trial in state of California, Canada, the central U.S. and the state of Florida (or other southern locations) to determine the limitations of their introductions. With these results, it is indicative how the plant will perform throughout North America.

- Propagation and quality limitations. Once a plant has passed all the visual tests, it then has to meet all the production performance requirements. If a new introduction excels in the garden, it may be dropped if it cannot be produced easily with a quality cutting or seed.
- Production or cultural issues. Once a new cultivar has been selected, it is placed in production tests to identify crop time, photoperiodic requirements for flower bud initiation/development (if any), nutrient and pH requirements, etc. This is especially true for new crops that have not been previously grown. For instance, until petunia 'Purple Wave®' was introduced, seed-propagated petunias could be grown in a number of different containers. However, while Wave® Purple performs best in 24 cm (4 in.) and larger pots, the plants grow together too quickly in a cell pack or flat and it is difficult to separate them.
- Market segment and acceptance. There are times when breeders develop new selections but the market either won't accept them or consumers have moved away from that class of products. For instance, 'Dragon Wing®' begonia is an ideal example. There were other large-leaf, large-flowered begonias (*Begonia x tuber-hybrida, B. x hybrida*) on the market in the 1970s when 'Dragon Wing®' was first developed, but these quickly fell out-of-favor with home gardeners. The begonias at any trialing station in N. America in the early 1990s were dwarf flowering plants (primarily *B. x semperflorens)*, which grew less than 48 cm (8 in.) tall. There were only three series that had plants taller, growing to a maximum height of 72 cm (12 in.). While 'Dragon Wing®' was first developed in the 1970s, it wasn't until the late 1990s that the market accepted this cultivar (PanAmerican Seed Co., 1999). 'Dragon Wing®' is now a favorite that grows 84 cm (14 in.) or taller by the end of the summer and is loaded with big, colorful flowers (PanAmerican Seed Co., 1999).

All-American Selections (AAS) is an public organization closely involved with the introduction of new seed-produced products for the N. American market (http://www.all-americaselections.org/). Numerous cultivars have been introduced since this organization's founding in 1932. The AAS trials rating system is a good example of a uniform numeric (quantitative) system. Working with seed companies, universities, growers and a number of other qualified trialing locations, AAS is one of the leading agencies dedicated to bringing new cultivars to the market. Numerous AAS judges throughout N. America rate everything from bedding plants (varieties planted to the garden in bloom from cell packs or pots) and flowering plants (landscape, cutflower and other selections that are planted green or with flower color just starting), to vegetables. AAS developed an evaluation form that is used at all locations to standardize the results and select potential winners (http://www.all-americaselections.org/).

At commercial trials, a two-tiered rating system is used based on the introduction of an improved versus a new cultivar (Nau, unpublished data). The first system rates perceived improvements within an existing, or previously seen, class of plants. Specifically, if a new introduction is promoted that expresses one trait that is unique, but is not entirely different from the current cultivars already on the market, it is treated as an updated or marginal change in the market (termed 'improved'). These may include improved or new colors in already-existing crops; changes in flower size, flower shape or type; or differences in height, habit or garden performance than what is already available. Regardless, these improvements seldom make a significant impact on the market.

The second system of measurement is used to evaluate plants of superior performance (Nau, unpublished data). These are new cultivars that that can change the market. In most cases, they don't possess just one of the traits as mentioned above but several. In these cases the evaluator has to be a visionary, imagining what is possible and whether the plant can be successful. If a new plant appears to be truly better than anything else, it is rated in what may be termed the "Five Points of Success" (Nau, unpublished data). In an average year there can be as many as 400 new seed products. Adding this to the number of new vegetatively propagated cultivars, it is easy to determine that the number of new and previously released items to be trialed can overwhelm any trialing location. However, the "Five Points of Success" rates plants that are already visually acceptable and measures an assumption as to what level of interest these plants will have to the marketplace. The new cultivar is evaluated against current plants based on the following:

1. Is it an entirely new type of plant, not previously seen, known, or marketed?
2. Does it have a unique, interesting or new flower color, form, shape, and/or size?
3. Is it noticeably different in habit, height, or other garden attribute not previously seen?
4. Does it perform through the appropriate growing season, e.g. all summer, throughout winter (regional) or all year? Is it reliable and weather-tolerant in the regional trials (including a wide range of conditions across the intended market areas)?
5. Does it meet or exceed the needs of the home garden and fit reasonably within the cultural needs of the professional grower?

This "Five Points of Success" rating system is used when an opportunity presents itself. A plant possessing just one of these features does not mean an exciting new product. Instead, when a new introduction meets two or more of these criteria, it is possible that this product is something truly different or unique.

4. A NETWORK OF TRIAL GARDENS

The trial gardens of private companies are just one of the many across N. America and around the world for testing herbaceous plant material. Some are maintained and managed by private horticultural family greenhouse or nursery operations. More are located at botanical gardens, arboreta and wholesale seed and plant companies or at colleges and universities with horticulture baccalaureate educational programs. Klett and Courtney (2001) of Colorado State University state that the basic premise of such trial gardens "…were established to allow students, researchers, industry representative, homeowners and extension personnel to learn, teach and evaluate horticulture research and demonstration projects in the Rocky Mountain High Plains Region". The latter is responsible for much of the independent trialing and data collection on herbaceous plants, especially annuals, that is used by many in the professional horticultural industry. Some examples include Colorado State University (http://www.ext.colostate.edu/pubs/columngw/gr010409.html), the University of Georgia (http://www.uga.edu/athensselect/oldnewsarchive.htm), the University of Tennessee (http://www.ca.uky.edu/agc/pubs/pr/pr450/pr450.pdf), Oklahoma State University (http://osu-ns03.cis.okstate.edu/), the University of Minnesota (http://www.florifacts.umn.edu), Pennsylvania State University (http://hortweb.cas.psu.edu/research/trial.html), and Michigan State University (http://web1.msue.msu.edu/iac/florflor.html). Many of these institutions have open houses to invite growers, the garden press, Master Gardeners, allied trade, home gardeners and other interested groups to spend a day or two reviewing new plants and to evaluate performance. Some divide their open houses between two or three dates to invite home gardeners on different days than the seed and vegetative companies. Regardless, visiting several of these open houses provides a wealth of information on the performance of plants, especially with regard to how they are cared for and managed, as well as which plant introductions are more seasonal, regional, or temperamental in garden performance.

Some, like the University of Minnesota, have a number of trialing locations, each with an open house and each evaluating some of the same plants to see their regionality within one state. Colorado State University is an excellent testing location for trialing drought tolerant plants but it, along with Pennsylvania State University's Landisville site has an excellent potted plant or container trial (108-144 cm or 18-24 in.) to evaluate how plants perform when grown all summer in containers. Southern latitude trial locations (e.g. the University of Georgia; Dallas, Texas Arboretum, Oklahoma State University, the University of Tennessee) are excellent locations for exposure to high heat and relative humidity and their effects on herbaceous plants. In addition, such sites can test for late summer/early autumn pansy (*Viola x wittrockiana*) cold tolerance.

Many trial sites record their results and publish these in gardening journals (both home garden and professional grower magazines) and a number provide an individual booklet that provides a numeric or visual rating for the plants that they tested. The results of these trials are often published in the seed and plant catalogs by the professional industry. In addition a number of the university programs provide a 'Best of the Best' rating. This encapsulates the growing season into just a handful of top performing plants. For instance, the University of Georgia promotes its 'Georgia Gold Medal Winners'. Such winners have to be "not only good performers, …[but] the best performers over the entire season in the entire garden. To win this award the winners had to be outstanding all season, defying heat, humidity, drought, rain, little old ladies, students, weekend football games, bugs and disease" (Armitage, et al., 1998). To understand the scope of the trials, Pennsylvania State University reported that "the University Park garden contained a total of 849 entries; 569 of which were annual flowers, 199 were perennial flowers and 81 vegetables. The Landisville trials consisted of a total of 403 entries" (Shumac, 1999). Without the objective trialing data and annual reports of these programs, it would take much longer for new cultivars to impact the market and gain acceptance by gardeners throughout N. America.

5. SUMMARY

Trial gardens are the testing ground for the introduction of new flowers and vegetables. Many of the final decisions prior to the introduction of a plant are based on the outcome of their performance in this arena. However, the first sight a consumer (the home gardener) has of a new cultivar (in the garden center, the box stores, or the parking lot of the seasonal garden shop) is long after the trialing process. While no one intentionally plans on this, the results are the same. If there are three varieties of white-flowering petunias on the retailer's bench, the gardener selecting a plant will choose the one that interests them the most. If the gardener is a seasoned veteran, knowledgeable on what they have tried in the past and well versed on what is new, then they may be very selective about what they purchase. However, many 'new' gardeners and 'non-gardeners' select and purchase plants that are visually appealing with colors, habits and forms that serve their gardening purpose. Thomas Bridgeman (Bridgeman, 1847) thus noted:

To raise your flowers, various arts combine;
Study these well, and fancy's flight decline.
If you would have a vivid, vigorous breed
Of every kind, examine well the seed:
Learn to what elements your plants belong,
What is their constitution, weak or strong?
Be their physician, careful of their lives,

And see that every species daily thrives;
These love much air, these on much heat rely,
These, without genial moisture, droop and die.
Supply the wants of each, and they will pay
For all your care through each succeeding day.

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Chapter 4

PROTECTION

Plant patents, Utility patents, Plant breeders' rights, Trademarks, Branding, Royalties

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Abstract: Plant Breeders are certainly in a better position than ever to gain recognition and financial support for their work. The information provided is intended to aid in the understanding of the issues surrounding plant variety protection and to provide information relative to the various types of protection that are applicable to plant breeders.

Key words: Plant breeders' rights, plant patents, plant variety protection, trademarks.

1. INTRODUCTION

An understanding of intellectual property rights for plant breeders has become increasingly important over the last few decades. It is also a confusing topic to many in the industry. Confusion is understandable when we are presented with various types of protection, many acronyms, terms used interchangeably and incorrectly, regulations that vary internationally and a lack of understanding or consistent interpretations of laws that govern these rights.

Plants are unique from other marketed products due to their complex nomenclature, the ease of their propagation, and the many levels of distribution and promotion involved in their marketing. The protection required for a plant cultivar needs to be considered in relation to all of these factors in order to be correctly implemented and maintained.

Breeding and commercialization of new ornamental plant varieties is an economically important activity both for the industry and the well being of our society. Legal protection for new plant varieties provides the means by which

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companies, institutions, and independent plant breeders can receive a return on their investments of time and money as well as justify their involvement in research and new plant development. In addition, legal protection enables plant breeders to be recognized for their craft and skill and encourages plant breeding as a profession. Plant variety protection provides an incentive for continued breeding and to ensure the availability of new varieties that meet future demands and keep the industry thriving.

Protecting the marketing rights of a new plant variety requires the application of various types of intellectual property protection and activities that may include: keeping trade secrets, marketing only F1 hybrids, obtaining plant breeders rights and/or patents, and using trademarks as brands for marketing purposes. An understanding of the various types of protection and their uses is especially important when world--wide rights is a goal.

2. HISTORY AND BACKGROUND OF PLANT VARIETY PROTECTION

The systems that we currently have to protect the rights of plant breeders took come time to evolve. The "Plant Patent Act", was enacted in 1930 in the United States and by "an Act of Congress" and considerable lobbying by fruit and ornamental plant breeders led by Luther Burbank (Janick et al., 1983). Plant patents were, and remain today, limited to vegetatively propagated plants (except potato and Jerusalem artichoke) and thus benefited only breeders of fruit and ornamental plants. The U.S. is the only country that specifically issues plant patents. Forty years later, in 1970, the Plant Variety Protection Act was enacted in the United States to provide intellectual property rights for sexually produced plants and tuber--propagated plants.

Europe, struggled for years to find a method to protect plant breeders' rights. Attempts were made to modify patent laws and use trade organizations, however these systems failed to offer any secure, satisfactory results (Heitz 1999). To overcome these difficulties, ASSINSEL, the Association of Plant Breeders for the Protection of Plant Varieties convened between 1957 and 1961 to develop a unified system for plant breeders' rights. Negotiations led to the adoption of the International Convention for the Protection of New Varieties of Plants, "The UPOV Convention" on December 2, 1961. Minor revisions were made in 1972 (the 1972 Act) and major revisions were made in 1978 (the 1978 Act) and 1991 (the 1991 Act).

The UPOV Convention sets the basic rules and principles for plant variety protection for those states that are ratified to the Convention; there are currently 49 member States. States that were ratified by a previous act, have an option of being further ratified by more recent acts. They are bound to whatever act they were ratified under. However, new members must be ratified to the 1991 Act. The 1991 revision was significant and one of the changes was to allow intergovernmental organizations to become ratified. This provision will enable the Community Plant Variety Office (CPVO), that grants plant breeders' rights in fifteen European

countries (The European Union), to initiate procedures towards membership in UPOV (UPOV 2002c). In addition, members of the World Trade Organization (WTO) are now obligated under the Agreement on Trade--Related Aspects of Intellectual Property Rights (TRIPS), to "provide for the protection of plant varieties either by patents or by an effective *sui generis* or by a combination there of." WTO members lacking any current system will undoubtedly devise protection systems based on the UPOV convention to be in compliance (Heitz 1999).

Utility patents for new plant varieties may be obtained in some countries. In the U.S., a landmark case was decided December 10, 2001, that solidified the use of utility patents for new plant varieties. Most likely, there will be an increase in their use, especially by large companies.

3. THE UPOV CONVENTION AND PLANT BREEDERS' RIGHTS

The International Union for the Protection of New Varieties of Plants, UPOV, is an intergovernmental organization with headquarters in Geneva, Switzerland. The acronym, UPOV, is derived from the French name of the organization, *Union internationale pour la protection des obtentions végétales*, as the convention was signed in Paris in 1961; it is also referred to as the "Paris Convention of 1961". UPOV, although a separate intergovernmental organization, works closely with the World Intellectual Property organization (WIPO). UPOV is undoubtedly the most influential organization in determining future directions and regulations for plant protection worldwide.

The aim of UPOV is to promote the protection of plant breeders' rights and offer a means towards uniformity and harmony on an international level. It defines explicit rules on the conditions for granting protection, provides rules for the scope of protection, and other regulations that must be included in the laws of its members. It also sets the general principles concerning the examination of plant varieties and provides specific guidelines for some 160 genera and species to enhance harmonization of applications and allow for exchange of examination results (UPOV 2002c).

Breeders that are aware of the basic rules and principles outlined in the UPOV Convention will better appreciate the reasoning behind the laws of the States, in which they seek plant protection and gain insight into the rules that govern international protection. It is important to know that, although the UPOV Convention can decrease some administration details, a breeder must make separate applications in each State. Although UPOV recognizes patents as well as plant breeder rights (PBR) and plant variety rights (PVR), the regulations and guidelines are more applicable to understanding the systems for PBR and PVR.

As of December 2001, about 50% of the member States are party to the 1991 Act, with the remaining States party to the 1978 Act with the exception of Spain and Belgium (1961/1972 Act). Some basic provisions and features of the UPOV Convention will be addressed below. More complete details, including the complete texts of the Acts and contact information for each member State can be obtained by

contacting UPOV (UPOV 2002c). The convention sets up a minimal scope of protection but allows for member States to take national circumstances into account when they formulate their legislation. Laws differ according to which Act a member State is ratified. It is therefore wise for breeders to familiarize themselves with the laws and rules of each individual State in which protection is sought.

3.1 Basic Provisions of the UPOV Convention

The following overview of the provisions and basic features of the UPOV Convention address both the 1978 Act and the 1991 Act, where they differ. It is advised that any person who is interested in or affected by any of the topics discussed below, consult the UPOV website for more details and the complete texts of the Acts (UPOV 2002c). "Member States" include all members of UPOV, whereas "Contracting Party" is used for identifying members that are party to the 1991 Act.

3.1.1 Persons Entitled to Protection

Under the 1991 Act, protection is granted to: (i) the person who bred, or discovered and developed, the new variety: "the breeder", (ii) an employer of the "breeder", that is entitled through contractual agreement, or (iii) the successor in title to the parties in (i) or (ii). It is significant to note that in the 1978 Act, protection is granted to the breeder whatever the origin (artificial or natural). Thus mere discovery was sufficient. In the 1991 Act, the breeder must also have developed the variety. Discoveries from the wild are, therefore, cannot be protected under the set of conditions of the 1991 Act.

3.1.2 Basic Rights of the Breeder of a New Plant Variety

Authorization from the breeder is required for the following activities concerning the new plant variety under the 1978 Act: (i) the production of the propagated material for commercial purposes, (ii) the offering for sale of the propagating material, (iii) the marketing of such material, (iv) the repeated use of the new plant variety for commercial production of another; and (v) the commercial use of ornamental plants or parts thereof as propagating material in the production of ornamental plants or cut flowers. Protection under the 1978 Act allows for the use of a new variety as a genetic source for the creation and marketing of other new varieties without any authorization or obligation to the breeder of the original variety. However, States are allowed to grant more exclusive rights to breeders such as extending the rights of the 1991 Act.

The scope of protection was redefined in the 1991 Act and specifies seven acts of exploitation in respect to the propagating material that require the breeders' authorization: (i) production or reproduction (multiplication), (ii) conditioning for the purpose of propagation, (iii) offering for sale, (iv) selling or other marketing, (v) exporting, (vi) importing, (vii) stocking for any of these purposes. These acts also relate to harvested material (whole plants and parts thereof) provided the material

was obtained through unauthorized use of propagated material and when the breeder has had no reasonable opportunity to exercise his/her rights in relation to the propagating material. Furthermore, the 1991 Act extends rights to four subject matters: (i) the protected variety itself, (ii) varieties which are not clearly distinguishable from the protected variety, (iii) varieties which are essentially derived from the protected variety and (iv) varieties whose production requires the repeated use of the protected variety.

The significance of the rights to "essential derived" varieties remains vague due to a lack of its application to date. Under the 1978 Act, others may freely use any protected variety as the source of initial variation to develop new varieties, without any obligation to the breeder of the original variety. The 1991 Diplomatic Conference addressed this situation, as there was great concern that it hindered incentive for plant breeders, particularly when genetic manipulation was considered. In essence, the 1991 Act states that a variety is only essentially derived when the new variety retains virtually the whole genetic structure of the earlier variety. Implementation of this ruling will more clearly define it, but it is narrowly defined. The 1991 Act does, however, retain the ability to freely use the underlying genetic resource in protected varieties for breeding purposes without the need for authorization from the original breeder. Additionally, varieties that are essentially derived and fulfill the protection requirements, may be protected, but can only be exploited with the authorization of the breeder of the original variety.

Activities that a may be exempt from Breeders' Rights, include: (i) acts done privately and for non--commercial purposes; (ii) acts done for experimental purposes and (iii) acts done for the purpose of breeding and exploiting other varieties, provided they are not essentially derived. It is optional to except farm--saved seed if they reasonably safeguard the rights of the breeder.

3.1.3 Right of Priority

Any breeder (national or resident of a member State) may file an application for the same variety in any other member State within 12 months after filing his/her first application and benefit from the right of priority. A State is required to examine an application if it is filed by the priority deadline. This may be confusing in comparison with the filing of a U.S patent. Although the United State Patent and Trademark Office (USPTO) honours this right, as opposed to PBR that grants a right on "first to file", this may not always the case with a U.S. patent (see *"U.S. Plant Patents"* below).

3.1.4 Right of Protection of the Denomination

Member States must recognize the breeder's right to protection of a new plant variety. When a denomination is registered in one of the member States, it may not be used, in any member state, as the denomination of another variety of the same or closely related botanical species. Member States must also ensure that the denomination can be used in connection with the variety even after the protection period has expired.

3.1.5 Genera and Species to Which the Convention Applies

The Convention may apply to all genera and species. States must make an effort to provide protection to the largest number of genera and species possible. There are requirements for increasing the number of species that they protect and it is dependent on which Act they are party to and when they became bound by the Act. A breeder may encounter States that do not provide protection for their particular plant. All States party to the 1991 Convention must grant and protect breeders' rights for all genera and species after five or ten years if also bound by the 1978 Act or 1991 Act, respectively.

3.1.6 Conditions for the Allowance of Protection

The Convention provides conditions that must be fulfilled for protection in detail and forbids States from imposing other conditions except for formalities and payment of fees. Protection can be granted only after it has been determined by an authority of the member State that the new variety fulfills the following conditions: (i) **Novelty:** The variety must not, where the law of a State so provides, have been offered for sale, marketed, or otherwise disposed to others for more than one year with the consent of the breeder in the State where protection is sought, nor more than four years (six in the case of grapevines and trees, including rootstocks) in any other States. The conditions for "novelty" for U.S. patents does not follow this rule (see "U.S. Plant Patents" below); (ii) **Distinctness:** The variety must be clearly distinguishable by one or more important characteristics from any other variety whose existence is a matter of common knowledge; (iii) **Uniformity:** Subject to the variation that may be expected from the particular features of its mode of propagation, the variety must be stable in its essential characteristics; (iv) **Stability:** Subject to the variation that may be expected from the particular features of its mode of propagation, the variety must be stable in its essential characteristics; (v) **Denomination:** The variety must be given a denomination enabling it to be identified; the denomination must not be liable to mislead or to cause confusion as to the characteristics, value or identity of the new variety and/or the identity of the breeder.

3.1.7 Selection of the Denomination of a Variety

The denomination is the name of a new variety that is chosen by the breeder. An authority of a member State will ascertain that it conforms to the prescribed requirements and then register it when the title of protection is granted. It becomes the cultivar name, referred to as the "generic designation" that is to be understood in relation to trademark laws. The denomination that is chosen by the breeder must adhere to a set of criteria for acceptance. These criteria include, but may not be limited to: (i) it must enable the variety to be identified; (ii) it must not mislead or lead to confusion concerning its characteristics, value, or identity of the new variety or breeder; (iii) it must be different from every denomination that designates in any member State, an existing variety of the same or closely related species; (iv) third

party rights must not be affected (its use must not infringe on any trademarks); as a rule, the same denomination must be used in all member States (there are concessions if a name is unsuitable in a territory); (v) it is required that the denomination be used by all persons who offer for sale or market a variety that is protected in their territory and (vi) it is permitted to use a trademark or tradename in association with the variety, however the denomination must be easily recognizable. The selection of a denomination requires careful consideration and should be chosen only after considering trademark laws, marketing plans, and rules of nomenclature.

3.1.8 Examination of the Variety

In adhering to UPOV regulations, protection of a new variety can only be granted after examination of the variety shows that it complies with the requirements for protection. In particular, an examination of a new plant variety should establish that it is distinct, unique and stable, and novel. A common acronym used to describe these requirements is **"DUS".**

An examination is based on growing tests carried out by either the authority granting the PBR, an institution on behalf of the authority, or by the breeder in some cases. The methods and regulations concerning testing vary among member States. UPOV has adopted "Guidelines for the conduct of Tests for Distinctness, Homogeneity and Stability". These test guidelines are established separately for individual species. They currently have guidelines for 160 genera and species and are published in separate versions in English, French, German, and Spanish. These guidelines were created to provide uniformity and encourage cooperation between member States. As there are different means by which test results can be shared and division of labour accomplished, it is in a breeder's best interest to investigate ways to avoid multiple examinations.

3.1.9 Right of National Treatment

The Right of National Treatment is an important right that all States party to the 1978 and 1991 Acts of UPOV must recognize. In laws pertaining to the protection of plant breeders' rights, each member State must give the same treatment to the nationals and residents of all other member States as it provides for its own nationals. There is an allowance for complying with the formalities of a member State; for example, a State may require the use of a resident as an agent. However, the spirit of this right is to extend equal rights to all, regardless of which member state a breeder resides.

3.1.10 Support Functions of UPOV

The role of UPOV in the formation of laws and regulations that effect plant protection worldwide is well known, but there are other UPOV activities that support plant breeders as well. They provide the means for harmonization of administration procedures by preparing model application forms and technical questionnaires that many States have adopted or closely followed for preparation of

their forms. This makes multiple applications easier for breeders and helps governmental officials in member states understand and evaluate the forms of other States. They also established a model gazette so that the gazettes of different States are easier to understand. In addition, UPOV produces the *UPOV-ROM Plant Variety Database*, a CD-ROM of protection information from all member states that is updated bi--monthly and a periodic newsletter, *Plant Variety Protection*; both are available by subscription to any interested party. The of UPOV office also works to promote breeders' rights by assisting State and organizations in setting up protection systems, by keeping in close contact with many organizations throughout the world that have interests in the field of plant variety protection, and by presenting symposia and seminars to facilitate the spread of knowledge and interest in plant protection.

4. PLANT BREEDER'S RIGHTS VS. U.S. PLANT PATENTS

Plant breeder's rights (PBR), plant variety rights (PVR), plant variety protection (PVP) are all titles used by various States for plant variety protection. They are, however, commonly referred to as PBR, regardless of the formal title. PBR is not used to describe any protection in the U.S. however, when PVP is used to refer to protection of seed and tuber propagated plants and plant patents indicate protection of asexually reproduced plants. PBR systems adhere closely to UPOV regulations and guidelines, while the regulations for a U.S. plant patent is based on the Plant Patent Act and adherence to the general patent laws of the United States. UPOV guidelines are not very informative for understanding requirements for a U.S. plant patent. However, the statutes for the variety nomenclature, the DUS requirement, some of the rights afforded and recognition of the right of priority clearly correspond. In fact, the patent statutes in the U.S., whether for plant or utility patents, have never been amended for the U.S. to ratify and be bound by the 1991 UPOV convention. Only PVP is in compliance (Ward 2001).

There are other aspects that differ between PBR and a U.S. plant patent. PBR requires an examination of the variety whereas a plant patent application requires a detailed botanical description and the USPTO relies on the information provided by the applicant and the knowledge of the examiners and/or the United State Department of Agriculture (USDA) for determination of DUS and novelty. In this regard, PBR can be more difficult to obtain, especially if any reversion or instability is observed. The rights, however, that are afforded by UPOV regulations and PBR are broader and better defined. Another difference is that PBR applications are typically published, or at least a notice of the application is posted, shortly after filing, whereas a plant patent is not published until 18 months after filing. This can affect the term of rights which, if dependent on publication, both U.S. plant patents, due to the American Inventors' Protection Act of 1999, and European PBR (EUPBR or CPVR) allow for provisional rights after publication (CPVO 2002b, USPTO 2002a). Finally, cost of PBR varies greatly depending on the State, but typically an annual maintenance fee is required. No such fee is required for a plant patent.

PBR in each country and State are independent of each other. The exception is CPVR obtained through CPVO that affords protection throughout the European Union. Although it seems straight forward, it is common to find false and misleading information published in catalogues and other publications, most likely without intent. Often, there are plants that have EUPBR that are listed as patented, there are plants that are mislabeled as patented or PPAF and there are royalty amounts listed for plants that have no protection anywhere. From an industry perspective, it cannot be presumed that a plant that has PBR in a State is subject to a U.S. plant patent or protected elsewhere in the world. Neither can one assume that it is not protected if a label does not give any protection details. It is the responsibility of a nursery when offering new plants, to investigate origins and protection status, especially when a plant is picked up in one country and brought to another with the intent of production and sale. It is wise to track the source of the plant or perhaps subscribe to the UPOV CD-ROM. U.S. plant patent status can be difficult to determine, as applications are kept confidential for 18 months. It is prudent to investigate, however, as production of a protected plant can be a costly error. For example, even if propagation of a protected plant was done prior to a grants of rights or notification of publication (infringing circumstances), an entire crop may need to be destroyed, once rights are granted. It is the responsibility of breeders' and companies that introduce new plants to ensure that protection information is correctly stated. Catalogues, tags, and marketing material must be monitored as well. The number of protected plants is increasing rapidly and the industry has been quite supportive. It is important that they can trust the information that is published in order for them to remain breeders' allies.

5. PROTECTION OF NEW PLANT VARIETIES IN NORTH AMERICA

The type of protection available for a new plant cultivar in the United States is dependent on its mode of reproduction, the type of protection desired and the degree that the requirements of each can be met. Plant patents provide protection of an asexually reproduced new plant variety, plant variety protection (PVP) affords protection for sexually reproduced (by seed) and tuber propagated plants, and utility patents can be obtained for protection of new plant varieties, regardless of their mode of reproduction. It is even possible to have double protection, where a plant variety is protected by both a utility patent and by plant variety protection or a plant patent. All three types of protection will be discussed in this section. Plant patents, however, are the most common type of protection sought to date for ornamental plants and will be addressed in more detail. On August 26, 1997, a special event was held to honour the issuance by the USPTO of the 10,000th plant patent (USPTO 1997).

The USPTO administers plant and utility patents, while PVP is administered through the Plant Variety Protection Office (PVPO) of the United States Department of Agriculture (USDA). Additional information concerning U.S. patents can be

obtained from the USPTO (USPTO 2002c) and more information on plant PVP is available from the PVPO (PVPO 2002).

The National Association of Plant Patent Owners (NAPPO) is a useful resource for those interested in plant protection in the U.S. NAPPO is a trade association that is dedicated to promoting the development, protection, production and distribution of new and improved plant varieties. The American Nursery & Landscape Association (ANLA) administers NAPPO and membership is open to firms and individuals interested in plant breeding, introduction, and intellectual property protection. NAPPO works to educate both governmental policy members and the industry on issues concerning plant patents and trademark and sponsors research activities in related areas. Members receive periodic newsletters and bulletins and a monthly list of new patents issued. Information about NAPPO can be obtained from their website (NAPPO 2002).

Many breeders who seek protection for plant cultivars in the United States are also interested in protection in Canada. Canada offers both PBR and a voluntary protection system through the Canadian Ornamental Plant Foundation (COPFb). Both opportunities should be explored if a new variety protection is to be grown or sold in Canada.

5.1 U.S. Plant Patents

5.1.1 Determining the Eligibility of a New Plant Variety for a U.S. Plant Patent

U.S. plant patents are granted by the U.S. federal government and provided for by Title 35 of the United States Code, section 161 (35 U.S.C. § 161) that states: *"whoever invents or discovers and asexually reproduces any distinct and new variety of plant, including cultivated sports, mutants, hybrids, and newly found seedlings, other than a tuber propagated plant or a plant found in an uncultivated state, may obtain a patent therefore, subject to the conditions and requirements of this title. The provisions of this title relating to patents for inventions shall apply to patents for plants, except as otherwise provided."*

To further clarify this statement: tubers refer to tubers that also sold as food, such as Irish potatoes and Jerusalem artichoke. Therefore strictly ornamental plants propagated by tubers are patentable; asexual reproduction can be any means other than seeds, including, but not limited to root or stem cuttings, layering, budding, grafting, and tissue culture. An"uncultivated state" refers to plants collected in the wild. The reference to the provision of this title refer to the fact that except for specific requirements or exceptions that pertain to plant patents, the general patent laws, codes, and regulations that apply to utility patents, also apply to plant patents. It is this element of adhering to patent laws that makes U.S. plant patents unique. The attributes, requirements, formalities and even the protection afforded is distinctive compared with PBR.

The provisions that must be satisfied for a plant to be eligible for a plant patent include:

1. The plant may be bred or discovered; if discovered, the discovery must have been from a cultivated (not wild) site.
2. The plant must have been found to been stable by asexual reproduction.
3. Plants propagated by tubers must not also be food tubers.
4. The person or persons filing the application must be the inventors of the plants, whereby he/she or they discovered, developed, identified, or reproduced the plant.
5. The plant must be unique and differ in at least one distinguishing characteristic from known, related plants.
6. The invention must not have been obvious to one skilled in the art at the time of the invention.
7. The plant must not have been sold or released in the United States more than one year prior to the date of application.
8. The plant must not have been enabled by a description in a printed publication with an offer to sale in the U.S. or anywhere in the world, more than one year prior to the application for patent.

5.1.2 Significance of 35 U.S.C. § 102 Rejections

Conditions for patent eligibility based on novelty of *any* invention are subject to the laws of 35 United States Code Section 102 (U.S.C. 35 § 102). This section covers the implications of aspects such as dates of invention, foreign filing, publication, prior public knowledge, sales, and more. These laws can be complex on their own, but adding to the complexity, is the fact that over the last few years, interpretations by the patent office in regards to plant patents has been like a roller coaster ride for the industry.

Prior to October 2000, the industry, the plant patent office and practitioners were working with the understanding that if all other requirements were met, plants were patentable in the U.S. if they had not been sold or described with an offer for sale in the U.S. more than one year prior to filing a patent application. Foreign sales and PBR activity was of any consequence. In October 2000, the patent office started rejecting patents that had been granted a PBR certificate if the PBR application was made more than one year prior to the U.S. patent application. As it was not a change in law, just an interpretation, there was no warning to the industry and understandably, many people were upset.

In January 2001, after a review of matter, the USPTO decided that the rejections under 35 U.S.C. § 102(d) that were based on a PBR certificates were not appropriate and all rejections were withdrawn (Kunin 2001). However; the situation led to a closer look by the USPTO of interpretations of §102(b) as well, that relates to the publication and sale of the invention: §102(b) states that the right to patent is lost if: "the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States". The patent office began questioning the applicant if a plant was previously published in a PBR gazette. Plants are unique, however, compared with other inventions as asexual reproduction of the plant is part of the invention and cannot be accomplished without access to the plant material. The patent office has been basing final decisions concerning

"enablement" on whether the plant was offered for sale or publicly available at the time that the plant was described in a publication. PBR publications alone therefore, will most likely not result in loss of patent rights, as publications of PBR application filings typically do not mention availability or on offer for sale.

While future interpretations or rulings are uncertain, representatives of the industry have been working towards a solution. As of April 2002, the provisions stated above (Numbers 7 & 8) seem to be the current position held by the USPTO. The implications of this situation are significant to the industry and patent owners and applicants are advised to keep up--to--date on the matter. The rights of hundreds of issued patents are in jeopardy. For more information, refer to a comprehensive treatment of the entire saga that includes pertinent correspondence compiled by patent attorney Vincent Giola (Giola 2000a).

5.1.3 Rights Granted by a U.S. Plant Patent

The right that is granted with a U.S. plant patent is provided by 35 U.S.C. § 161 that states: *"...the grant shall include the right to exclude others from asexually reproducing the plant, and from using, offering for sale, or selling the plant so reproduced, or any of its parts, throughout the United States, or from importing the plant so reproduced, or any parts thereof, into the United States."* The reference to "any of its parts" and "any parts thereof" were added by the Plant Amendment Act of 1998, with the purpose of protecting patent owners against the unauthorized sale of plant parts taken from plants illegally reproduced.

The right is given as a right to "exclude" even though the right allows the patent owner (inventor(s), assignee, or heir) to exploit the patented plant and license others to do so, the patent owner must adhere to commerce laws of the United States. For example, rights as a patent owner could be limited if he or she violates anti--trust laws, infringes upon another's patent, or lacks a particular license required to sell the product (Bouchoux 2001). Plant patents protect only one variety of plant and plants that rise from asexual reproduction of the claimed plant. Further clarification of this point will be made in the section below *(*see *Patent Enforcement and Infringement)*. The term (length of protection) of a patent filed after June 8, 1995 begins on the date of issue and ends twenty years from the date the patent application was filed.

5.1.4 The U.S. Plant Patent Applicant

By law, only the actual inventor(s) may apply for a patent (or a legal guardian if the inventor is deceased, insane, or legally incompetent). Even if the rights of a new variety are assigned to another party, the inventors' signatures are still required for the initial application. For plant patents, there are two steps that constitute the invention: the identification of the novel plant and the asexual reproduction showing stability of its unique characteristics. There may be multiple inventors. If one person made the parental cross, another selected the plant, and another asexually reproduced the plant; all three could be considered co--inventors. However, an inventor can direct a custom propagation service, such as a tissue culture lab, to perform the asexual reproduction and they would not be considered co--inventors.

5.1.5 Assistance in Drafting and Filing a U.S. Plant Patent

Patent applications may be prepared and prosecuted (processed) by the inventor, however securing the services of a patent agent or patent attorney is advisable in many cases. Agents and attorneys must be registered with the USPTO. Although either may prepare, file, and prosecute a patent application, only an attorney can represent an inventor in litigation proceedings. The USPTO website has a list of registered practitioners. NAPPO can assist in finding an agent or attorney that has experience with plant patents.

Inventors who choose to handle their own applications should have or obtain some knowledge of patent law, the technical requirements of application preparation, and patent office procedures. In addition, one must be able to botanically describe the claimed plant and know how it differs from closely related plants. The USPTO offers a publication, *General Information About 35 U.S.C. 161 Plant Patents*, which provides a basic overview of plant patents and information concerning the preparation and content of the application (USPTO 2002b). Although it is very informative and useful, it may not be up to date and lacks the details for preparing and filing a formal application. The patent office should be contacted concerning new requirements and further details can be found in other publications on the USPTO website. There are three publications that govern all patent matters: The Patent Statures; 35 United States Code (35 U.S.C), 37 Code of Federal Regulations (37 C.F.R.), and the Manual of Patent Examiner's Procedures (MPEP); which is followed by patent examiners in reviewing applications. All three can be accessed through the PTO website and copies can be ordered from the Government Printing Office (GPO 2002). The MPEP is not needed to draft a patent application, but access to it may be useful for prosecuting the application. A copy of the MPEP can also be found at Patent and Trademark Depository Libraries; a list of locations can be found on the USPTO website. Those individuals that previously filed patents should become familiar with recent changes in the laws due to the American Inventors Protection Act of 1999 (USPTO 2002a).

5.1.6 Basic Requirements of a U.S. Plant Patent Application

The four primary components of a plant patent are: (i) a specification that describes the invention, (ii) an oath or declaration of the inventor, (iii) a drawing (usually one or more photographs) of the claimed plant, and (iv) a filing fee. There are also application and fee transmittal forms, an Information Disclosure Statement (IDS) and documents concerning assignment of the patent that may be submitted at the time of application, if applicable. An IDS is voluntary but should be submitted if there are any matters, publications or other patents that may effect patent eligibility; it is the duty of all persons involved with a patent application, to disclose pertinent information. The specification, claim, and drawings become part of the granted and published patent.

The specification is the most critical element. It is a written document that discloses information concerning the claimed plant to include: distinguishing characteristics from related varieties, a complete botanical description, location and manner of asexually reproduction and if it was discovered, the location and character

of the area of discovery. As plants are not tested against other varieties as they are for PBR certificates, the specification and drawings alone are used to determine the characteristics and uniqueness of a plant. **The elements of the specification include:**

1. **The tile of the invention**: must include the name of the plant used for international recognition, fall within the requirements of the *International Code of Nomenclature for Cultivated Plants, 1980,* and the cultivar name must be in single quotes (Trehane et al., 1995).
2. **Cross--Reference to related applications (if any):** those related to the claimed plant, such as a utility patent on the same plant or co--pending applications from the same breeding program.
3. **Statement as to rights to inventions made under federally--sponsored research and development (if any).**
4. **Background of the invention:** to include a comparison of the claimed plant to prior art (parents or closely related plants), information on how the plant was attained and asexually reproduced, and a statement concerning the stability of the propagated material.
5. **Summary of the invention:** a list or narrative of the major characteristics of the plant.
6. **Botanical description of the plant:** the description should be as complete as reasonably possible and include all aspects of the plant, its growth habits, and propagation, including non--distinguishing characteristics. Colours should be identified by reference to a designated colour chart (cf. The Royal Horticultural Society or RHS Colour Chart is typical used: RHS 2001). The description should be in enough detail as to distinguish the plant from known varieties.
7. **Claim:** A plant patent can have only one claim and must make reference to the plant as shown and described in formal terms. It must be a single sentence and drawn to the plant as a whole. No products or parts may be claimed.
8. **Abstract of the disclosure:** a brief description of the plant and its most notable or novel characteristics.

The declaration or oath is a truth and disclosure document that must be signed by all of the inventors of the claimed plant. This document is also used to claim a right of priority based on a foreign application. Priority can only be claimed if the application is filed within one year from the date of the first foreign application for the plant. Claiming priority may afford patent eligibility that would otherwise be lost due to lack of distinction from another applicant's plant, however; a priority claim cannot be used to overcome any restrictions imparted by 35 U.S.C § 102 concerning the enabling of the invention to the public. The actual filing date will be used for those determinations.

Plant drawings are typically photographs and they must be in colour if colour is a distinguishing characteristic of the plant. Colours depicted in the drawings must correspond as close as possible to the colour values given in the specification. All distinguishing characteristics that can be shown in a drawing must be shown and at least one drawing must depict the entire plant, even if only a single plant part is considered novel. If the plant is a sport of a known variety, every effort should be

made to photograph the claimed plant along side of the parental variety with the parental variety identified as "Prior Art". There are detailed paper requirements for both the specification and the drawings that are defined in the 37 Code of Federal Regulations (37 C.F.R.). New regulations also became enforceable as of October 1, 2001.

5.1.7 U.S. Plant Patent Fees

The basic cost of a U.S. Plant Patent, in terms of the USPTO, consists of a filing fee payable when the application is made and an issue fee that is paid after allowance of the patent. Two different fee structures are assessed by the USPTO: a standard fee and a reduced fee called a small entity fee in which fees are reduced by 50 percent. Small entity status is given to individuals, small business concerns, and non--profit organizations that either own all rights to the invention or have not assigned, granted, conveyed, or licensed any rights to the invention to any party that would not qualify as a small entity. A small business concern is one with fewer than 500 employees. Any fees due subsequent to conveying a license to a business that does not qualify as a small entity, even if non--exclusive, would be accessed at the standard rate. As of January 1, 2003, the filing fee is US$520 (US$260 for a small entity), the issuance fee is US$630 (US$312 for a small entity). A publication fee of US$300 is also accessed. Fees for assignments, extensions of time, and other matters may be necessary, if applicable. The charges or fees of an agent or attorney for drafting and processing the application would be in addition to the USPTO fees.

5.1.8 The U.S. Plant Patent Application Process

Plant patent applications can be filed in person or mailed; they cannot be faxed or filed electronically. If the application is mailed via "U.S. Express Mail", the date mailed will be the filing date; otherwise it is the date of receipt by the USPTO. Once the application clears the application branch as complete, a filing receipt with a serial number is sent to the applicant and the application is assigned to a patent plant examiner. The examiner completes a review of the application and sets forth his or her opinion of the application in an "Office Action" that is typically sent out about twelve months after filing. Nearly all applications are initially subject to rejection and raised objections. The reasons for the rejections and objections are given in the Office Action, along with any action required of the applicant. An applicant may amend the patent application in response to the examiner's suggestions. New material cannot be added to the specification with the exception of descriptive information specifically requested by the examiner. The applicant must respond within six months, but a response within three months is required to avoid additional fees. If the examiner is satisfied with the amendment and response, a notice of allowance will be issued and after the issue fee is paid, the patent should be granted within four months. If the examiner is not satisfied, a "Final Office Action" will be sent and an additional response may be filed. If allowance is not granted after a response to the Final Office Action, there are options, such as an appeal, that may be pursued if the applicant does not wish to abandon the patent application. Typically, the entire patent process takes about two years.

5.2 Plant Variety Protection in the U.S.

The Plant Variety Protection Act (PVPA) was enacted in 1970 to encourage the development of novel cultivars of sexually reproduced plants. The PVPA was further amended in 1994 to comply with the 1991 Act of UPOV convention and added protection for F_1 hybrids, essentially derived varieties, and tuber--propagated plants that were barred from obtaining plant patents. The PVPA is administered by the PVPO, a division of the United State Department of Agriculture. Although the majority of PVP certificates granted to date have been for agronomic crops, the use of PVP for seed strains of ornamental crops is increasing. A database of current genera that have PVP applications or certificates is available on the PVPO website (PVPO 2002).

The requirements and elements of the 1994 PVPA very closely follow the current 1991 UPOV convention guidelines. There are no examinations however, as deposited material, trial data from the breeder, and database searches are relied upon to determine distinctness and stability. PPVA provisions require that a variety be new, uniform, stable and distinct. Proof of these requirements lies with the applicant. The novelty requirement adheres to UPOV guidelines, i.e. a variety is eligible if it has not been sold or offered for sale for more than one year prior to application in U.S. and for more than four years (six for a vine or tree) in a foreign country. In contrast to a U.S. patent, where invention rights can be established by first to conceive or reduce to practice (propagate and test stability), PVP entitlement is based on first--to--register.

Under the 1994 PVPA, the owner of a protected variety has the right to prohibit others from: selling, marketing, delivering, offering for sale, shipping, consigning, exchanging, importing, exporting from the U.S., importing into the U.S., conditioning for sale, stocking for a prohibited use, transferring of title or possession and sexually multiplying. The act of using the variety for producing a hybrid is also prohibited. Use for the *development* of a new hybrid is allowed. These rights are extended to essentially derived cultivars, indistinct cultivars, harvested material (obtained from authorized propagation), and cultivars that require repeated use of the variety.

Although the protection is quite comprehensive, exemptions do exist. A farmer (or home owner) can save seed for their own use. Under the 1994 PVPA, a farmer may no longer sell the seed for reproductive purposes. The variety can also be used for research and breeding to develop a new variety, thus preserving the UPOV tradition that the underlying genetics of a protected variety should be freely available. For this reason, utility patents are sought in cases where a breeder wants to prevent the use of the protected variety in another's breeding program. Finally, a single plant selection made from a protected variety and *asexually* propagated (only) would not be an act of infringement and may be eligible for a plant patent.

PVP certificates are granted for a period of 20 years from the date of issue for non--woody plants and 25 years for a woody plant. Neither the PVPO nor the USDA will take any action against infringing activity. As with patents, the owner of the variety must enforce the protective rights. The owner of the variety may bring civil action against persons infringing on their rights and can ask a court to issue an injunction to prevent another from further violations.

The basic cost of PVP as of December 2002 is US$3,025 for the application, examination and issuance fee. More detailed information on fees can be obtained from the PVPO website (PVPO 2002). An applicant is required to complete four exhibits that provide the details of the breeding history, descriptions of the variety and its closest comparison varieties, and a statement of ownership. A deposit of 2,500 untreated seeds or a verification of a viable cell culture, if a tuber--propagated plant (for later deposit), and payment of the application and examination fees are required. Additional information on all aspects of PVP can be obtained from the PVPO (PVPO, 2002). Strachan (1999), an examiner for the PVPO, provides a more comprehensive discussion of the application requirements.

5.3 U.S. Utility Patents

Utility patents cover any new and useful process, machine, article of manufacture, composition of matter, or any new and useful improvement thereof. The U.S. statute that very broadly defines the patentable subject matter is 35 U.S.C. §101. The costs of obtaining and maintaining a utility patent if greater than those of a plant patent. As of January 2003, the filing fees are US$750 (US$375 for a small entity) and the issuance fee is US$1,3000 (US$650 for a small entity). The maintenance fees due at three and one half years, seven and one half years, and eleven and one half years are US$890, US$2,050, and US$3,150 respectively. Small entities are given a fifty percent reduction in maintenance fees.

Utility patents in the U.S. regarding plants have, until recently, been typically used to protect inventions with aspects other than the propagation or use of a new variety. Although utility patents have been issued by the USPTO since 1985 that afford protection to hybrid plants; this form of protection has been relatively unrecognised or used for ornamental plants. Controversy existed because some reasoned that with the existence of U.S. plant patents for asexually reproduced plants and PVP for tuber and seed--produced plants, utility patents under 35 U.S.C. §101 were not applicable for protection of new plant varieties. Two U.S. Supreme Court decisions clarified the use of utility patents for plants. In *Diamond v. Chakrabarty*, regarding a patent for a microorganism, it was decided that living things were patentable if it arose as the result of human ingenuity and research (Findlaw 2002a). In December 2001, in *J.E.M. AG Supply Inc. v. Pioneer Hi--bred International, Inc*., the issue was challenged once again (Findlaw, 2002b). J.E.M. Ag Supply Inc., in business as Farm Advantage, challenged an infringement suit by Pioneer Seed arguing that sexually reproduced plants, such as Pioneers' corn plants, were not patentable subject matter within §101 and therefore, the patents were invalid. Pioneer prevailed in the case as the court held the decision that Congress never removed plants from patentability under §101 and that newly developed plant breeds do fall within the subject matter of §101.

The requirements for a utility patent are greater and more stringent than those for a PVP certificate or a plant patent and are more difficult to obtain. The preparation of the specification and prosecution of the application is more complex and costly. In addition to the "new and distinct" requirements of PVP and plant patents, a utility patent requires that the plant must be "useful" and described with sufficient specificity to enable other to "make and use" the plant after the patent term expires.

Typically, there is a requirement for a deposit of biological material, such as seeds or tissue, in an accessible depository to satisfy the enablement and description requirements (Peet 1995a).

One of the most important differences between a plant and a utility patent is that multiple claims can be made in the latter (thirty is not unusual). In a plant patent only one claim is allowed, which refers to the specification. Writing of the claims, which must be backed up by the specification, is critical and defines the level of protection that the patent is afforded when issued. The primary advantage of obtaining a utility patent is the level of protection attained. If claims are properly constructed, the use of the claimed plant for breeding activity by others *could* be considered infringement of the patent.

The majority of utility patents granted to date have been for inbred lines and hybrids. The patent for an inbred line protects the inbred line and the hybrids produced using the inbred line. A hybrid plant patent typically protects the plant, its seeds, variants, mutants, and trivial modification of the hybrid. Utility patents may also cover other aspects pertaining to the invention, such as breeding methodologies and engineered gene constructs. Examples of utility patents that have been granted for ornamental plants are bicolour petal patterns in Impatiens (PP 5,986,188), ornamental characteristics of scented geraniums by genetic transformation (PP 5,648,598), flower form and method of breeding of New Guinea Impatiens (PP 5,986,188), and the plant hybrid germplasm of the My Favorite® Chrysanthemums (PP 13,387).

PVP now protects any variety that requires repeated use of a variety and has some, yet to be defined, provisions for essentially derived material, but it also allows farmers to collect seed for their own use and use of the variety for research. There are no exemptions for these activities with a utility patent. There is no protection whatsoever of the underlying genetics with a plant patent. Protection under utility patents is theoretically a broader form of protection than that afforded by PVP or plant patents (Peet and Bent 2002).

5.4 U.S. Patent Enforcement and Infringement

Infringement aspects for plant patents, utility patents, and PVPA certificates are highly dependent on facts with only a few legal decisions to allow definitive statements. An attorney should always consulted in these matters. However, there are some general aspects and ideas that are useful for understanding of the parameters of protection afforded by plant patents and encouraging activities that prevent or correctly identify infringement.

Plant patents are not self--enforcing and a patent owner needs to monitor the market and take action to stop any infringing activity. It can save time and money if efforts are made to avoid patent infringement as well. Making sure that protection information is present on all tags and marketing information, and using advertising or other means to inform the trade of its protection status is good practice. This is particularly important if a plant is on the market while the patent is pending, as U.S. patents are held in confidence until they are published 18 months after filing.

Direct (literal) infringement occurs when a party offers for sale, sells, uses, or imports (see *Rights Granted by a U.S. Plant Patent* above) a patented plant without

authority to do so. A nursery that has purchased material from a licensed propagator may of course, resell the plant without risk of infringement (the first sale doctrine), provided that proper tags are used. In 2002, Proven Winners® prevailed in a lawsuit against Jernigan's Nursery (Dunn, NC) for failure to insert a plant tag with every plant in hanging baskets (Greenhouse Grower 2002). All plants should be labeled with appropriate tags using the correct plant name (name on patent) and either the word "patent" and the patent number or PPAF (Plant Patent Applied For) or patent pending, if the patent is still pending. Either way, the tag should state that unauthorized propagation is prohibited. Neither PPAF nor "patent pending" have any legal effect, however, false use of these phrases is illegal if a patent application has not been filed. This is often a problem when catalogues are printed well in advance of sales but the patent drafting is still in process. Some individuals and firms use the acronym PPIP for "plant patent in process" or "plant patent rights asserted". These words may not be legally appropriate either as they imply that a patent application has been filed. Certainly, it is in the best interest of all to know when there is an intention to file a patent. Keeping a plant a trade secret under secure trials until a patent is filed may be the best option.

There are two other types of infringement: indirect infringement and infringement under the "Doctrine of Equivalents". Indirect infringement occurs if one actively induces or contributes to another's direct infringement. The doctrine of equivalents comes into play when a party sells a plant that is indistinguishable, to a reasonably skilled person, from the patented plant (Battle 1997). Although a carefully drafted utility patent *may* cause a similar plant, in which the characteristics are essentially the same, to be considered an infringing product, the scope of protection for plant patents is quite different because a plant patent is only enabled through asexual reproduction of the patented plant. A Federal Circuit court ruled in the *Imazio Nursery, Inc. v. Diana Greenhouses* case, that only propagation from the original patented plant would be considered infringement (Emory 1995). The *Imazio* decision requires a plant patent owner prove that the accused variety was derived from the patented variety, typically by a "chain--of--possession". Biochemical data such as from DNA or isozyme analyses may prove useful to resolve a dispute. It is clear from the *Imazio* case that simply providing phenotypic evidence of similarity of an independently derived variety would not make a strong case for infringement (Peet and Bent 2002). In other words, in the case of a plant patent, a similar plant in existence may prevent the granting of a patent due to lack of novelty but propagation of a similar independently derived plant *may* not be considered infringement.

If infringement is suspected, a party should first obtain as much information as possible and a patent attorney should be consulted. Patent litigation is expensive and patent suits are litigated in federal courts. Fortunately, settlements are often made without litigation. An attorney should carefully consider infringement charges, as the threatened party may try to prove that the patent is invalid, possibly resulting in costly litigation and/or loss of the patent rights.

Companies and nurseries are advised to do some research to avoid infringement before producing and marketing new plants because even innocent infringement may be prosecuted. If a company becomes aware that they have engaged (perhaps unknowingly) in such activity, contact a patent attorney and immediately cease the

activity. Patent laws allow the awarding of up to three times the amount of actual damages and perhaps attorney fees in exceptional cases, such as those involving willful infringement.

Patent rights cannot be enforced until a patent is granted even though the term is calculated from the filing date. However, propagators are usually respectful of pending patents. Both the propagator(s) and their customers, would be at risk for infringement as soon as the patent is granted and if done knowingly, they may not be in a favourable position for obtaining a license from the patent owner. In addition, now that patents are published 18 months after filing, there are provisions in the laws for collecting fair royalty payments between publication and issuance. A copy of the publication should be sent to any party that a patent owner would like to charge a royalty fee, as knowledge of the publication is required to exercise that right. Once a patent is granted, monitoring the industry for proper use of the patent is essential and companies involved in managing a patented plant should have either their own system or a contract with an outside firm for that purpose.

5.5 Plant Protection In Canada

Plant protection may be obtained by PBR or through the Canadian Ornamental Plant foundation (COPF). COPF is a non--profit organization that was started in 1964 to promote new ornamental plant development and served as the only protection means until PBR became available in 1990. COPF has a network of grower/breeder members and royalties are collected on a voluntary basis, with a percent of the royalties retained by COPF for their services. When PBR became available, COPF expanded their services to include managing license agreements and royalty collection for breeder clients with PBR, but have continued representation of plants without PBR as well. COPF registration costs $50 Canadian per variety, whereas PBR rights costs approximately $1,500 Canadian with a $300 Canadian annual renewal fee, plus the cost of an agent's services for processing the application and setting up examination trials. In most cases, it is advantageous to register a plant with COPF even if licenses are administered outside of COPF, as they keep the industry informed about the protection status of registered plants through the *COPF Newsletter*. Additional information concerning COPF can be obtained from their website (COPF 2002a).

At first consideration, the costs of PBR in Canada may seem unrealistic in terms of the market size for a particular plant. Canada, however, has a growing industry and many growers have trade relationships with the U.S. Obtaining PBR in Canada allows for greater awareness of the protection status in the U.S., a factor that should be considered when analysing the merits of obtaining PBR. Canadian PBR is administered through the Plant Breeders' Rights Office as part of the Canadian Food Inspection Agency. The PBR system in Canada closely adheres to 1978 UPOV regulations, although there are a few particulars that warrant mentioning:

1. A foreign resident is required to assign a Canadian agent (resident of Canada) to submit the application and correspond with the PBR office on their behalf.
2. Examination trials in Canada are required. Even if the trial results are supplied from another UPOV member, a one--year trial with a comparison to varieties currently grown in Canada is necessary.

3. An option exists to file a "protective direction" to serve as a means to protect a variety while PBR is pending. If elected, however, sales of the variety are not permitted by any party until rights are granted, unless the protective direction is removed.
4. Under current regulations, a PBR application for a variety cannot be filed if an offer for sale is made anytime prior to filing an application. This requirement is surely to be changed; the PBR office recognizes the restrictive implications to the industry and legislation is currently being sought to correct the situation.

For obvious reasons, the latter point is an important consideration when seeking protection in Canada. If a grower in the U.S. is licensed to sell in Canada and their catalogue arrives in Canada before a PBR application is filed, the application will be rejected. Changes to regulations to would enable ratification of Canada to the 1991 UPOV convention and alter this requirement to the one--year rule and affect other aspects as well (COPF 2002a). Additional information concerning the PBR application process and a copy of PBR regulations can be obtained from the Canadian PBR office (CPBRO 2002).

6. PROTECTING THE RIGHT TO OBTAIN PROTECTION FOR A NEW PLANT VARIETY

There are measures that a breeder can take to ensure that protection rights are not jeopardized. Although these suggestions are also useful for asserting and protecting proprietary rights in other countries as well, they are geared towards U.S. patent protection because particular precautions are needed.

There are two important factors to keep in mind for conserving your right for a U.S. Patent. The first concerns the situation where two parties claim the same plant or plants too similar to both acquire patents. In most cases, the party that can provide evidence that they were the first to discover or select and propagate the plant will be granted the patent. As asexual reproduction is an indivisible component of a plant patent, it is advisable to propagate the plant and establish stability as soon as possible. This situation is unique to the U.S. as ownership is generally determined by "first to file" for patents and PBR in other countries (Elias and Stim 2001). A second precaution that must be taken is to avoid an offer for sale of the plant in the U.S. or a description with an offer for sale anywhere in the world more than one year prior to applying for the patent; a violation of this condition is an absolute statutory bar!

It is advisable for breeders to record information concerning their work in a notebook. When a selection or discovery is made, a description of the plant should be written and dated photographs taken. If possible, a witness should sign the entry. All activity related to the plant should be recorded in the notebook. This should include any notes concerning propagation and market testing endeavours. This method is much preferred to that of mailing a disclosure to oneself as a record. If a breeder wants does not a want to use a witness; a disclosure can be filed with the

USPTO for a small fee. Many experts consider witnessed disclosure preferable (Elias and Stim 2001).

Protection, whether patents or PBR, protects your rights to a plant but it does not market, or in any way provide compensation without the breeder or another party marketing the plant. Often, breeders will want to discuss the plant with a perspective nursery, promotion company or a breeder agent that can assist them in both analyzing the market potential prior to paying for protection. It is perfectly acceptable in regards to both patents and PBR to set up experimental trials, however precautions must be taken to avoid public disclosure of the plant during this process. It is imperative to have any and all third parties sign a confidentiality agreement prior to presenting them with a complete description and photograph of the plant of interest. The owner of the plant should make it clear, that the purpose is to preserve his or her proprietary rights. If there is an interest to trial the plant, a testing and non--disclosure agreement should be put in place with any party that is involved in the testing process. A testing and non--disclosure agreement should at a minimum contain an agreement clauses stating that the plant will not be distributed or sold to any party and that they will not disclose any information about the plant to an outside party. It is also wise to assign a code name to the plant during the discussion and testing stage to avoid premature public disclosure of the cultivar name or assigned breeder identification number.

These measures have more than one effect. The party involved in testing and analyzing the plant will be less likely to cause a public disclosure and if the party decides that they are not interested in the plant, a breeder ideally should be able to get a non--biased opinion from another party as they should not know of the prior party's disinterest. Testing a variety is useful for determining its commercial potential before the expense of patenting is incurred. Precautions in this stage cannot be overemphasized. Even if a plant were publicly enabled by sale or publication without a breeder's consent or knowledge, a bar to patentability would still be made. There may be legal recourse based on your agreements, but not under patent law.

There is one other possible action that can be taken if a publication or sale date is a concern to patentability. A provisional patent can be filed. Provisional patents require a complete specification, but without the formalities and the filing fee is only US$80. The advantage is that a filing date is obtained to guard against rejections and bars due to publication or prior art, you may use "patent pending", and you can gain some additional time to assess commercial potential. A regular, non--provisional patent must be filed within one year or the application will be considered abandoned. The patent term, however, will be extended as if filed on the later date.

7. INTERNATIONAL PATENTS

Plant patents are unique to the U.S., although that does not preclude the possibility of obtaining a utility--type patent in other countries for a new variety of plant. New plant varieties are patentable in the U.S., Australia, Japan, and in some circumstances, Europe (Ward 2001). Patents for plants has been problematic in many countries as the authorities have argued that plant characteristics are too

variable in response to external conditions and that it is impossible to definitively describe a plant. The U.S. has circumvented this problem by allowing deposits of plant material to in part meet the descriptive requirements. In Europe, there are complications relating to obtaining both a community plant variety right and a patent on the same plant material. It is problematic because of the different regulations and requirements of the European Patent Convention and the CPVO; if a CPVR is granted on a plant, any national PVR or patent in Europe would be suspended for the duration of the CPVR (CVPO 2001). There may be patent opportunities in countries other than those listed above and the individual patent offices of countries of interest should be contacted.

Applicants interested in obtaining protection in multiple countries may want to file a Patent Cooperation Treaty (PCT) application. Priority rights in all member countries can be obtained with a single international filing. Separate applications (national applications) must be subsequently filed in each member country according to their individual requirements. The advantage of a PCT application is that an international search is conducted, paperwork is reduced, and more time is allowed to complete all of the national applications as compared to filing the applications separately without a PCT application. A source of additional information concerning the PCT is available from The World Intellectual Property Organization (WIPO) website (WIPO 2002).

8. TRADEMARKS

There is probably no other area of intellectual property rights related to plants that is more controversial or misunderstood than the use of trademarks. Trademarks are familiar to everyone with almost every product we use. There are aspects pertaining to plants that are unique and do not exist with other products. A plant may be produced by many different growers, marketed through a complex chain of distribution and then promoted, written about, and labeled by many different parties with many different agendas. The most unique feature of plants is their nomenclature. The fact that plant has a cultivar name (fancy or nonsensical), a common name, a scientific name, and possibly a selling name or trademark associated with it, adds a dimension that does not exist in other industries. The use of trademarks in connection with plants is poorly understood, particularly in the U.S, and there are conflicting opinions in regards to how they should be used. There is also a lack of consistency in presentation of a trademark in relation to the cultivar name. Consistent of nomenclature alleviates confusion and it may require that the industry work together to develop mutual policies.

Trademarks can be of great benefit to a plant breeder. They may be used to heighten the reputation and exposure of a breeder and enhance the marketing of a breeder's plants. Trademarks that develop into well--known brands are valuable and powerful assets. Proper use, marketing, policing, and ingenuity are required to protect them and make them meaningful. Where trademarks are concerned, the more knowledge and expert advice that one obtains, the greater the chance of realizing the benefits that they can provide. Examples of effective trademarks in the industry include: Flower Fields®, Proven Winners®, and My Favorite® that

indicate both a source and a collection of plants that undergo a selection process, Pride of the Prairie® that is used to promote native plant cultivars selected by Neil Diboll of Prairie Nursery (Westfield, WI), and Athens Select® to promote plants selected by the University of Georgia based on good performance in the South Eastern region of the U.S.

8.1 Trademarks Defined

The term trademark is defined as any word, phrase, symbol, or device, or any combination thereof that is used to identify and distinguish one's goods or products from those of others and indicates the *source* of such goods or products. Quite simply*, a trademark is a brand.* The Merriam-Webster dictionary's definition of the word *brand* in this context is "a class of goods identified as the product of a particular firm or producer" (Merriam-Webster 1995). A meaningful trademark or brand should identify a particular source to the consumer (or trade) and provide an assurance of quality and consistency of the product. Quality assurance may be related to the selection of the plant, health and vigour, or to any other standard that has meaning and helps one make informed purchasing decisions.

It is improper to use a trademark, if the trademark becomes the name by which a product is known from a customer's point of view. This is called "generocide" and is the reason that Kleenex, Escalator, and Aspirin all became invalid trademarks. When using trademarks in connection with a single cultivar of plant, it is very important to use the generic or cultivar name whenever the trademark is used. Even when steps are taken to always use the cultivar name, often, they remain unknown to customers and theoretically the trademark may have no legal merit.

The word "trademark" is commonly used to refer to service marks and all types of marks. The various types of marks are mentioned below in connection with specific uses that are applicable to the marketing of plants.

8.2 Specific Uses of Trademarks in the Horticulture Industry

It is critical to identify the intended use of a trademark prior to selection and use. Specific uses within the industry in connection with plants include:

1. **To identify plants from a particular breeder, breeding program, or plant selection program**. Marks used for this purpose indicate to the end user that the plants under this mark have merit; they have been bred and selected by experts that they can trust.
2. **To identify a series of plants with a common characteristics.** Marks associated with a series indicate to the consumer (or trade customer) that all the plants in the series have particular qualities or characteristics. A series may be created based on criteria such as plant habit, flower size, length of bloom, or disease resistance.
3. **To indicate a grower or nursery as a source.** This mark informs a purchaser that the plants are grown by a particular nursery. If they produce healthy, well--developed, vigorous plants, their mark becomes meaningful as a source of

quality plants. A business name can be used as a trademark as long as goods (or services) are associated with the name.

4. **To identify the product from a group of growers.** A mark for this purpose can be registered as a "Collective Mark". Filing of this type of mark seems to be underused in the U.S in the horticulture trade and is an effective way to "share a mark".
5. **To indicate a level of testing or approval.** This type of mark can be registered as a "Certification Mark". It is useful to identify products that have withstood a level of testing or won the approval of a selection committee. This type of mark is also underused in the industry.
6. **To identify a particular service.** Often referred to as a trademark, but correctly it is considered a "Service Mark". An example would be a service that provides marketing support.
7. **To use as the selling name for a single cultivar.** Used to identify a particular plant. If used for this purpose, it should always be used in addition to the cultivar name, a point clarified below.

8.3 Trademark Use for the Marketing of a Cultivar

Most trademark owners around the world use their trademarks as a general marketing tool, rather than to identify a particular cultivar (Tehrane 2001). However, in some countries, including the U.S., the practice is quite common, especially by introducers of woody ornamentals. There are both advocates and opponents of this use of trademarks. Those who dislike the practice, argue that it is constitutes improper use of trademarks because of *genericide*. Secondly, the spirit of the codes and ethics of nomenclature are violated because universal recognition of a plant name is hindered (Avent 1999). The primary reasons that trademarks are used to market a particular cultivar are as follows.

8.3.1 Reasons for Using a Trademark for an Individual Cultivar

1. **Used as an attractive selling name.** A trademark is used because the cultivar name that is registered and used as a denomination for PBR and patenting is unattractive and perhaps unpronounceable (e.g. a breeders code). If a plant has only a nonsensical name, some sort of selling name is needed. It is common standard practice to use code names with PBR applications. By using a code name, one is less likely to violate another's trademark and it is easier to use different selling names in different countries. There is some validity to this reasoning. One could also select a universally accepted cultivar name and perform a trademark search for the cultivar name. Worldwide recognition of the cultivar name is more pleasing to the industry and may be beneficial in policing and marketing a plant. In the case of annuals, a code name for a cultivar is typical, as they are often sold under a selling name (often a colour) that allows for easy replacement by an upgrade or improvement, without the need to change the selling name (Chisholm 1999). This is called "superization", "silent

introduction" or "silent replacement" and is not a deceptive as it sounds. Typically, it is the series to which the plant belongs that bears a trademark and both the replacement plant and the plant replaced are patented with the new cultivar name used in marketing materials. The other reason for the use of a code name is because of the limitation of the patent term (addressed below).

2. **It is a way to protect a plant without the expense of patenting or when a plant is unpatentable**. As only the use of a trademark name is an act of infringement, a plant can be freely propagated as long as it sold under the cultivar or another name. A patent, PBR, or PVR is the only way to prevent others from propagating a plant and trademarks should not be confused with serving the same function. It can be argued by the trademark owner that the unknown status of the cultivar name will prevent any interest by other growers, however, that will not prevent others from coining a different name altogether, even a trademark, creating greater confusion. It can be expensive to defend and protect a trademark, however and may not be justified for a single cultivar. The strength of using a trademark for this purpose is effective only to the degree to which it is respected.
3. **It is a way to gain protection of a variety after the patent term (or PVR) expires.** The rationale follows that, since a patent expires in twenty years from the filing date while a trademark can be renewed indefinitely, the protection period is extended. A trademark becomes well--known and other growers are not interested in marketing the plant under the unknown cultivar name. This practice is now a reality for certain patented trees, as tree growers argued that the patent term was too short to realize an adequate royalty return. The fault of this system is that that the trademark name has become the identifying name for the plant (which was the intent), but the mark is in danger of being invalid. The term length may be a valid argument for woody crops, but this solution may not be the best choice. For annuals and perennials, market life beyond twenty years is less likely.
4. **To obtain double protection.** There are some legal experts that provide counsel that trademarks are cheaper to defend and enforce than patents and that greater protection is afforded from foreign competitors if a trademark is used. If a plant is known and in demand, based only on the trademark name, then protection is enhanced by having both patent and trademark protection (Giola 1999).

8.3.2 Use of a Trademark in Connection with a Plant Name

If a plant is patented or has PBR, it is required that the generic denomination is used when the plant is marketed. When using a trademark, the trademark should be used in a way as not cause confusion. In particular, it should not appear to be the cultivar name by appearing after the genus or be put in single quotes. Ideally, it is distinguished by some means, such as using italics, bold type, or capital letters (Giola 2000b). Piers Trehane pointed out an example that is a good example of trademark use by Lake Country Nursery in Ohio, USA (Tehrane 2001). They market *Malus* 'Sutyzam' in association with the Trademark Sugar Tyme®, clearly stating that Sugar Tyme® is registered with the USPTO. The common name given

is Sutyzam Crab. As it is industry practice to use the cultivar epithet preceding the common name for the species, it only follows that it would be inappropriate to use a trademark in a common name. It is even more detrimental to trademark a cultivar name, as that name will then become the *de facto* generic designation for that variety and the trademark would fail. Others may argue that a trademark used with a genus or common name is proper use, whereas Kodak® film and Mybrand® maple are similar situations. This opinion fails to recognize that cultivars names are considered the generic designation for plants: as stated by UPOV, the USPTO, and the International Code for Botanical Nomenclature.

8.3.3 Trademark Use from a Breeders' Perspective

From a breeder's perspective, one needs to consider the merits, or pros and cons, of having their plant marketed under a trademark name. If a plant is to be marketed under a well--known brand, tremendous benefit can be realized. If a plant is marketed under another's (not the breeder's) trademark and the plant in unknown by the cultivar name, it may not be in his or her best interest should the relationship cease for some reason. On the other hand, a selling name may be necessary if the cultivar name is not marketable or a breeder may be convinced by that added protection is afforded. An additional option would be for the breeder to obtain his or her own trademark. Again, legal advice is prudent.

8.4 Trademark Selection

Selection of a trademark should not be taken lightly, particularly if you plan to seek registration. A trademark needs to adhere to certain rules, not infringe on another's trademark, be defendable, and be attractive for marketing purposes. These general aspects of trademark use apply to any field of trade. There are many references on the subject of trademark selection (Elias and Stim 2001, Tramposch 1999, Wilson 1998). The strength of a trademark and potential costs of defending it are highly dependent on selection of a mark. If a mark is not distinct enough, it may prove costly to defend. If it is too distinct, it will require more promotion to gain recognition. A thorough search of trademarks already in use is also prudent as a poor selection can result in a lawsuit (Wilson 1998). Trademark law is complex and managing a trademark can be quite costly. The advice and services of an attorney specializing in trademarks should be seriously considered prior to trademark selection and use.

8.5 Establishing and Registering a Trademark in the U.S.

The use of trademarks is a common law right that is obtained simply through use of the mark by applying them to goods used in commerce. Under common law, the use of "TM" is permitted to indicate to others that a trademark is intended. In the U.S., if the trademark is used on a product that crosses state lines, then federal registration of a trademark with the USPTO is possible. Registration is beneficial as

it notifies others that you are using the mark and increases the strength of the trademark should a dispute arise. Registration does not grant any rights. How one uses a trademark governs the right to use it. Once a trademark is registered, the symbol "®" may be used in connection with the trademark. The mark must be properly used and in continuous use to retain the rights to use it. Trademark registration involves filing a simple form, which can be done online, and a payment of an application fee (January 2003) of US$335 (USPTO 2002c). The application requires the submission of proof of its use in commerce, such as a photo of a plant labeled with a tag using the trademark. If a specimen is not available, an intent--to--use--application can be filed, with submission of a document showing proof of use filed later with an additional US$100 fee. The specimen must be submitted before the USPTO will issue the mark. If the USPTO examiner has no objections to the mark, the mark is then published in the *OG,* a weekly publication by the USPTO to allow others to oppose the mark. Generally, if there is no opposition, the USPTO will issue a registration in about 12 weeks after publication (Bouchoux 2001).

Trademark registration can be renewed indefinitely if the trademark remains in use. The first renewal is due between five and six after registration and every ten years thereafter. An affidavit of use must be filed and the current renewal fee is US$400 per class of goods. It is not required that the registration be renewed in order to use it, although one could no longer use "®", the owner can rely on common law rights. A cancelled mark may still be in use. This is one of the reasons that one should not depend solely on a search of registered marks in determining if a mark is "clear" for use.

8.6 International Trademarks

Trademark rights in a given country are dependent on the laws of that country. Separate applications must be made in each country, with the exception of the European Union. A trademark registration in the 1996 European Community Trademark System (CTM System) currently covers 15 member nations. The Paris Convention of 1883, however, does provide for priority rights for nationals of the 130 countries that adhere to it. If an application is filed in a member country within six months of the filing in his or her own country, the application will be given the priority filing date of the earlier home application (Bouchoux 2001).

Trademark registration in most countries is on a first--to--file basis, as opposed to the system in the U.S. where "first to use" decides trademark rights. In addition, most countries do not require prior use for registration. Although there is usually a requirement to use it in a reasonable amount of time, this registration system leaves trademarks open to piracy. People that keep up on emerging trends register trademarks for ransom, similar to "cybers--quatters" that file domain name registrations. It follows that a delay in international trademark registrations should be avoided. The U.S. does allow registration of marks used in foreign countries prior to use, however, an "intent--to--use--application" must be filed. Trademark attorneys can assist with registration in other countries, as many work with foreign associates.

Trademark owners from nations that are members of the Madrid treaties may also submit their national registrations to WIPO (WIPO 2002). WIPO administers

the Madrid treaties and after a member submits a registration, member countries have one year to reject the trademark. If a rejection is not received, the mark is eligible for continued registration. The European community and 16 other countries are members. The U.S. is not party to the Madrid treaties (Elias and Stim 2001).

9. LICENSING AND ROYALTIES

License agreements are used to extend rights of use for patents, trademarks, or plant breeders rights to another party. The construction and content of a license agreement is dependent on the laws governing each. A licensor may grant an exclusive license to one party or license many parties on a non--exclusive basis. In all cases, the owner of the rights retains all ownership. If ownership is to be transferred, then an assignment of rights is needed. In many countries, licenses as well as assignments require registration of user agreements. A license may require a lump--sum payment, periodic royalty payments based on sales, or both.

The owner of a variety issues license agreements to specify the terms and conditions for use of the protected variety. Some elements that are typically addressed for a patent license are: (i) variety ownership and protection status, (ii) proper use of the denomination including policies on tag information and nomenclature, (iii) license term, (iv) royalty rate and payment terms, (v) field of use (geographic or type of activity), (vi) rights afforded and forbidden activities, (vi) conditions for revoking the license. A license must adhere to rights allowable by patent or PBR laws and be free of antitrust violations. Selection of quality licensees is critical to success in marketing a new plant variety and reputation, enthusiasm for the plant to be licensed, and a successful track--record for launching new varieties are qualities to consider.

Royalty payments are typically accessed on a per--plant--sold or other unit basis (unrooted, rooted propagules) basis. A patent owner will need to determine a fair royalty amount or analyze an offer from a prospective licensee. A breeder agent, experienced industry professional familiar with your product, or even a prospective licensee can offer good advice on an appropriate royalty amount. There are several factors that should be considered when setting a royalty rate: (i) typical royalty rate for the class of plant (e.g. annual vs. an herbaceous or woody perennial plant), (ii) the ease of propagation (e.g. tissue culture vs. stem cuttings), (iii) the prospective number of plants that can be produced and sold, (iv) market price, (v) the type of market (e.g. specialty retail vs. large outlet), (vi) uniqueness of plant (vii) services provided (e.g. marketing support provided by licensee), and (viii) geographical market (e.g. Europe vs. U.S.). Breeders should be cautious not to think that a higher royalty rate is always in their best interest as a larger numbers of plant sold at a lower royalty rate may be more profitable. Currently in the U.S., typical rates for vegetative annuals are in the range of $0.04 to $0.10, for perennials $0.10 to $0.50, and shrubs $0.20 to $1.00. Higher rates are typical when a plant is difficult to propagate, demand is greater than supply and a premium price is acceptable to the consumer. Rates are also higher when a portion of the royalty collected is used to support a promotional program set up by the licensor. Royalty payments are typically collected once or twice annually. Most breeders either work with a

distributor directly that may or may not license other propagators and/or growers or contract with a company that offers administration services that include license management and royalty collection.

License agreements for trademark use should always include provisions for quality control. The licensor must retain control over the quality and consistency of the products bearing the mark and should require that the mark be properly used. A license that does not stipulate any standards of quality or mark usage is considered a "naked license" and the trademark owner could lose all rights to the mark. Where other aspects pertaining to trademarks may be done without aid, consulting with a trademark attorney is critical for drafting trademark licenses.

Disclaimer: Topics of interest should be more thoroughly investigated from authoritative sources and individuals with expertise in a given topic. Intellectual Property is a legal field and breeders' should obtain legal advice to assure that their rights are maximized and preserved.

10. SUMMARY

Intellectual property rights for breeders of ornamental plants has come to the forefront of the nursery industry in the last decade and new varieties are becoming increasingly more exquisite with every release. It is anticipated that industry leaders, companies, institutions, and breeders will become increasingly savvy, knowledgeable in the field and be able to communicate and work with peers and legislative bodies. This will enable enacting laws and practices that will enhance the floriculture industry and continue to benefit plant breeders.

The increased support of breeder's rights by the entire industry cannot go unnoticed, but it is the talented flower breeders themselves that deserve the credit. It is their magnificent skills, hard work, and creativity that creates products for the industry in the first place and provides a more enjoyable environment for all.

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Chapter 5

HERBACEOUS ORNAMENTAL PLANT GERMPLASM CONSERVATION AND USE

Theoretical and practical treatments

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Abstract: Preservation of herbaceous ornamental crop germplasm has been traditionally accomplished by private and public sector flower breeding programs, rather than through a publicly funded government agency. The recent founding of the Ornamental Crop Germplasm Center by the U.S. Department of Agriculture, as part of the U.S. National Plant Germplasm System, has brought attention to the critical needs of preserving floricultural crop germplasm for worldwide research and development. Preservation of a crop's germplasm *in toto* is a complicated but critical scientific endeavor, which will ensure future flower crop development. There are numerous challenges in germplasm preservation and accessibility, including collection of germplasm, determining crop centers of origin, conservation methodologies, genepool creation, conservation concepts, genebank procedures, adherence to governing international conventions for germplasm collection & conservation, and use of plant protection mechanisms. Future development possibilities and global networking opportunities are important for continued *ex situ* and *in situ* conservation of floriculture crop germplasm.

Key words: Conservation, genebanks, genepools, genetic diversity, priority genera.

1. INTRODUCTION

Crop germplasm is any genetic material that can be used by plant breeders for the improvement of a crop. The International Plant Genetic Resources Institute (IPGRI) defines plant germplasm as 'a set of genotypes that may be conserved and used' and plant genetic resources as 'the genetic material of plants, which determines their characteristics and hence their ability to adapt and survive' (IPGRI,

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2004). The total genetic diversity of a crop, therefore, includes its related wild species, weedy companion species, subspecies, botanical varieties, landraces, ancient and heirloom cultivars, genetic stocks, inbred lines, obsolete and modern cultivars that make up the total genepool of the crop. In some cases, such as the Orchidaceae where intergeneric crosses are possible, this variability is extended to genera level. These genetic materials, therefore, could be a gene and its alleles, a series of loci, quantitative trait loci (QTLs), linked genes, an epistatic set of gene combinations, a combination of different genomes, an addition or lack of whole chromosomes (polyploidy and aneuploidy series), and their combinations. The task of a flower breeder is to combine these building blocks into marketable cultivars. Plant germplasm provides the genetic variability and the essence of a crop improvement program. Without adequate germplasm a plant breeder's successes will be deficient. In flower breeding this could mean the development of a new crop, e.g. New Guinea impatiens (*Impatiens hawkeri);* a new innovative form, e.g. the 'Wave™' petunia (*Petunia x hybrida);* new uses of existing crops; insect- or disease-resistant cultivars that require lesser pesticide application; cultivars with resistance and tolerance to physiological and environmental stresses.

Harlan (1975) divided a crop total genepool based on the ease of natural (sexual) gene exchange from the donor plant to the crop into three categories: primary, secondary and tertiary genepool. The primary (1º) genepool consists of all taxa including related wild species that can readily hybridize and are fully inter-fertile with the concerned species. There is also no hybrid breakdown in the F_1 and later generations and, thus, are equivalent to the concept of biological species. The secondary (2º) genepool consists of taxa that can be hybridized using conventional breeding methods but the F_1 and/or later generations are subjected to hybrid breakdown where some of the progenies are sterile or would not come to maturity. Whereas in the tertiary (3º) genepool, intercrosses are difficult to make and might only be achieved with special techniques such as embryo rescue or bridging species. The hybrids are usually non-viable or sterile, but gene transfer may be achieved through such techniques as amphidiploid breeding.

With the recent advancements in molecular genetics and gene-transfer technology, flower breeders/geneticists are able to isolate genes and transfer them to unrelated species. For example, genes of microorganisms and animals have been successfully introduced into plants, such as *Bt* genes from bacteria to maize and cotton; a jellyfish fluorescence gene to different plants in gene expression research, respectively. Thus, Harlan's 3° genepool boundary of a species expands to encompass other species and organisms due to our ability to introduce genes of one species into the genome of another. The scope in plant germplasm conservation suddenly broadens creating a need to collect and conserve a wider range of species in order to capture greater genetic diversity. In ornamental plants where taxonomy is not well documented, the application of the genepool concept in germplasm collections will significantly complement the selection of taxa for breeding.

Plant germplasm conservation is the safekeeping of the genetic diversity of a targeted crop and its related species as seeds or living plants for future use. It has developed in the past fifty years into a recognized discipline. A typical germplasm conservation program includes activities in plant exploration, plant conservation, characterization, evaluation, documentation, and distribution. Genebanks with seed-storage vaults maintained at -18ºC for long-term storage of base collections and 2ºC for medium-term storage of active collections of seed, and liquid nitrogen cryo-preservation tanks at –196ºC for storing seed, tissue cultures, and dormant buds have been established. Additionally, many vegetatively propagated species and clones are planted and maintained in the field and greenhouse, called field genebanks. In food, forage and industrial crops, an estimated 6.1 million accessions have been collected and conserved globally in some 1,500 genebanks of different countries and the Consultative Group on International Agricultural Research (CGIAR) international research centers, such as the International Rice Research Center in the Philippines and the International Potato Center in Peru (FAO, 1998). However, in herbaceous ornamental plants the systematic conservation of these resources is in its nascent stage. The U.S. National Plant Germplasm System (NPGS) Collection of more than 450,000 accessions of 4,474 species has only about 3,000 accessions of herbaceous plants, a mere 0.7% of the collection (Tay, 2003a). Presently, international agencies including IPGRI and the Food and Agriculture Organization of the United Nations (FAO) have no mandate in conserving ornamental plants and most national programs have no or limited focus. Its conservation occurs mainly in botanical gardens and arboreta, private collections of seed companies, plant nurseries and individual plant aficionados. However, the goal of most botanic gardens is to hold a few specimens of each taxa and to possess as many species and genera as possible rather than conserving the entire genepool of a species and its related species as in a genebank. For example, the Millennium Seed Bank Project of the Royal Botanic Gardens, Kew, England, aims to conserve over 24,000-plant species worldwide against extinction but not to collect the whole range of variation in each species (Linington, 2000). The establishment of the Ornamental Plant Germplasm Center (OPGC) of the United State Department of Agriculture (USDA) National Plant Germplasm System (NPGS) at The Ohio State University, Columbus, Ohio in 2001 gave rise to the first specialized genebank for the conservation of herbaceous ornamental plant germplasm (Tay et al., 2004).

2. CENTERS OF ORIGIN OF HERBACEOUS ORNAMENTAL PLANTS

The center of origin of a flower crop is the place where its progenitor is endemic (Vavilov, 1935). Knowledge on the center of origin of a crop and how it was

domesticated and evolved are essential in the planning and preparation of plant exploration missions so that the taxa and field sites can be targeted. N.I. Vavilov (1935), in his extensive plant exploration of the world, observed that related wild species and botanical crop varieties occur in greatest diversity in their native endemic habitats at the center of origin for each crop. This has become one of the main criteria to postulate the place of origin of a crop. However, secondary centers of diversity may be formed when a species is moved to a new area and if the eco-environment is suitable, naturalized populations will be established and continue to evolve outside their place of origin (Zohary, 1970). In herbaceous ornamental species, this phenomenon is frequent and some species may become invasive, e.g., *Lantana camara* in Australia (ARMCANZ et al., 2000). When this occurs in places where a related endemic species of the same genus is present, natural hybridization and introgression occurred, and 'hybrid-swarms/populations' will result to create a new center of diversity.

The origins of heirloom cultivars in herbaceous ornamental plants are even more complex. Many are direct selections of natural variants in wild populations in or outside the countries of origin. Again many are the result of hybridization and selection of two or more species endemic to different countries or continents outside their natural habitats. In this case they do not have a specific geographical origin and are commonly described as of garden origin. In the case of ancient flowers, such as the chrysanthemum and peony cultivars of China; the tulip and carnation of Turkey, they were domesticated in their countries of origin and thus have a definite center. On the contrary, most modern seed cultivars are F_1 hybrids of complex origin, e.g. *Impatiens wallerana*, *Petunia x hybrida*, *Begonia x tuberhybrida*, etc. For species where F_1 hybrid seed technology is not applicable, vegetative-propagation is increasingly being used to propagate clonal cultivars of complex origin. A noted example is the breeding and development of New Guinea impatiens (*Impatiens hawkeri)*, the success story of ornamental plant germplasm introduction and new crop development. Longwood Gardens (Kennett Square, Pennsylvania, U.S.A.) and the USDA derived this crop from several accessions of a joint plant collecting expedition to New Guinea in 1970. Due to the vast and distinct variations in these accessions, they were originally given separate specific epithets until later cytogenetic work showed they belonged to one species (*Impatiens hawkeri*). Crosses with additional species from Java and the Sulawesi islands led to the cultivars in production today (Cathey, 1995).

The process of new plants being selected from wild germplasm and then introduced directly into commerce is still practiced. Table 5-1 shows a total of 431 taxa being researched or listed as being researched in 24 countries in the proceedings of the ISHS Symposia I to IV on New Floricultural Crops (Christensen, 1989; Roh and Lawson, 1993; Considine and Gibbs, 1998; Maloupa, 2000). However, this represents only a small fraction of the 2,000 genera and 15,000 taxa used as

ornamentals as described in the American Horticultural Society A-Z Encyclopedia of Garden Plants (Brickell and Zuk, 1997).

Table 5-1. A summary of the number of taxa being researched (Res) and under research in the respective countries (List) in the ISHS I-IV International Symposia on New Floricultural Crops.

Country	1988		1991		1996		1999		
	Res	List	Res	List	Res	List	Res	List	Taxa
Australia			3	24	20	19	9	34	81
Belgium		1							1
Brazil					2	3			5
Cyprus								10	10
Denmark	30	4	1		4				39
Estonia								20	20
Germany	8				2				10
Greece					1		18		19
Indonesia					2				2
Ireland	4							3	7
Israel	10				1		1	16	27
Italy	2		2		2		3	7	15
Japan					20		3		22
Korea			1				3	2	6
Malaysia					3				3
Netherlands	2	9	2				1		14
New Zealand			1		1				2
Poland	1								1
Portugal							3		3
S. Africa			1	27	1		1	13	45
Spain			5	2			4		11
Taiwan			1						1
Thailand						20			20
U.S.A.	8	7	11	26	1	22	2	9	68
Total No. of Taxa									432

N. I. Vavilov (1935) found that the origin and domestication of food crops tend to concentrated in certain geographical mountain-valley regions in the world and he identified 8 primary centers (Chinese, Indian, Inner Asiatic, Asia Minor, Mediterranean, Abyssinian, South Mexico and Central American, and South America Andes Center) and 3 sub-centers (Indo-Malayan, Chilean and Brazilian-Paraguayan Subcenter) of origin for crop plants. A preliminary survey of 30 common flowers and their related species shows a similar pattern of concentrated

occurrence of species in certain geographic regions (Figure 5-1). Some 15 major centers can be demarcated, namely, (1) the North American region; (2) Meso-American and the Caribbean region; (3) South-western South American region including Chile and Peru west of the Andes; (4) Northern South American region including Columbia, Venezuela, Guyana, Surinam, French Guyana and northern Brazil and part of northern Peru; (5) South-eastern South American region including southern Brazil, Bolivia, Paraguay, Uruguay and Argentina; (6) European region including Scandinavia; (7) North African and Mediterranean region; (8) Balkan and Anatolia region including central Asia; (9) East and Central African region; (10) South African region; (11) Far Eastern region; (12) Southern China to Eastern foothill of Himalayan regions including northern Burma, Thailand and Vietnam; (13) South Asian region; (14) Southeast Asian region; and (15) Australian region. These centers coincide to a significant extent with the regions of the Conservation International's biodiversity hotspots in the world (Table 5-2). One could postulate that the abundant plant diversity provides greater probability for the finding of plants with esthetic potentials for domestication. In a situation like this, as in food crops no one single country is self-sufficient in all the germplasm of flowers and the situation is even more complex because unlike food crops where crops are grown mainly within their climatic adaptation ranges, many tropical ornamental species are grown in temperate countries in heated greenhouse or as indoor plants.

The centers of origin of ancient flowers from China and Turkey coincide with the Vavilov's food crop centers of origin. This may indicate that the domestication of food crops might also lead to the domestication of those flowers. These flowers were part of the crop-weed complex right from the beginning and those with beautiful flowers would have been selected for domestication. Archaeobotanical research (Hillman et al., 1989; Colledge, 1994) showed weed assemblages in the beginning of crop domestication commonly include the following taxa: *Adonis, Aegilops, Astragalus, Avena, Bromus, Bupleurum, Camelina, Centaurea, Centranthus, Coronilla, Fumaria, Galium, Glaucium, Hordeum, Lathyrus, Lithospernum, Lolium, Malva, Papaver, Polygonum, Reseda, Silene, Valerianella* and *Vicia.* This shows the likelihood of co-domestication of ornamental plants and in this case 16 taxa of the total of 24 on this list are herbaceous plants and used today as ornamentals.

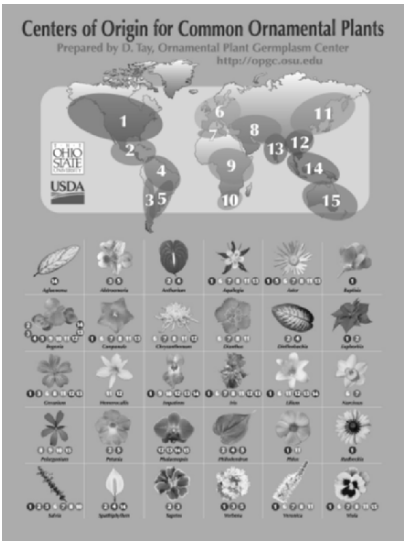

Figure 5-1. The centers of origin for some common flower crops.

Table 5-2. Plant species in the Conservation International identified biodiversity hotspots in the world in 2005 (http://www.biodiversityhotspots.org/xp/Hotspots).

Region	Original Area (km^2)	Remaining Area (km^2)	(%)	Endemic Plant spp.	Ex-tinct spp. *
N. and Central America					
California Floristic Province	293,804	73,451	25.0	2,124	2
Caribbean Islands	229,549	22,955	**10.0**	**6,550**	38
Madrean Pine-Oak Woodlands	461,265	92,253	20.0	3,975	1
Mesoamerica	1,130,019	226,004	20.0	2,941	7
Subtotal	2,114,637	414,663	19.6	15,590	48
S. America					
Atlantic Forest of Brazil	1,233,875	99,944	**8.1**	**8,000**	1
Cerrado of Brazil	2,031,990	438,910	21.6	4,400	0
Chilean Winter Rainfall-Valdivian Forests	397,142	119,143	30.0	1,957	0
Tumbes-Chocó-Magdalena	274,597	65,903	24.0	2,750	4
Tropical Andes	1,542,644	385,661	25.0	15,000	2
Subtotal	5,480,248	1,109,561	20.2	32,107	7
Europe and Central Asia					
Caucasus	532,658	143,818	27.0	1,600	0
Irano-Anatolian	899,773	134,966	15.0	2,500	0
Mediterranean Basin	2,085,292	98,009	**4.7**	**11,700**	5

Region	Original Area (km^2)	Remaining Area (km^2)	(%)	Endemic Plant spp.	Ex-tinct spp. *
Mountains of Central Asia	863,362	172,672	20.0	1,500	0
Subtotal	4,381,085	549,465	12.5	17,300	5
Africa					
Cape Floristic Region	78,555	15,711	20.0	6,210	1
Coastal Forests of Eastern	291,250	29,125	**10.0**	**1,750**	0
Africa	1,017,806	106,870	**10.5**	**2,356**	1
Eastern Afromontane	620,314	93,047	15.0	1,800	0
Guinean Forests of West	1,659,363	82,968	**5.0**	**2,750**	1
Africa	600,461	60,046	**10.0**	**11,600**	45
Horn of Africa	274,136	67,163	24.5	1,900	0
Madagascar and the Indian	102,691	29,780	29.0	2,439	1
Ocean Islands					
Maputaland-Pondoland-Albany					
Succulent Karoo					
Subtotal	4,644,576	484,710	10.4	30,805	49
Asia-Pacific					
East Melanesian Islands	99,384	29,815	30.0	3,000	6
Himalaya	741,706	185,427	25.0	3,160	0
Indo-Burma	2,373,057	118,653	**5.0**	**7,000**	1
Japan	373,490	74,698	20.0	1,950	7
Mountains of Southwest China	262,446	20,996	**8.0**	**3,500**	0
New Caledonia	18,972	5,122	27.0	2,432	1
New Zealand	270,197	59,443	22.0	1,865	23
Philippines	297,179	20,803	**7.0**	**6,091**	2
Polynesia-Micronesia	47,239	10,015	21.2	3,074	43
Southwest Australia	356,717	107,015	30.0	2,948	2
Sundaland	1,501,063	100,571	**6.7**	**15,000**	4
Wallacea	338,494	50,774	15.0	1,500	3
Western Ghats and Sri Lanka	189,611	43,611	23.0	3,049	20
Subtotal	6,869,555	826,943	12.0	54,569	112
TOTAL	23,490,101	3,385,342	14.4	150,371	221

*Note: Recorded extinctions since 1,500; figures in bold indicate biodiversity hotspots most affected by human activities.

3. CONSERVATION OF HERBACEOUS ORNAMENTAL PLANTS

3.1 The Industry and its Needs

Floriculture is an important global horticultural industry with an estimated value at US$50 billion dollars in 1995 with US$31 billion dollars in cut flowers and US$19 billion dollars in potted plants (de Groot, 1998) and the trend is going up. The U.S.A. floriculture and plant nursery industry, a leading agricultural sector, has an annual wholesale value of US$4.88 billion in 2001 (USDA, 2003). Ornamental crops play a leading role in agricultural diversification initiative in many countries including developing countries like Colombia, Costa Rica, Ecuador in Latin America, and Kenya and Tanzania in Africa, and this underscoring a critical need to collect, conserve and utilize ornamental plant germplasm to support breeding programs and sustain the growth and development of the industry.

3.2 Genetic Erosion

Plant genetic erosion is the process of losing the genetic diversity of a species. The accelerating rate of human disturbance in our natural environments, such as urban expansion, deforestation, mining, changing land-use practices, the expansion of industrialized agriculture and environmental pollution, and sometimes natural disasters are displacing and reducing natural genetic diversity. For example, wild species of African violet, *Saintpaulia ionantha*, in their natural habitats in East Africa are facing environmental decline because of forest clearing and urban and farm development. In Ohio, U.S.A. there was a mosaic of more than 300 remnant prairies mostly in the western half of the state where the first European pioneers arrived in the Ohio Valley wilderness in 1780s. Currently, limited pockets remain due to the pressure of farm and urban developments and the State government only have nine sites under its protection. Similar prairie degradation reports are found in other states (Figure 5-2). Similarly, the harvesting of wild populations of American ginseng especially in central Appalachians is of increasing concern (Gagnon, 1999). The over-exploitation of species through excessive uncontrolled harvesting from the wild for trades, e.g. orchids in the world has caused species extinction and endangered and threatened many other species. This concern had given rise to the establishment of an international convention – the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITIES) to protect these plants and animals.

Figure 5-2. A restored remnant of the N. American Prairie in Dane County, Wisconsin. This illustrates the pressure of urban development on the Ice Age National Scenic Trail, which runs along the edge of the last Ice Age glacier 15,000 years ago.

Human impairment on natural habitats is serious, widespread and on-going in all inhabited continents. An estimated 85.6%, i.e. 6,042,612 km^2 of the original area of 23,490,101 km^2 identified as biodiversity hotspots of the world by Conservation International are affected (Table 2). There are only 10-12% remaining original habitat areas in Africa, Asia-Pacific, Europe and Central Asia. In the Americas, it is a bit better but is a low 20%. The figures also show that areas with high plant diversity of endemic species also are areas that are facing greater loss of natural habitats. They also consist of areas with larger surface areas. In term of number of extinct species from 1,500 year onward, the island regions, namely, Caribbean Islands, Madagascar and Indian Ocean Islands, New Zealand, Polynesia-Micronesia, and Western Ghats and Sri Lanka appear to be more vulnerable to the survival of a species. The preservation of these hotspots is therefore critical.

The globalization of the floriculture industry is contributing to the genetic erosion process because the rapid adoption of few modern flower cultivars worldwide is rapidly replacing of many traditional heirloom cultivars and other species causing them to disappear. Most ornamental plant breeding programs are focusing primarily on aesthetic qualities such as flower and plant characteristics resulting in an inadvertent narrowing of genetic base of many modern flower cultivars. As the result cultivars of popular species such as the F1 hybrid cultivars of impatiens, marigold, pansy and petunia of many of the major international flower seed companies look alike as evidenced in seed catalogs of these major flower seed companies. This narrowed genetic base has reduced our capability for breeding innovation and increased the potential for epidemic disease. Access to a diverse germplasm pool is therefore fundamental for the successful identification and

incorporation of novel traits in the improvement of commercial ornamental crops. Herbaceous ornamental plant genebanks are, therefore, crucial to collect and conserve the disappearing heirloom genetic materials, to explore, collect and conserve new genetic materials and species with more than just aesthetic characteristics, and to evaluate and distribute these germplasm. The OPGC is the first major undertaking to establish a specialized genebank for herbaceous ornamental plants (Tay et al., 2004).

3.3 Which Genera and Species to Conserve?

Ornamental plants encompass a vast number of plant genera and species from all climatic regions and ecosystems of the world. Brickell and Zuk (1997) cataloged some 2,000 genera and 15,000 taxa in the American Horticultural Society A-Z Encyclopedia of Garden Plants. Many of them have thousands of years of domestication history with complicated well developed hybrid-complexes and on the other hand many new species are continuously being evaluated and introduced into our gardens. Every conservation program has a fixed limited operational budget. An efficient genebank is one that is able to put the maximum amount of genetic diversity of targeted crops into safe storage without losing the individual accession genetic integrity but allowing for a tolerable shift in the genetic composition of an accession. The conservation steps include seed exploration, multiplication, drying, cleaning, packaging, storage, viability monitoring during storage, characterization, evaluation, documentation and distribution. These activities are tedious and routine. The main concern of a plant germplasm conservation program is therefore to collect the most representation of a crop with the least number of accessions. An accession is a plant sample, strain or population held in a genebank or breeding program for conservation and use.

However, in the case of ornamental plants the foremost decision is the genera and species to be collected and conserved because of the large number of taxa presented. For example, the Kew's Millennium Seed Bank Project decided to focus on 24,000 species in the temperate dry zones of the world. The NPGS/USDA Herbaceous Ornamental Crop Germplasm Committee (HOCGC) in preparation for the establishment of OPGC established three recommended priority genera lists in 1995, 1999 and 2001 with 24, 48 and 30 genera, respectively (Table 5-3). The three lists tally a total of 64 distinct genera. The 37-member Committee is made up of plant breeders, university professors, and seed and plant nursery industry and botanical garden representatives. The lists thus reflect the importance of the genera in the US floriculture industry in different time periods. The OPGC adopts the 2001 list and the 30 priority genera consist of about 6,700 species based on Hortus Third (The Staff of the Liberty Hyde Bailey Hortorium, 1976), which are too many for the center to focus on at this initial phase of its development. The genera were ranked

in the order of importance by the US floriculture industry and university researchers in 2002 using eight criteria as follows (Table 5-3):

1. Market Potential: 1-least potential to create new forms of existing crop or new crop; 5-highest potential
2. Market life phase: 1- declining; 3 - peaked; 5 - increasing/new market
3. Bottle-neck genes need: 1 - little need for disease, pest, stress and other genes; 5 - strong need
4. Researchable: 1 - already sufficient information or difficult to get good research results; 5 - not much research has been done and potential to get good results
5. Other uses: 1 - no knowledge that it could be used as nutraceuticals, pharmaceuticals, essential oils, etc.; 5 - good potential for other uses
6. Genetic diversity available to be collected: 1 - little; 5 – plenty
7. Urgency for conservation: 1 - not endangered and threatened; 5 - endangered and threatened
8. Country of origin: 1 - not possible to get germplasm out of the countries; 5 - freely exchange

Table 5-3. Development of the USDA Herbaceous Ornamental Crop Germplasm Committee (HOCGC) priority genera list of herbaceous ornamentals.

Year	No. of Genera	Genus
1995	24	*Ageratum, Alstroemeria, Aster, Begonia*, *Caladium, Catharanthus*, *Chrysanthemum, Dianthus*, *Dieffenbachia, Euphorbia*, *Eustoma, Hemerocallis, Hippeastrum, Impatiens*, *Kalanchoe, Liatris*, *Lilium*, *Lobelia, Pelargonium*, *Petunia*, *Spathiphyllum, Verbena, Zantedeschia* and *Zinnia*
1999	48	*Alstroemeria*, *Antirrhinum*, *Aster*, *Begonia*, *Campanula*, *Catharanthus*, *Chrysanthemum*, *Crocus*, *Cyclamen*, *Delphinium*, *Dianthus*, *Echinacea*, *Euphorbia*, *Eustoma*, *Freesia*, *Gazania*, *Gentiana*, *Geranium*, *Gloxinia*, *Gypsophila*, *Hemerocallis*, *Heuchera*, *Hosta*, *Impatiens*, *Iris*, *Lantana*, *Liatris*, *Lilium*, *Lobelia*, *Orchidaceae*, *Osteospermum*, *Paeonia*, *Papaver*, *Pelargonium*, *Penstemon*, *Petunia*, *Phlox*, *Primula*, *Rudbeckia*, *Saintpaulia*, *Salvia*, *Senecio*, *Solidago*, *Tagetes*, *Verbena*, *Veronica, Vinca* and *Viola.*
2001	30	*Aglaonema, Alstroemeria, Anthurium, Aquilegia, Aster, Baptisia, Begonia, Campanula, Chrysanthemum, Dianthus, Dieffenbachia, Euphorbia*/Poinsettia*, Geranium, Hemerocallis, Impatiens, Iris, Lilium, Narcissus, Pelargonium,*

Year	No. of Genera	Genus
		Petunia, Phalaenopsis, Philodendron, Phlox, Rudbeckia, Salvia, Spathiphyllum, Tagetes, Verbena, Veronica and *Viola*
Total	64 distinct genera from the three lists	

As the result, the top 15 genera in descending order of importance were *Begonia*, *Impatiens*, *Geranium*, *Petunia*, *Salvia*, *Pelargonium*, *Viola*, *Dianthus*, *Campanula*, *Hemerocallis*, *Alstroemeria*, *Lilium*, *Verbena*, *Rudbeckia* and *Phlox* (Tay, 2003b). At this early development of the center germplasm acquisition is concentrating on the top five genera. In spite of this, efforts should also be taken to collect and conserve germplasm judging to be valuable and are facing threats of disappearing or destroy. For example, the OPGC in collaboration with the Ohio Department of Natural Resources collected 280 accessions of American native prairie flowers in the last prairie remnants in Ohio in 2004. There is, therefore, no fixed set of genera and species that have to be collected and conserved. The decision on taxa to be included on a priority list depends on the needs of the user groups.

In the trade, the distinction between herbaceous and woody species is not solely based on the biology. The definition is a combination of both the biological and trade characteristics. An herbaceous species anatomically should not have a secondary woody thickening. However, a crop such as poinsettia is usually considered an herbaceous by the industry but the species in its natural state is a tall shrub with thick woody stems. There are some specific requirements in the setting up of an herbaceous versus a woody species genebank especially in term of field and greenhouse spaces and these have some significant consequences on the efficiency of a genebank.

3.4 Harlan's Genepool Concept

Harlan's primary (1º), secondary (2º) and tertiary (3º) crop genepool concepts was used to define the targeted germplasm for collection and conservation. In assembling crop germplasm, the first step is to define the priority species (1º and 2º genepools) and heirloom varieties (1º genepool) to be collected. Efforts are concentrated on taxa of the 1º and then of the 2º genepool. It is difficult to circumscribe the taxa of the 3º genepool. This is especially so when genes from a species can be transferred to another using gene transformation breeding. The boundary of Harlan's 3º genepool has grown to encompass all living organisms (Figure 5-3). Advancements in DNA sequencing and synthesizing technologies have allowed artificial genes to be constructed by altering the DNA sequences to create novel genes. An indefinite variation of genes and their alleles can thus be

constructed. These developments present both a potential and challenge in the future of plant genetic resources conservation and utilization.

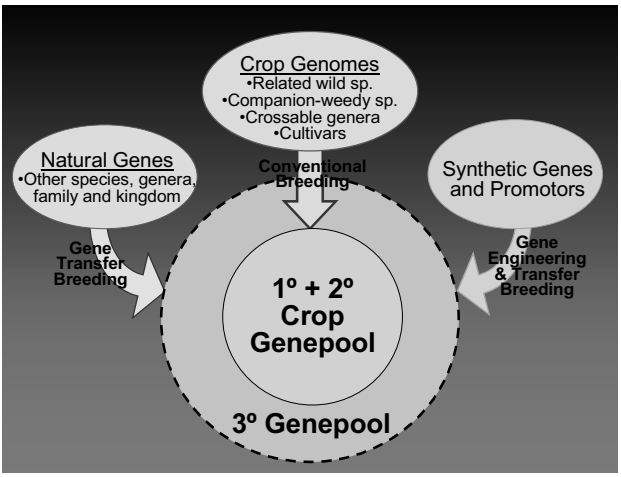

Figure 5-3. The primary (1º), secondary (2º) and tertiary (3º) genepools of a crop illustrating the growing boundary of tertiary genepool to encompass genes from all living organisms and human-made synthetic genes based on Harlan's Genepool Concept.

4. CATEGORY OF GERMPLASM

Taking into consideration the goal of a genebank to maximize the amount of genetic diversity to be conserved with a minimum number of accessions of targeted species and to conserve genes for traits of current interest and for future unknown needs, the classification of germplasm into different biological categories will helps genebank managers and curators to make plan on the species, ecotypes, and number of accession of each categories to be collected and conserved. A practical germplasm category system in descending order of importance based on Harlan's Genepool Concept is as follows:

1. Related wild species. This category includes the progenitors and related wild species of a targeted crop, i.e. species in the 1º and 2º genepool. Often wild relatives are weedy companion species that grow with the crop in and around the field. Occasionally, natural hybridization occurs and resulted in gene introgression from one species to another. Some of these crop relatives may also function as 'bridging' species where genes from distant related species (3º genepool species) can be passed on to the crop via them. Collected accessions should represent populations across the original ecogeographic distribution

range of these species, subspecies and botanical varieties. In herbaceous ornamental plants, many crop and cultivars are direct selections of wild populations. This indicates the importance of this category.

2. Landraces, primitive and heirloom cultivars. This category represents genotypes that are proven in time in cultivated environments. It is therefore an important category. Priority should be given to conserve well-documented accessions from known ethno-geographic sources. Landraces and primitive cultivars are the products of location-specific natural selection and adaptation, and they often carry introgression genes of valuable traits from weedy companion species that co-evolve with the crop cultivars in their natural growing environments. Again, representative samples of the total genetic diversity of as many landraces and primitive cultivars should be collected and conserved. In herbaceous ornamental plants, ancient and heirloom cultivars should be given the same consideration, and judged by the same criteria of uniqueness and/or potential utility.
3. Other wild species. This category consists of other wild species of the same genus as the targeted crop, i.e. 3º genepool. However, in some plants such as many orchids and gesneriad (Gesneriaceae) inter-generic hybridization is common. In this case, species of related genera of the crop may also have to be considered for conservation. These species often are the only source of genes for resistance to diseases and pests, and other environmental stresses. It is therefore an important category in this sense.
4. Modern cultivars and germplasm releases. In general, this category is given low priority and is only being considered when access to germplasm in category 1 and 2 are limited. The focus is mainly on obsolete or discontinued cultivars with specific uniqueness and importance, and that are not direct descendants of cultivars already under conservation. Higher priority has to be given to those developed from more unique or genetically divergent sources with broader genetic base. Cultivars that are used frequently as industry standard comparison in research experimentation should be maintained to prevent genetic shift. Germplasm releases are treated in a similar way.
5. Genetic stock collections and breeding lines. This category represents a highly specialized group of genetic resources with identified genes. Its conservation is important. However, some of the lines are difficult to maintain because of the unique genes that their have and genebank managers have to determine the value of the genetic stocks in relation to the cost of maintenance. Similarly, breeding lines have to be assessed accordingly and only those with special uniqueness should be maintained.
6. Genetically engineered lines. This is a recent concern in relation to the possibility of genetic contamination of the original genepool in a collection with engineered genes. Otherwise, it can be treated as genetic stock or breeding lines as in category 5.

The total genepool of a crop is thus complex especially when the species concerned is of hybrid origin. Germplasm of direct progenitors and all the contributing parents and related species have to be collected and conserved. Table 5-4 provides a summary of the species of all levels of Harlan's genepool that need to be conserved in some of the common herbaceous plants.

Table 5-4. The primary (1º), secondary (2º) and tertiary (3º) genepools and origins of some common herbaceous ornamental plants (Herbaceous Ornamental Crop Germplasm Committee Report, Sept. 1, 1995, http://www.ars-grin.gov/npgs/cgc_reports/herbscgc1995.htm.).

Crop	Origin	1º Genepool	2º/3 º Genepool
Ageratum (43)	Central & South America	*A. houstonianum* (Mex), *A. conyzoides*	~41 spp.
Begonia (~1,500)	Latin America, Asia, Africa		~1,500 taxa
Fibrous-rooted	Latin America, Asia, Africa	*B.* x *semperflorens-cultorum* (*B. cucullata* var. *hookeri* x *B. schmidtiana*)	
Tuberous-rooted	Andes of Peru, Bolivia	*B*. x *tuberhybrida* (*B. boliviensis*, *B. clarkei*, *B. davisii*, *B. pearcei*, and *B. veitchii* cross)	*B. rosiflora*; ~1,500 taxa
Hiemalis		*B*. x *hiemalis* (*B. socotrana* x *B. tuberhybrida*)	
Cheimantha		*B.* x *cheimantha* (*B. dregei* x *B. socotrana*)	
Catharanthus (7)	Madagascar, India	*C. roseus, C. trichophyllus*	*Catharanthus ovalis* (Extinct?)
Chrysanthemum (40)	Eastern Asia to Europe	*C*. x *grandiflorum* (derived from *C. indicum*) (*=Dendranthema x grandiflorum)*	*C. pacificum, C. weyrichii, C. zawadskii* var. *zawadskii*, *C. zawadskii* var. *latilobum*.

Crop	Origin	1º Genepool	2º/3 º Genepool
Dianthus (300)	West Asia, Europe		
Florist's/Border Carnation	Mediterranean	*D. caryophyllus, D. arboreus*, *D. chinensis*, *D. knappii*	
Sweet William		*D. barbatus*	
Allwood's Pink		*D. caryophyllus* x *D. plumarius*	
Alpine Pink		*D. alpinus*	
Cheddar Pink		*D. gratianopolitanus*	
China Pink		*D. chinensis*	
Cottage Pink		*D. plumarius*	
Deptford Pink		*D. armeria*	
Maiden Pink		*D. deltoides*	
Dieffenbachia (30*)*	Tropical America	*D. amoena, D. maculate*	
Eustoma, Prairie Gentian	N. America	*E. grandiflorum, E. exaltatum*	
Hemerocallis (20), Daylily, 2,600 B.C.		*H. flava* (= *H. lilioasphodelus*), *H. thunbergii*, *H. middendorfii*, *H. minor*, *H. dumortieri*, *H. aurantiaca*, *H. fulva*	*H. altissima, H. forrestii, H. multiflora, H. nana,* +10 spp.
Hippeastrum	Eastern Brazil, Central & Southern Andes	*H. vittatum, H. leopoldii, H. pardinum, H. reginae, H. puniceum, H. aulicum*	*H. papilio, H. blossfeldiae* + 60 spp.
Impatiens	Tropical Africa and Asia		600-1,000 spp.
I. wallerana, Busy Lizzie	Tanzania to Mozambique	*I. usambarensis, I. cinnabarine, I. auricoma*	*I. nzoana*, *I. tincoria, I. serpens, I. cinnibatina, I. decipiens*
I. hawkeri, New	New Guinea &	*I. aurantiaca*, *I.*	

Crop	Origin	1º Genepool	2º/3 º Genepool
Guinea impatiens	S.E. Asia	*hawkeri;* possibly others	
I. balsamina, Balsam impatiens		*I. platypetala, I. hawkeri, I. niamniamensis, I. repens, I. sodeni*	
Liatris (32)	N. America	*L. aspera*, *L. pycnostachya*, *L. spicata* + 10 spp.	*L. helleri*, *L. ohlingerae*, *L. provincialism* (Endangered)
Lilium (70)	N. America, Asia, Europe		
American	N. America	*L. Parryi, L. pardalinum*	
Asiatic	East Asia	Complex hybrids	
Canditum (Madonna)	Europe	*L. candidum*	
Longiflorum (Easter Lily)	East Asia	*L. longiflorum*	
Martagon	Europe & East Asia	*L. martagon, L. Hansonii*	
Oriental	East Asia	*L. auratum*, *L. speciosum*	
Tiger	East Asia	*L. lancifolium, L. tigrinum*	
Trumpet	East Asia	As Asiatic	
Lobelia (375), Cardinal Flower	N. America	*L. cardinalis*	
Edging *Lobelia*	S. Africa	*L. erinus*	
Blue Cardinal Flower	N. America	*L. syphilitica*	*L. cardinalis*
L. splendens	Mexico	*L. splendens*	
L. tenuior	Australia	*L. tenuior*	
Pelargonium			~250 spp.
Zonal Geranium	S. Africa	*P.* x *hortorum* (*P. inquinans, P. zonale*)	
Ivy Geranium	S. Africa	*P. peltatum*	
Regal Geranium	S. Africa	*P.* x *domesticum* (*P.*	

Crop	Origin	1º Genepool	2º/3 º Genepool
		cucullatum, *P. fulgidum*, *P. grandiflorum,* + other spp.)	
Petunia			~30 spp.
Garden *Petunia*	S.E. South America	*P*. x *hybrida* (*P. axillaris*, *P. inflata*, *P. parodii*, *P. violacea*) (*P. integrifolia* var *integrifolia* = *P. inflata* and *P. violacea*)	
P. parviflora	Latin America	*P. parviflora*	
Spathiphyllum			~35 spp.
S. 'Mauna Loa'		Unknown, *S. wallissii*	*S. floribundum*, *S. cannifolium, S. phryniifolium*
Verbena	Tropical N. & S. America		~200 spp.
Dakota Vervain	N. America	*V. bipinnatifida*	
V. bonariensis	S. America	*V. bonariensis*	
Rose Verbena	N. America	*V. canadensis*	
Garden *Verbena*	Argentina, Brazil, Chile	Interspecific hybrid: *V. peruviana* x other species	
Vervain	Argentina, Brazil	*V. rigida*	
Moss Vervain	S. America	*V. tenuisecta*	
Zantedeschia	S. Africa		6 spp. + 2 subspp.
Common Calla	S. Africa	*Z. aethiopica*	
Spring Calla	S. Africa	*Z. rehmannii* (Pink/Red); *Z. elliottiana* (Golden/Yellow); *Z. jucunda*; *Z. pentlandii*; *Z. albomaculata* (Spotted) - ssp. *Albomaculata*; ssp. *Macrocarpa*; ssp.	

Crop	Origin	1º Genepool	2º/3 º Genepool
		Valida	
Zinnia	N. America; S. America (1 spp.)		~20 spp.
Z. violacea	Mexico	*Z. violacea*, *Z. angustifolia* var. *angustifolia*	
Z. haageana	Mexico	*Z. haageana*	
Z. angustifolia	Mexico	*Z. angustifolia*; *Z. angustifolia* var. *angustifolia*	

4.1 The Species Concept

In ornamental plant germplasm conservation, species is the principal working unit. A species is usually defined as a group of individuals with a set of phenetic appearance and can interbreed and remain true-to-type in its natural environment. However, when different species concepts are used by different taxonomists and evolutionists the resulting situation becomes unclear and much controversy has been created. There is not a single satisfactory species concept that can accommodate all kinds of situations. A practical definition of a species is essential for genebank curators, field botanists and plant collectors to recognize targeted and related species.

The traditional morphological and topological species concept draws a species boundary based mainly on a common segregating set of morphological characters so that a given species name can be related to a group of plants within a genus in its natural environment. The purpose is for identification and thus its diagnostic value. Its major weakness is immediately revealed when a vast amount of variability is presented for classification. Each unique variant is named as a Linnaean species, variety or form. Thus the very purpose of classification is defeated. Instead of bring order it creates confusion. An example is the genus *Begonia* where some 1,500 species have been described, many of which inter-fertile, thus sharing the same genepool. It is, therefore, obvious that the morphological concept by itself is inadequate. This becomes even more convoluted when variants below species level are presented for classification.

A modern extension of the morphological concept is the phenetic species concept. It is based on numerical analysis techniques, known as numerical taxonomy (Sneath and Sokal, 1973), of large set of mainly morphological characters to calculate similarity or distance coefficients between individuals to derive their relationships and to groups them using algorithms such as cluster analysis. However, no consideration is given to their interbreeding ability because it has no attempt to associate the taxonomic classification outcome to their evolutionary

relationships and this leads to some major shortfalls. In spite of this, the methodology is considered useful for studying intra-specific variations especially cultivar groups in many herbaceous ornamental plants. In addition, chemical characters such as those obtain from electrophoresis and chromatography, and DNA markers can be combined with morphology and anatomy characters and subjected to this analysis to produce a more 'natural' classification.

On the contrary, the biological species concept (Mayr, 1940) defines a biological species as "groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups". Dobzhansky (1950) rephrased this as "the largest and most inclusive reproductive community of sexual and cross-fertilizing individuals which share a common genepool". Using the pure biological species concept, many of the species in *Begonia* may have to be 'lumped' together. Lumping of many morphologically distinct species together in this case will cause a serious oversimplification and therefore negate the purpose of classification. The objectiveness of the biological species concept, for various reasons is not shared by all plant taxonomists. Some would rather see taxonomy separated from evolutionary considerations (Sokal and Crovello, 1970) and think of species as "utilitarian mental constructs and that species in practice do not comply with sets of prescribed rules" (Levin, 1979). The species unit is therefore empirical and the resulting classification may be more "natural" as in the phenetic classification.

The current trend is to apply the phylogenetic and cladistic species concept to manifest both the diagnostic and ancestry nature of a classification scheme as defined by Eldredge and Cracraft (1980) as 'a diagnosable cluster of individuals within which there is a parental pattern of ancestry and descent, beyond which there is not, and which exhibits a pattern of phylogenetic ancestry and descent among units of like kind'. Nixon and Wheeler (1990) refined it as 'the smallest aggregation of populations (sexual) or lineages (asexual) diagnosable by a unique combination of character states in comparable individuals (semaphoronts)'. The Angiosperm Phylogeny Group classification is gaining popularity in the world and a working classification scheme is available on the Missouri Botanical Garden website (http://www.mobot.org/MOBOT/Research/APweb/welcome.html). Mabberley (personal communication) in revising his Plant-book (Mabberley, 1997) is adopting this scheme. The end result is a phylogenetic tree, also known as a tree of life or simply a phylogeny, which describes the branching relationships among taxa.

A combination of the species concepts including phenetic, biological, ecological and cladistic (phylogenetic) concepts, steering a middle course between the two extremes of over splitting and lumping, and reflecting their breeding system and the eco-geographical distribution in their natural environments may result in a more useful and applicable classification. It should also be simple to use and flexible for amendment and addition if called for. Evolutionary viewpoint will only be reflected when explicit evolutionary path of the concerned taxon has been agreed upon. It

will thus contain information on existing natural variation as well as evolutionary information and the system will be ever evolving with new information being added upon discovered. The International Code of Botanical Nomenclature (IAPT, 2001) and International Code of Nomenclature for Cultivated Plants (Brickell et al., 2004) should be followed to name and rename taxa to avoid unnecessary potential confusion.

5. CONSERVATION OF GENES VS. GENOTYPES

A fundamental theoretical question facing genebank managers is whether it is adequate to preserve a set of representative genes of a targeted species rather than specific genotypes, i.e. useful gene combinations or linkage groups. There is no recommended guideline available, currently, even for the most well researched crops such as corn, rice and wheat. Usually, a balance approach is applied taking into consideration both the scientific knowledge and the amount of genebank operational resources available. The decision is species/crop specific and it depends on whether the goal in the conservation process is to preserve a range of useful gene combinations and therefore be able to use them directly for plant breeding and in other research, or to preserve a set of all the known genes and to use them individually one at a time using breeding techniques such as backcrossing breeding and modern bioengineering techniques. The question is what type of germplasm will best serve our plant breeders or rather what are the future trends in plant breeding methodologies.

With the advancement of molecular and DNA analysis, gene isolation and gene transformation technologies, and the decreasing cost of doing these types of research the preservation of individual genes may become more and more important. In future germplasm preservation may not be focusing on specific crop/species like what is now but rather it will be trait specific genes, i.e. a collection of resistant genes for *Phytophthora* from different plant species or even from microbes and animals. These genes could be maintained either *in vivo* as seed of a plant species/variety/line (i.e. genetic stock collections) or *in vitro* as isolated genes attached to its gene transformation bacteria. The mandate will be to assemble the widest range of genes and loci and their combinations so that these germplasm could be used in direct transformation breeding. This system of breeding may appear focus and efficient but is heavily dependent on the presence of already existing well-adapted good cultivars. If this is the case the conservation of genotypes is essential to provide the “well-adapted good cultivars” and this is what all modern genebanks are doing. The strategy is therefore to select the widest representation of genotypes possible for preservation and this leads to the concept of “core collection”.

6. CORE COLLECTION CONCEPT

The core collection concept was proposed in the 1980s (Frankel and Brown, 1984; Brown 1989) with the goal to minimize the cost of germplasm conservation while ensuring the preservation of maximum genetic diversity due to the rapid increase in the number of accessions in collections of major food crops like wheat, rice, corn, potatoes, etc. The proposal is to select a subset of say 10% of the base collection so that it represents the base collection with the aim to reduce the number of accessions for large scale germplasm regeneration and evaluation programs and more focus germplasm research. The concept is broadly accepted and attempted. However, the selection of which accessions to include in a core collection depends on the amount of information available on the collection. A set of criteria must be established to select a "core" collection of species that captures the maximum amount of genepool of the genus. Some of the selection criteria proposed are:

1. Specific unique genotypes
    - Pest and disease resistance
    - Stress tolerance, e.g. drought, frost, heat tolerance
    - Adaptation characteristics, e.g. cultural input efficient genes
    - Physiological characteristics, e.g. photoperiod response
    - Specific marketing/commercial traits, e.g. aesthetic genes and shelf-life genes.
2. Cultivar groups and genetic diversity
    - Morphological and DNA data
3. Geographical and ethnological distribution
4. Balance representation of related species (secondary and tertiary genepool as described in the above section on "genepool concept")

Hintum (1996) modified the concept and defined it as "a germplasm collection optimally representing specific genetic diversity" to allow flexibility in the assembly of core collections, and to justify the formation of multiple core collections of a target species in space and time. This definition is equivalent to breeder collections where individual breeders assemble and manage their own distinct collections. In recent years, many approaches including random sampling, stratified sampling, phenotypic analysis, genetic markers and coefficient of parentage have been proposed to establish core collections. In the recent review of the subject by IPGRI (Johnson and Hodgkinew, 1999) new information is available for making better decision.

This concept should be very useful in ornamental plant conservation because it is common for genebank curators to encounter many thousands of registered cultivars/clones for conservation in many species through centuries of breeding and hybridization. A case in point is the daylilies where in the U.S.A. alone there were 48,538 registered varieties in Year 2000 and out of these about half of them are still available for distribution and trade (American *Hemerocallis* Society, pers. comm., 2001). In a situation like this the establishment of a core collection becomes essential from germplasm conservation point of view. For example, the US National

Arboretum maintains mainly species collected by specific collecting expeditions to East Asia and the annual winners of awards. Similarly situation occurs in other ornamental crops in both seed and vegetatively propagated species such as begonia, chrysanthemum, dianthus, lily, marigold, narcissus, pelargonium, petunia, zinnia, etc.

The proposed conservation strategy to handle large volumes of genetic materials is as follows:

- For wild related species – use seed of individual accessions
- For traditional seed cultivars – use seed of individual accessions
- For modern seed cultivars – use seed of polycross within groups
- For non-seeding species – use in vitro and live plants
- For non-seeding traditional cultivars – use in vitro and live plants

7. *IN SITU* AND *EX SITU* CONSERVATION

In situ conservation is the "conservation of plants in the areas where they developed their distinctive properties, i.e. in the wild or in farmers' fields" and *ex situ* conservation is the "conservation of a plant outside of its original or natural habitat" (IPGRI, 2004). Brown (1999) defined *in situ* conservation of agricultural diversity as "the maintenance of the diversity present in and among populations of the many species used directly in agriculture, or used as sources of genes, in habitats where such diversity arose and continues to grow". In cultivated plants, *in situ* conservation is found in home gardens and subsistence farms where many cultivars or lines of a range of crops are grown either together or in separate plots. The cultivars are distinct, have their traditional names and are selected by their owners to maintain their distinctness. However, hybridization between them occurs and new distinct forms will be selected to create more diversity. Introgression of genes between the cultivated forms and their companion weedy species growing in and around the fields helps to broaden the crop genepool and thus new variations. The system is dynamic and the germplasm under conservation continues to evolve in its entire agroecossystem. As for the wild related species, special land reserves in their natural habitats such as the world heritage preservations, national forest reserves, state parks, private reserves and alike have been established to allow the co-evolution of the species and their ecosystem. The advantages of *in situ* conservation are that the germplasm is being utilized and it continues to evolve. Its main weakness is the uncertainty on whether the custodians will continue to farm their fields in the traditional way with the genetic diversity. Similarly, nature preserves may be destroyed by human development or natural disaster. In recent years, the concept of ecotourism (Egan, 2001; Garen, 2000,) e.g. in Costa Rica and the co-use of preserves for farming, e.g. the Gray Ranch of Nature Conservancy in Malpai Borderlands Group in southern borderlands of New Mexico and Arizona, USA

(Schumann, 2003) have added on new dimensions to this complex conservation system.

Ex situ conservation has been the main thrust in crop plants. Plant germplasm are maintained in genebanks and botanic gardens. In genebanks, storage of seed is the preferred method and of the 6.1 million accessions of the crop plants about 90% are kept as seed in cold storage (FAO, 1998). The remainders are clonal germplasm and recalcitrant seed species and are kept in field plantings as field genebanks, in tissue culture as *in vitro* genebanks and in liquid nitrogen as cryopreservation collections. The main advantages of *ex situ* collections are the stability of the conservation if genebanks are well management with well documented germplasm information and have plant materials for immediate distribution. The drawbacks is the high expense in building and operating a good genebank and as the result only limited species are to-date conserved in this way. Some of the poorly run genebanks are facing serious germplasm erosion while in storage due to the lack of proper seed viability and regeneration program. Table 5-5 provides the IPGRI compilation of issues encountered in the four types of genebank and the proposed solutions to resolve them (http://www.ipgri.cgiar.org/regions/ssa/useandrestoration/Table1.htm).

The existing challenge is to interface between the *in situ* and *ex situ* system. Up till now, the two systems are more or less implement independently by two different groups of people and institutions with different basic conservation philosophy. The *in situ* group is habitat and ecosystem orientated and, in contrast, the *ex situ* group are species and crop orientated with very confined objectives. There is an increasing effort to bring the two together and in fact they complement and strengthen each their efforts and benefits, and provide better solutions to combat each other weaknesses.

Table 5-5. IPGRI proposed measures to resolve issues as found in the four types of genebank (Source: International Plant Genetic Resources Institute - http://www.ipgri.cgiar.org/regions/ssa/useandrestoration/Table1.htm).

Type of Gene bank	Characterization & Evaluation	Monitoring	Data Mgt.	Storage	Dissem-ination of infor-mation
Seed	Development of descriptor lists	Reference to guide books	Harmonization of data	Research and applicat-ion of low cost technol..	Guide-lines
	Morphological and molecular characteriza-tion	Embryo rescue	Data analysis		Tech-nical bulletins
		In vitro germination	Providing expert system	Research on seed	CD-ROMS

Type of Gene bank	Characterization & Evaluation	Monitoring	Data Mgt.	Storage	Dissem-ination of infor-mation
	Agronomic and horticultural evaluation		Information sharing mechanisms	technolo gies	Descrip-tors
Field	As above	Rescue of threatened accessions Research on sample size	As above	Explore seedling banks	As above
In vitro	As above	Somaclonal variation	As above	Research on storage media Develop ment of new collec-tions Expand research on new plants	As above
Cryopres-ervation	As above	Research on genetic integrity	As above	Expand research on new plants	As above

8. ORTHODOX VS. RECALCITRANT SEED

Orthodox seed are seed that can withstand drying to low seed moisture content (MC) of about 5% and survive in dormant state in optimum storage conditions for decades or even thousands of year as in the case of the Canadian arctic lupin (Black, 1967). Seed is thus the prefer plant organ for long-term storage in a genebank (Hong et al., 1996). On the contrary, recalcitrant seed cannot survive dehydration (Robert, 1973). The critical moisture content of many tropical fruit and tree seeds is about 30% and on drying they die (Hor et al., 1990). Storage of recalcitrant seed using techniques such as desiccation and fast drying of excise embryos (Chin, 1988; Pritchard, 1991; Fu et al., 1993) are still under investigation. In between these two categories there is an intermediate group with species, e.g. papaya and some citrus that can survive moderate drying to 7-12% MC range (King and Roberts 1979; Chin 1988). However, the different between this intermediate group and the orthodox seed is that they cannot survive in cold temperature of less than 10°C (Hong and Ellis, 1992 and 1995).

The physiology of why a seed is recalcitrant is still under investigation and it may be related to the under or over development state of seed and embryo physically and physiologically. Berjak et al. (1989) reported that an important characteristic of recalcitrant seeds is that there is no arrest in their development, as with orthodox seeds. In the latter, it has passed the threshold for the seed to reverse back to dormant stage. This phenomenon is seen in some orthodox seed species where seed of some genotypes with no seed dormancy begin to germinate immediately after the seed have reached physiologically maturity when the fruit containing the seed is still physically and/or physiologically not ripen. In this case, the germinated seed with radical beginning to protrude and others with enlarged plumule or cotyledons are physiological a seedling and therefore can not withstand seed desiccation. The fact that many embryonic axes have higher MC than their storage tissue supports this view (Grabe, 1989).

Fortunately, most of our major herbaceous ornamental species have orthodox seed. However, seed storage ability varies between genera, species within a genus and cultivars within a species. Table 5-6 lists the storage life of some common flowers. This is an approximation as limited information is available. For example, *Antirrhinum*, *Capsicum*, *Coreopsis*, *Dianthus*, *Helianthus*, *Lupinus*, *Petunia*, and *Tagetes* store well (personal experience).

Table 5-6. Storage life of some flower seeds; Short = less than 1 year, Medium = less than 3 years and Long = more than 3 years (McDonald, 2005).

Short	Medium	Long
Anemone, Aquilegia,	*Achillea, Ageratum,*	*Brassica, Calendula,*
Arabis, Asclepias,	*Alyssum, Antirrhinum,*	*Celosia, Centaurea,*
Asparagus, Aster,	*Brachycome, Campanula,*	*Chrysanthemum,*

Short	Medium	Long
Begonia, Bellis, Browallia, Calceolaria, Callistephus, Catharanthus, Cleome, Consolida, Coreopsis, Echinacea, Echinops, Fuchsia, Gaillardia, Gerbera, Geum, Helichrysum, Hippeastrum, Iberis, Impatiens, Iris, Lantana, Liatris, Lilium, Limonium, Nemesia, Penstemon, Phlox, Primula, Salvia, Sinningia, Thunbergia, Veronica, Vinca, Viola	*Capsicum, Cineraria, Clarkia, Coleus, Cyclamen, Dahlia, Delphinium, Dianthus, Euphorbia, Gaillardia, Gomphrena, Helianthus, Heuchera, Hibiscus, Lathyrus, Lavandula, Lisianthus, Lobelia, Lobularia, Lotus, Lupinus, Matthiola, Nicotiana, Paeonia, Papaver, Pelargonium, Petunia, Portulaca, Rudbeckia, Saintpaulia, Scabiosa, Schizanthus, Sedum, Senecio, Tagetes, Torenia, Verbena*	*Convolvulus, Cucurbita, Dendranthema, Gypsophila, Leucanthemum, Lycopersicon, Lathyrus, Mimulus, Zinnia*

8.1 Seed Science and Technology in Germplasm conservation

Seed quality is believed to be the best at seed physiological maturity, i.e. the stage when a seed has the heaviest dry weight. After that seed started to loss weight and deteriorate. However, for most species there is no information on the exact days after pollination is this stage. In most flowers, we have limited knowledge on how the growing and early seed development environments affect seed physiological maturity and thus quality. In field situation, seed at physiological maturity is often too wet for harvesting as the flower head and fruits are usually not ripen or sufficiently dry for safe harvesting. During germplasm regeneration the aims are to provide the best growing environments for seed formation and to slow down the rate of deterioration after seed physiological maturity. A checklist of some of the factors that should to be taken into consideration during germplasm regeneration is as follows:

- Seed production location – cool and dry with good quality water for irrigation and free from pest and disease sources;

- Growing environment – balance fertilizer in term of both macro- and micro-elements and optimal fertilization regime and rate, and pest and disease control;
- Uniform and synchronized flowering – vernalization, photoperiodism and fertilization and water withdrawal treatments;
- Pollination – Present sufficient appropriate insect pollinators or hand pollination when pollen and stigma are most receptive, and proper isolation;
- Early seed development environment - balance fertilizer in term of both macro- and micro-elements and optimal fertilization regime and rate; pest and disease control, and provide cool and dry environment;
- Physiological maturity and after-ripening of seed – pest and disease control, and provide cool and dry environment to prevent weathering damage;
- Harvesting time and method – optimal seed head and fruit maturity, discard pest and disease infected heads and fruits, and prevent mechanical contamination;
- Stage I drying – dry off free water on seed heads and fruits due to dew and rain, and prevent mechanical contamination;
- Threshing – perform at right seed moisture content to prevent seed bruising in wet seed and cracking in over dry seed, and prevent mechanical contamination;
- Seed cleaning – remove all empty and damaged seed, and prevent mechanical contamination; and
- Stage II (final) drying – slow drying at low temperature and relative humidity.

A practical problem facing seed technologists in most herbaceous ornamental species is that we know very little about most of the above requirements. The recent book on flower seeds (McDonald and Kwong, 2005) managed to fill some of these knowledge gaps. However, science is just beginning to recognize the important relationship of early seed development, seed physiological maturity, after-ripening, dormancy and seed quality. Tay (2005) made an attempt to summarize the seed science and technology applied in germplasm conservation. On the other hand, the application of proven protocols used in storing seeds of major food crops has shown promising result, e.g. at OPGC seeds of *Petunia* accessions in the NPGS collection that have been stored for more than 40 years have been germinated using tissue culture techniques.

9. GENEBANK PROCEDURES

The operation of a genebank, an *ex situ* conservation program, entails knowledge in many disciplines of both biological and physical sciences including botany, taxonomy, plant genetics, plant physiology, biochemistry, seed technology, agronomy, plant pathology, entomology, weed science, computing, office automation, agricultural engineering, etc. An efficient genebank is one where its staff is able to regenerate the highest quality seed (genetic, physiological and physical) for storage because this helps to lengthen the period between regeneration cycles. In every cycle of seed multiplication and storage some changes in the genetic composition occurred in an accession, thus causing the loss of genes, i.e. genetic erosion in a genebank. To reduce genebank genetic erosion it is essential to have a well designed genebank physical facilities including the building, the seed drying, processing and packaging system and the cold storage system, and secondly, a simple efficient genebank operational protocol from germplasm acquisition, regeneration and conservation. It is therefore important to integrate knowledge from multiple disciplines during genebank design (Tay, 1991) and formulation of the genebank management system and operational manual (Tay, 2005).

The activities in a genebank are germplasm exploration and acquisition, regeneration, conservation, characterization, evaluation, distribution and documentation. Figure 5-4 shows a flow diagram of how these activities are linked together and the flow of germplasm materials and information that is vital for the management of a genebank, and the required disciplinary knowledge and skills need for genebank staff to implement each steps. A good plant germplasm conservation program is one where all the desired accessions are regenerated with acceptable genetic and seed quality as compared to another where only a portion of the desired accessions is regenerated with very high quality seed, but some of them are lost completely (Figure 5-4).

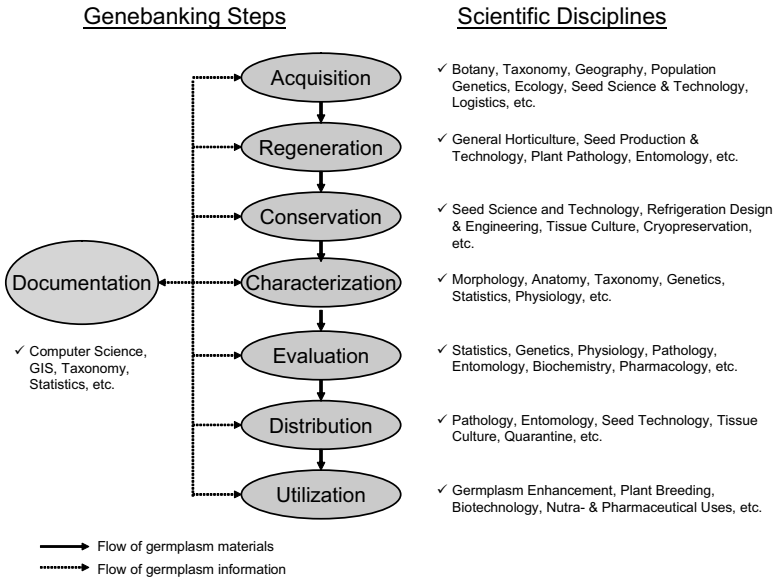

Figure 5-4. Genebanking steps from germplasm acquisition to utilization in relation to knowledge in scientific disciplines required.

9.1 Germplasm Acquisition & Exploration

All germplasm acquisitions and explorations must abide by: 1) the principle of national sovereignty over plant genetic resources as stated in CBD; 2) protection of threatened and endangered species in CITES; 3) global partnership to conserve, protect and restore the health and integrity of the Earth's ecosystem in UN Agenda 21 - Rio Declaration on Environment and Development; 4) the Principles for a Global Consensus on the Management, Conservation and Sustainable Development of All Types of Forests. CBD and CITES requirements are treated in detail later. The UN FAO International Treaty on Plant Genetic Resources (ITPGR), which has no direct declaration on herbaceous ornamental species, may also have to be consulted.

An economic means of acquiring germplasm is the exchange and donation from seed and plant nursery companies, botanical gardens, university researchers, crop-specific societies, seed savers' groups, and private plant enthusiasts. The number of seed required per accession depends on a seed lot viability and the aim is have enough seed to grow about 50-250 plants for seed regeneration. A representative seed sample can be withdrawn as done by official seed inspectors for seed

certification (AOSCA, 2003). All the passport information relating to the germplasm has to be collected. IPGRI crop descriptors, e.g. *Capsicum* descriptors (IPGRI, 1995), have a standard set of recommended passport descriptors which are applicable for herbaceous ornamental plants. Collection of diseased seed and plants should be avoided so that the new acquisitions will not introduce diseases and pests into the collection. During acquisition it is often difficult to avoid the collection of duplicates due to unknown names. Duplicates are usually identified during characterization. If an accession has a donor number and other identification numbers they should be recorded, entered into passport database to reflect its origin to avoid confusion in future years.

Plant explorations may have to be launched when new germplasm (especially wild relatives) are targeted. This is costly and demands advanced planning, organization, and logistics. The first step is to determine the taxa and traits to be targeted; then the countries and locations to explore based on existing germplasm already in conservation. The preparation of a mission frequently starts with detailed survey of available published floras and herbarium specimen to establish the geographical regions, genetic variability, accessibility of the regions, microenvironment of targeted sites, proposed exploration route and timetables. An exploration team usually consists of 2-4 persons including botanists, horticulturists, and at least a local field botanist who is knowledgeable on the targeted taxa. All the necessary detailed maps of the targeted area; collecting tools and materials including a compass, GPS unit, a camera, herbarium presses; CBD / quarantine related permits; and a detailed field collection timetable should be prepared before-hand in collaboration with the local partner. Collection forms (Table 5-7) are essential to document collected accessions. In addition, all the on-site logistics including access to a four-wheel drive vehicle, shipping and transit procedures of the collected germplasm have to be planned ahead. IPGRI published a crop genetic resources field collection manual describing the planning process (Hawkes, 1980).

Table 5-7. Example germplasm collection form to be used on-site (Hawkes, 1983).

Category		Category	
Expedition Organization			
Country			
Team Collector(s)		Collector's Number	
Date of Collection		Photo Number(s)	
Species Name			

Category			Category		
Vernacular/Cultivar Name					
Locality					
Latitude		Longitude		Altitude	
Material (circle)	Seeds	Inflorescences	Roots / tubers	Live Plants	Herbarium
Sample (circle)	Population	Pure line	Individual	Random	Non-Random
Status (circle)	Cultivated	Weed	Wild		
Source (circle)	Field	Farm Store	Market	Shop Garden	Wild vegetation
Original Source of sample					
Frequency (circle)	Abundant	Frequent	Occasional	Rare	
Habitat					
Descriptive Notes					
Uses					
Cultural Practices (circle)	Irrigated		Dry		
Season	Approximate sowing dates			Approximate harvesting dates	
Soil Observations	Texture		Stoniness		
	Depth		Drainage		

Category			Category		
	Color			pH	
Land Form	Aspect			Slope	
Topography (circle appropriately)	Swamp Hilly Mountain-ous	Flood Plain Hilly Dissected	Level Steeply Dissected Other (specify)	Undula-ting	
Plant Community					
Other crops grown near in rotation					
Pests/Pathogens					
Name and Address of farmer					
Taxonomic Identification	by		date		
Name of Institution			Accession No.		

Sampling strategies and methods are formulated based on knowledge of population distributions and genetics of the targeted taxa in order to capture representative samples of the collected populations (Marshall and Brown, 1975; and Hawkes, 1980). The coarse and fine grid sampling (Random) method (Marshall and Brown, 1975) is used when a population is uniformly spread over a large distribution area with relatively same eco-environment. The grid can be tightened or loosened according to the genetic diversity of the targeted taxa and populations in the field and eco-edaphic variation, and at each grid a set number of accessions are collected. Embedded in the grid, additional samples are collected in light of greater genetic variation. This strategy allows the selective sampling and collecting of distinctive variations.

Random and selective sampling strategies are applied based on the variation presented in the field. Table 5-8 summarizes the importance of random and selective sampling strategies to be applied based on the genetic composition of the different category of plant germplasm. In the case of wild and weedy species, both

methods have to be considered objectively, depending on the spatial distribution of the population presented. Traditional cultivars are often maintained distinctively by farmers and thus selective sampling is applied to collect all the recognizable variations. Genetic stocks and improved and clonal cultivars are all distinctive and stable. Selective sampling can thus be applied easily.

There is limited information on the optimum sample size of an accession that should be collected. Based on experience in crop plants, Hawkes (1976) and Marshall & Brown (1975) suggested the number of plants required to obtain, with 95% certainty, all the alleles at a random locus occurring in the target population with frequency >0.05. Hawkes (1980) proposed ~50 seeds/plant and N=2,500 to 5,000 seeds / accession, i.e. 50-100 plants collected, particularly in highly variable crops where more plants have to be sampled for self-pollinated than cross-pollinated species. In species with few seeds/seed head or fruit, the suggestion is to take ≥five fruits from each of three adjacent plants every 'few' paces to make up the total of 50 seeds. In species with many seeds/head or spike, take only part of each head to provide the 50 seeds required/plant. Sampling of fleshy fruits, e.g. *Passiflora,* is basically similar. However, many wild populations are represented by few plants; thus limited seed are available. In other cases, only a few plants of a population flowered and set seed at any one time. In such cases, about 20% obtainable seed may be collected in order to ensure the survival of those small populations in their natural habitats, i.e. without threatening natural plant populations. Rare, threatened and endangered plants will be collected only with proper permits and under strict conservation standards. For example, this is often the situation when collecting in Ohio prairie remnants for prairie flowers such as *Rudbeckia*, *liatris*, *Baptisia*, etc. (personal experience). Wherever possible, matured seed free from pest and disease damage should be collected. Otherwise, more seed have to be collected to make up for damaged and immature seed. Healthy seed are required to clear quarantine inspection and to prevent potential contamination of existing collections.

Table 5-8. Category of germplasm and the sampling strategies suggested.

Sampling Strategy	Wild/Weedy Species	Traditional Cultivar	Genetic Stock	Improved Cultivar	Clonal Cultivar
Random	+++++	+++	+	+	+
Selective	+++	++++	+++++	+++++	+++++

Every sample collected is given a collection number and this identification number remains with the accession. Seed collected have to be dried immediately and thus paper or nylon-netting bag is a better container than plastic. It is good practice to write the collection number on the seed bag and to have a label with the number in the bag in case the outside number is soiled and unreadable. Recalcitrant seed have to be kept moist during shipment to sowing. If plant propagules have to

be collected mature bulbs may be allowed to dry up slowly, and rooted plants, cuttings and immature bulbs have to be planted and kept moist. In very specialized collecting mission, field tissue culturing techniques have been developed to collected embryos.

9.2 Regeneration

About 5,000 seeds/accession is required for long-term storage in at least two locations; another 5,000 seeds are kept in medium-term storage as active collection for germplasm distribution. The small amount of seed acquired or collected is not adequate for conservation storage. In addition, seeds deteriorate during storage and have to be regenerated. Seed regeneration is, thus, an important activity of a genebank. The aims in seed regeneration are: (1) to minimize changes of an accession's original genetic makeup so that the regenerated seed is similar to the original population and (2) to produce high quality vigor seed. The former is achieved by promoting random pollination, preventing genetic drift, and selection. The latter is likewise significant because it determines the storage life span of a seed lot where high quality vigor seed can be stored for longer period. Good storage condition cannot improve the quality of a seed lot and it only helps to slow down the deterioration. Seed production and technology knowledge and skills are, therefore, essential assets in the operation of a genebank.

Maintaining an accession original genetic composition is a population genetics issue. In genebanks, the number of randomly selected plants use for regeneration and the number of seed per plant contributes to the bulk seed lot are used to ensure minimal genetic shift. A rule-of-thumb is ~30-50 randomly selected plants for self-pollinated species and clonal accessions and 100-250 plants for cross-pollinated species. All recognizable off-types of pure lines, genetic stocks, or named cultivar accessions have to be removed to prevent genetic contamination. In heirloom cultivars there are often a tolerable range of variation (due to their nature) and should be maintained. However, when two or more distinct lines are observed in an accession they can be separated to form new accessions. Multi-line cultivars and segregating populations are maintained as such.

To obtain the required randomly selected plants and the contribution of the same amount of seed from each plant to the seed lot, the following steps are ensured:

- Seed have to be randomly withdrawn from a seed container to obtain a representative sample of the accession;
- All viable seed have to be induced to germinate relatively uniformly so that every viable seed has an equal chance to be selected for planting;
- All randomly selected seedlings have to be maintained so as to grow uniformly and vernalized to induce uniform flowering later and then randomly selected to constitute the required plant number for seed production;

- All randomly selected plants for seed production have to be treated so that all the plants will flower at the same time to allow random pollination; and
- Same amount of seed is harvested from all the plants to make the required bulk sample.

The amount of gene loss is significant if the above recommendations are overlooked. Table 5-9 illustrates how up to 88.5% of a genepool in an original seed sample could be lost in the process of seed regeneration of a single generation. The loss of rare recessive alleles is especially serious in this kind of scenario.

Table 5-9. Possible loss of genes in a genepool of an accession in a single cycle of seed regeneration.

Seed Production Step	No. of Seed/Plants[z]	% Loss of genepool[y]
1. Viable seed of a random sample	400 (s)	(100)
2. No dormancy breaking treatment (50% dormancy)	200 (p)	50
3. Only transplanted vigorous seedlings (50% vigorous and 50% weak)	100 (p)	75
4. Seed establishment and juvenile growth (90% survival)	90 (p)	77.5
5. No. of plants flowered (60%)	54 (p)	86.5
6. Pest and disease damaged (14.8%)	46 (p)	88.5
Resulting % of the original random sample genepool		11.5%

[z](s) = seed; (p) = seedlings or plants

[y](100) = the initial 100% genepool of the random sample

Many of the herbaceous ornamentals are open-pollinated with insect pollinators. Isolation in insect-proof pollination cages (Figure 5-5) or greenhouse compartments (Figure 5-6 and using insect pollinators such as honey bees, bumblebees, and flies have to be used. Optimal seed production sites, proper climatic conditions, and current seed technology practices are the basics to produce high vigor seed and prevent mechanical contamination between accessions (Tay, 2005). Efficient harvesting program and methods help to avoid seed weathering in the field, and prevent pest and disease infestation and physical cross-contamination of seed lines. Harvested seed heads and fruits should be dried immediately of free surface water

from rain or morning dew using high volume air-flow dryer at below 30°C or ventilator to prevent fungal growth and heat-producing composting effect.

Figure 5-5. Insect-proof pollination cages with honeybees as pollinators at the U.S.D.A. Ornamental Plant Germplasm Center.

Figure 5-6. Greenhouse compartments with bees as pollinators at the U.S.D.A. Ornamental Plant Germplasm Center.

Seed heads and pods are suitable for threshing when they are dried to the brittle state. A homemade threshing board (Figure 5-7) has proved to be efficient for small seed lots at OPGC. For bigger seed lots, a small thresher (Figure 5-8) running at appropriate speed is effective. Optimal thresher speed and clearance and seed MC have to be used to prevent mechanical injury to the seed. Wet seed in fleshy fruits, e.g. *Passiflora,* is extracted by squashing the fruits with their juice, allow to ferment to remove the mucilage layer around the seed and then wash in water to separate the seed from the pulp. Otherwise, the whole fruits are dried with the seed inside. Once dried, the seed are separated from the fruits and rubbed cleaning of the dried-up mucilage layer.

Figure 5-7. A homemade threshing board, measuring 39 x 39 x 10 cm, used at the Ornamental Plant Germplasm Center.

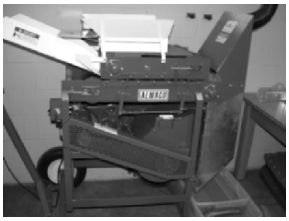

Figure 5-8. Modified small seed-lot belt thresher, showing the blocking of the original built-in winnowing component by a chute that direct the threshed seed and chaff down to a screened container at the Ornamental Plant Germplasm Center.

Seed cleaning and conditioning are done with a series of sieves, a laboratory seed blower (Figure 5-9), and a vibrating table (Figure 5-10). At the OPGC, a Faxitron Digital X-ray machine (Figure 5-11) is used to detect good and empty seed during the cleaning process in 20 seconds, in contrast to using the slow dissection or germination test (Tay, unpublished data).

Figure 5-9. Single column seed blower used at the Ornamental Plant Germplasm Center.

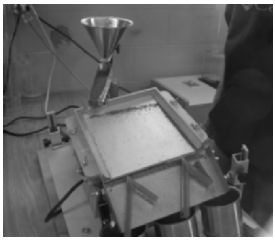

Figure 5-10. Small single deck vibrating table used at the Ornamental Plant Germplasm Center.

Figure 5-11. Faxitron digital X-ray machine used at the Ornamental Plant Germplasm Canter.

In diverse taxa and cultivars, there is an immense lack of information on seed germination, dormancy breaking treatments, cultural practices (e.g. pest and disease control during pollination), vernalization & photoperiodic treatments to induce and synchronize flowering, pollination biology (self incompatibility), isolation requirements, seed biology (e.g. after-ripening of seed), seed harvesting, drying & cleaning, and seed moisture content for long-term storage. Modern computerized greenhouses to some extent fulfill optimal regeneration environments. A multi-location regeneration program in both northern and southern hemispheres, like those practiced by floriculture seed companies, is an effective model. Alternatively, a decentralized model with a network of volunteers (individuals, institutions) to conserve what each participant is interested in growing, e.g. the British National Council for the Conservation of Plants and Gardens (NCCPG, 2005), American Begonia Society, and many other such crop-specific societies.

When seed regeneration is done in open fields, particular attention has to be taken into consideration regarding the potential invasiveness of many herbaceous species. The first step is to consult the national and local government prohibited or noxious weed and quarantine lists. Accessions of species with related species on the list, but not the species itself, should be closely monitored in the year of production and subsequently. Many species with hard seed coats can survive in cultivated fields for up to 5-10 years. An alternative is to regenerate seed in the confinement of a greenhouse and discard of plant materials safely at the end of the growing

season. Currently, there is no protocol for invasiveness evaluation in herbaceous ornamental plants. Continuous monitoring should also be built into protocols for germplasm characterization and evaluation. During germplasm distribution, caution should be documented to inform recipients of this risk.

9.3 Conservation

In a genebank, germplasm are stored as seeds in a seedbank, as living plants in a field genebank, as propagules such as bulbs and tubers in humidified coolers, as meristem culture in *in vitro* collections, and seed, dormant buds and tissue culture in liquid nitrogen cryopreservation banks. Seed is most widely stored propagule and the cold storage technology has had a proven success for 50 years. The system consists of a combination of long-term cold storage rooms at -18ºC, frost free for maintaining base and duplicate collections, medium-term storage coolers at 2ºC / 40%RH for maintaining active collections, and sometimes a short-term storage cooler at 15-20ºC / 40%RH for working collections and temporary storage. The design and layout of the system is such that the short-term cooler is the anteroom for the medium-term cooler and the medium-term cooler the anteroom for the long-term cold room. Usually, a seed packaging room at 20ºC and 40%RH is the anteroom for the short-term cooler. This is necessary especially in the tropics where the ambient RH is high. The short-term cooler is also the best place to locate the slow dryer set at 10-25ºC / 10-15%RH. This layout can be efficiently built with prefabricated insulated steel or aluminum sandwiched panels as described in Tay (1991).

Security of the stored germplasm is a top priority. Germplasm collections should be duplicated in at least another off-site location (a duplicate collection) so that if one site is destroyed due to machinery failure, fire, or natural disaster the germplasm is still available. The storage facilities at all sites should be protected with a series of alarm systems and a back-up electric supply (generators) to safeguard the collection. At the OPGC, the cooler is armed with three tiers of alarm-protection system where the first level alarm is the cooler in-built alarm to indicate high temperature, the second level of protection is the complete electric power cut-off relay switch (that will automatically kick-in if the cooler temperature continues to rise), and the third level alarm is a “Sensaphone” system which will activate a telephone call to an OPGC staff to physically come in to switch off the cooler manually. The “Sensaphone” system will also respond to the siren of the building fire alarm.

Further seed drying before packaging and storage is accomplished using a slow drying regime at 10-25°C / 10-15% RH as recommended by FAO/IPGRI (1994). Equilibrium seed MC of 3-7% w.b. can be achieved depending on the chemical composition in the seed concerned. Seed longevity is increased by a factor of ~3x if the storage temperature is reduced from 20ºC to 10ºC; by 2.4x from 10ºC to 0ºC; by

1.9x from 0ºC to –10ºC; but by only 1.5x from –10ºC to –20ºC (IPGRI, 1994). After final drying, seeds are immediately hermetically sealed in laminated aluminum pouches or canned in tins for long-term storage of base collection. Otherwise, due to the low temperatures, it is difficult to control to the low RH required for seed to stay dry.

Base collections consist of seeds for preservation only and not for distribution. The optimal seed MC of different species for -18ºC storage is unclear. At the moment, seed of all the major food crops store well at about 5%MC. Ultra-dry seed has been suggested but it is still under investigation. A seed viability monitoring system is vital in managing a base collection in order to detect seed deterioration (before they reach the fast deterioration phase of the seed viability curve, Figure 5-12) and to prevent genebank genetic erosion. A standard germination test is used (ISTA, 2005) and these very dry seed have to be equilibrated in moist room to allow seed MC to recover so as to prevent rapid imbibing injury in germination. The system includes an initial seed viability test and subsequent germination test at interval of 3-10 years depending on seed lot viability. For high quality seed lots, the early monitoring interval can be 7-10 years and then followed by 3-5 years later. A threshold germination value of about 80% is used to decide whether a seed lot has to be rejuvenated with a new lot of fresh seed. Randomly drawn seed samples for monitoring testing may be packed separately and kept with the base collection sample so that the base collection sample will not be reopened and resealed at each monitoring testing. To date, there is no non-destructible method for monitoring seed viability.

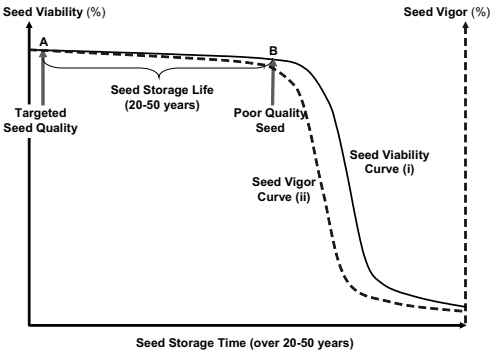

Figure 5-12. Seed viability and vigor curves showing the targeted seed quality (Point A) that is required for long-term storage in genetic resources conservation as compared to poor quality seed at Point B.

An active collection is often maintained in a low RH controlled cooler so those seed samples do not have to be hermetically sealed. Reclosable containers allow seed samples to be withdrawn for distribution because any moisture absorption by seed during seed withdrawal will be dried in the low RH environment in the medium-term cooler. In fact, at OPGC the medium-term cooler is used as the slow dryer once seed MC is less than 10% w.b. because of its low RH of 20-30%.

Accessions of species with recalcitrant seed and clonally propagated genotypes have to be maintained as living plants in greenhouses or fields as field genebank or *in vivo* collection. The method is costly and subjected to pest and disease attacks, and natural disasters. These collections have to be backed up by planting in another location, and for geophytic species with storage organs like *Dahlia*, *Narcissus* and *Tulipa,* their dormant propagules can be stored in high RH storage cooler. A more common method for backing up is to put them into meristem culture as an *in vitro* collection; the storage of dormant buds or tissue culture in liquid nitrogen at -196ºC is still under investigation. Several slow-growth tissue culture techniques have been used including the use of hard agar (Tay and Liu, 1992). These techniques are all less developed than in seed storage. Wild species of many vegetatively propagated herbaceous ornamentals where the conservation of genotypes is less important should be conserved as for seed producing species.

9.4 Characterization

Germplasm characterization is the description of each accession of a collection with a set of stable (non-plastic) plant characters that are unique to the crop. The aim is to promote germplasm utilization because potential germplasm users can then search through a database with these descriptions and select what they want based on a character description. It is also use in the identification of duplicates in a collection, for taxonomic analysis and decision, and in combination with GIS analysis to identify variation gaps in a collection. Experts familiar with the crop develop a crop descriptor. They select plant characters, which are stable and show variations among accessions in a collection or genus. Stable characters are those diagnostic features of the plant, e.g. leaf shape, that remain the same when grown in different environments. However, other characters such as plant height and number of days to flowering, which are affected by the environment and physiology, are also used. IPGRI (1995) and the International Union for the Protection of New Varieties of Plants (UPOV) have developed standardized descriptors for most food and industrial crops. UPOV standards also cover many ornamental crops, including: African Violet (*Saintpaulia*), *Alstroemeria*, *Amaryllis*, *Anthurium*, *Aster*, *Calibrachoa*, Carnation (*Dianthus caryophyllus*), *Catharanthus*, *Celosia*, Chincherinchee (*Ornithogalum*), Christmas cactus (*Schlumbergera*), *Chrysanthemum*, *Clematis*, Crown of Thorns (*Euphorbia milii*), *Cymbidium*,

Daffodils (*Narcissus*), *Dendrobium*, *Dieffenbachia*, Easter cactus (*Rhipsalidopsis*, *Epiphyllopsis*) Elatior Begonia (*Begonia elatior*), *Euphorbia fulgens*, *Eustoma*, Evening primrose (*Oenothera*), Everlasting daisy, *Exacum*, Firelily, Firethorn, Flax (*Linum*), *Forsythia*, *Freesia*, *Gentiana*, *Gerbera*, *Gladiolus*, *Guzmania*, *Hydrangea*, *Hypericum*, Ifafa lily, *Iris* (bulbous), *Pelargonium peltatum*, *Kalanchoe*, Kangaroo paw, *Lanchenaria*, *Lavandula*, *Leucadendron*, *Lilium*, *Limonium*, Ling, Lupins, *Nerine*, New Guinea Impatiens (*Impatiens hawkeri*), *Osteospermum*, *Pentas*, *Petunia*, *Phalaenopsis*, Poinsettia (*Euphorbia pulcherrima*), Potted azalea, *Protea*, *Pyracantha*, Regal Geranium (*Pelargonium domesticum*), *Rhododendron*, *Rosa*, *Scorzonera*, Scots heather, *Serruria*, *Spathiphyllum*, *Streptocarpus*, Sunflower (*Helianthus annuus*), Tuberous begonia (*Begonia tuberhybrida*), *Tulipa*, *Verbena*, *Weigela*, *Zantedeschia*, and *Pelargonium zonale* (UPOV, 2005). However, many herbaceous ornamentals are still without standard descriptors.

The characters of a crop descriptor can be categorized into numeric, ordinal, nominal, and binary characters. Numeric characters are those that are measured (quantitative), e.g. plant height, flower diameter, etc. Ordinal characters are actually numeric characters but instead of actual measurement they are given a score of a scale, e.g. 0 - 9 where a "0" indicates an absence of the characteristic, "1" the least expression of the character and "9" the most expression. Based on a normal "bell" shape curve the median is a "5", the 25% percentile is a "3", and the 75% percentile a "7". With the use of "3", "5" and "7", the 0-9 scale provides a span of flexibility when new extremes and in between variations are encountered. The individual scores of a descriptor are called descriptor states. Nominal characters are those that do not bear order of scale between descriptor states and the individual states are being described and nominated accordingly, e.g. flower color, petal shape, etc. Binary characters are those that exist only in two states, i.e. presence or absence (Sneath and Sokal, 1973).

Characterization is best done by growing a large number of accessions of a collection in a normal growing season specifically for this purpose (Tay, 1987). About twenty plants per accession in single or double rows, side by side, with or without replication in a well-prepared uniform field are grown and measured or scored. Scoring is based on the whole plot of an accession. Measurements are taken on 5-10 representative randomly selected plants. All recognized off-types are removed. Color descriptors such as leaf color, pigmentation and flower color are reference to color codes of the Royal Horticultural Society Color Chart. Color is affected by the lighting situation. It is therefore important to set the time of the day and note the angle of sun where scoring color characters.

Photography of a set of specific organs, e.g. leaf, inflorescence, flower, and fruit is a very useful method to record variations (Tay and Chen, 1993). Gray, blue and black backgrounds provided the greatest contrast. A meter stick should be included in a photograph to provide a size relationship. With the advancement in

computerized imagery, scanning technology, and personal computers, characterization could be automated using digital imagery analysis system.

DNA markers are increasingly replacing protein electrophoresis for germplasm characterization. It is a rigorous tool for duplicate identification, taxonomic and evolutionary relationship studies once a workable and repeatable system is available.

9.5 Evaluation

Germplasm evaluation is the screening of a collection for specific genes, such as resistance to important pests and diseases, abiotic stresses, adaptation and other commercial traits. These traits are included in all well-formulated standard crop descriptors, e.g. IPGRI (1995). The prioritization of the traits for evaluation depends on researcher needs and, thus, is location-specific. Specialized disciplinary knowledge and skills are required to implement most evaluation projects. For example, evaluation for *Pelargonium* ring-spot virus resistance requires a virologist, *Pseudomonas* wilt a bacteriologist, powdery mildew a mycologist, mites an entomologist, heat/cold/drought tolerance require plant physiologists, plant invasiveness a weed scientist, etc. Collaboration between researchers of genebanks and other research institutions is, thus, routinely forged.

Germplasm evaluation trials, such as those conducted by the floriculture industry and some U.S. Land Grant universities, consist of trialing new variants of existing ornamentals or completely new crops. The Athens Select™ program at the University of Georgia, Athens, Georgia (U.S.A.) is an example of how ornamental germplasm may be introduced, evaluated, and commercialized (Armitage, 2003). A network of trial sites to cover different geographical and ecological regions is ideal. Many of the multinational flower companies are doing this with sites in countries with major flower markets in both hemispheres.

Botanic gardens and arboreta are doing important work in germplasm exploration, introduction, and evaluation. They make new exciting germplasm known to the public to create the interest and markets. The botanic garden system is an important germplasm evaluation program and many of them have excellent annual germplasm exchange systems, known as *Index seminum*, where seeds or, occasionally, vegetative propagules are shipped across continents for planting.

Many herbaceous ornamentals are also used traditionally as medicines, functional food, herbs and biocides. For example, daylily flower buds are a delicacy in East Asia, *Salvia* is used as an herb, *Chrysanthemum* flowers are edible and medicinal, and *Tagetes erecta* flowers produce beta-carotene which is extracted for poultry feed and a biological control agent against plant pests. Evaluation of such uses should also be pursued.

DNA markers of desirable traits, genetic maps and gene expressions are increasingly being studied (De Vicente, et al., 2004). International groups like the Petunia Platform (http://www.petuniaplatform.net/), and the Snapdragon Home Page

(http://caliban.mpiz-koeln.mpg.de/~stueber/snapdragon/snapdragon.html) are building collaboration between researchers in different countries to share information, genetic materials, and research. Some of the flowers have become model plants for carrying out complex genetic, gene expression and developmental biology research.

9.6 Documentation

Germplasm documentation is an important activity in a genebank. It is the "nerve" that connects all the other activities together (Figure 5-4). Modern web-based databases of passport, characterization and evaluation, regeneration, seed quality, store inventory, distribution information and taxonomic information are weaved together so that users can search and see a wide range of information about an accession. A good example is the GRIN (Germplasm Resources Information Network) system (http://www.ars-grin.gov/npgs/) of the USDA/NPGS where its 25 decentralized active collection repositories can be accessed GRIN simultaneously via internet to input and manage their own data. Researchers with Internet access can log-on to search and request germplasm from any part of the world.

Digital images are beginning to be included as part of the information. In combination with image analysis technology and search algorithms, accessions from collections maintained in different countries may be compared via the Internet to streamline germplasm exchange and conservation. Other databases that have to be considered are: geographical and ethnological distribution (Szabo et al., 2003); center of origin and diversity; indigenous information; evolutionary relationships and taxonomy; species descriptors, characterization and evaluation methodologies; pest and disease evaluation methodologies; seed production, seed technology and seed treatment procedures of difficult species, cross compatibility, etc. Geographical information systems (GIS) and spatial analysis can then be performed with more layers (parameters) and thus better interpretation of a collection. Distribution gaps of a collection can be more accurately identified and mapped when planning for new germplasm exploration missions.

9.6.1 Distribution & Use

Germplasm distribution is a very important activity of a genebank because a collection not distributed and utilized is a museum collection. Lack of distribution defeats the purpose of conservation. A collection has to be value added to sustain use. Some of the ways to achieve this goal are to:

- Assemble germplasm collections with balanced genepool representations (1º, 2º and 3º);
- Provide full passport and characterization data;

- Provide evaluation data for important diseases and pests and important abiotic stresses including air pollution reaction;
- Organize field days on regeneration plots and elite germplasm accessions;
- Build communication linkage system between genebank and breeders e.g. user-friendly elite germplasm web-site, newsletter, workshops etc.;
- Provide clear policy on IPR/PBR on germplasm use and benefit sharing (see section later);
- Establish an efficient distribution system with phytosanitary certification and healthy germplasm to fulfill importing country quarantine laws and regulations.

10. RESEARCH

There is a lack of technical information in disciplines relating to germplasm conservation of herbaceous ornamental plants as described above. For example, the first book focusing specifically on flower seeds was published in 2005 (McDonald and Kwong, 2005). In-house research becomes critical to fill gaps of no information. Some of the research areas suggested are: evolution and taxonomy of targeted species; pest and disease evaluation methodologies; seed storage and cryopreservation techniques; seed production and seed technology of difficult species; formulation of crop descriptors, duplicate identification, DNA fingerprinting, DNA markers, and genetic maps; germplasm enhancement, tissue culture and gene transformation; databases with image-based search capability; and *in situ* conservation (crop-weed complexes and diversity creation and selection) through 'participatory breeding' methods incorporating the genebank, private sector and farmers.

At the OPGC, a checklist has been developed on key research areas and topics:

a. Evaluation of germplasm for commercialization
   - Trials – straight accessions
   - Trials – mixture of accessions and existing varieties
   - Trials – multi-location and photoperiodic responses
   - Photoperiodic and light intensity responses, and genetic and genomic study

b. Pest and disease evaluation
   - Identification of important pests and diseases of priority genera
   - Development of evaluation methodologies
   - Implementation of evaluation
   - Genetic and genomic study of tolerance and resistance

c. Abiotic and environmental stress evaluation

- Identification of important abiotic stresses including their physiological control system – cold, hot, flood, drought, heavy metals, air pollution, etc.
- Development of evaluation methodologies
- Implementation of evaluation
- Genetic and genomic study of tolerance

d. Other use evaluation

- Screening for pharmaceutical, nutraceutical, essential oil, pesticide use
- Biochemical and microbiological study
- Development of evaluation methodologies
- Implementation of evaluation
- Genetic and genomic study of identified traits

e. Conservation technology

- Seed production methods – pollination (bee and fly) and gene flow study, field and greenhouse and harvesting time and methods, seed shattering
- Seed post-harvest methods and seed technology – post-harvest maturation, threshing methods, drying regime and safe-storage methods
- Seed physiology – physiological maturity, seed vigor, germination, dormancy, vernalization, stratification, scarification, priming etc.
- Viability monitoring methods – seed testing, nondestructive methods
- Field/greenhouse genebank of clonal materials, storage of bulbs and tubers in cooler, *in vitro* conservation of meristem culture, cryopreservation and gene conservation
- Genetic and genomic study of seed traits

f. Germplasm use

- Transfer of useful genes to commercial genotypes for further breeding by the private sector
- Application of plant genomics and biotechnology

g. Taxonomic and crop evolution

- Taxonomic revision of individual species and genera
- Evolution of individual species and genera
- Identification of gaps in collection
- Sampling strategy in field exploration
- Individual crop descriptors

Germplasm enhancement

- Includes categories a-g above

Technology transfer

- Website
- Newsletter
- Tours, workshops

The purpose of the checklist is to provide a set of germplasm related research topics for the formulation of collaborative projects. It is arranged according to discipline and within a project the research will likely be multidisciplinary. The outcomes of the projects can then be adopted in the operation of OPGC.

11. INTERNATIONAL CONVENTIONS RELATING TO HERBACEOUS PLANT GERMPLASM CONSERVATION

In the last few decades, the United Nations has established various international conventions and agreements with significant impact in the protection and regulation on the sustainable exploitation of plant genetic resources in the world. These have included the Convention on Biological Diversity (CBD), Agenda 21, the Rio Declaration on Environment and Development; the Statement of principles for the Sustainable Management of Forests, the Convention on International Trade on Endangered Species of Fauna and Flora (CITES), and the International Plant Protection Convention (IPPC). Plant germplasm was shared and used as a common property of humankind before CBD. After CBD was implemented, plant germplasm became a country's natural resource. The use of a germplasm in another country now requires prior informed consents, collection permits, and specific material transfer agreements including benefit-sharing (CBD, 2002), and other mutual agreed terms of the owner national authority. The pre-CBD views on the open international exchange of germplasm have changed. The world is still learning and making a transition to adopt these changes.

11.1 Convention on Biological Diversity (CBD)

The objectives of CBD are "the conservation of biological diversity, the sustainable use of its components and the fair and equitable sharing of the benefits arising out of the utilization of genetic resources, including by appropriate access to genetic resources and by appropriate transfer of relevant technologies, taking into account all rights over those resources and to technologies, and by appropriate funding" (CBD, 2005). It is an important outcome of the 1992 United Nations Environment Programme's Earth Summit in Rio de Janeiro, Brazil where germplasm-rich countries were seeking a system to use their plant genetic resources in a sustainable manner and to share the benefits from their use. It can also be seen as the legacy of the 'North-South' debate on the ownership and use of plant germplasm and the recognition of traditional farmer contributions to the development of crop germplasm (Mooney, 1983). It is estimated that annually between $500 to $800 billion of genetic resources derived products are traded

globally (ten Kate and Laird, 1999). CBD is, thus, a response from germplasm-rich countries to the well-established intellectual property rights protection systems (IPRs) in place to protect developed germplasm.

CBD provides the procedures to recognize and implement Farmers' Rights as pronounced in the Agenda 21 and Resolution 3 of the Nairobi Final Act, to ensure a flow of benefits from the use of plant genetic resources to farmers and their communities now and in the future. Farmers' Rights are the "rights arising from the past, present and future contributions of farmers in conserving, improving, and making available plant genetic resources, particularly those in the centers of origin/diversity" in ITPGR Resolution 5/89 and Resolution 3/91 states that "Farmers' Rights will be implemented through international funding on plant genetic resources, which will support plant genetic conservation and utilization programs, particularly, but not exclusively, in the developing countries".

Currently, 188 countries have ratified CBD (CBD, 2005). However, various countries are in different stages of readiness in implementation from those where national laws are in place and under execution to those where laws have yet to be drafted and enacted. The 'Bonn Guidelines', CBD implementation guidelines, were published on sustainable access to genetic resources, and fair and equitable sharing of the benefits arising out of their utilization (CBD, 2002). Such voluntary guidelines were formulated for ease of use, practicality, flexibility and transparency, and the scope includes genetic resources, associated traditional knowledge, innovations and practices. The recommendations include the creation of a national focal point under a competent national authority to be responsible in CBD and to coordinate and solicit participation of all stakeholders. The steps cover prior informed consents which means that recipients have to disclose the intent use of the 'sourced' germplasm and then mutually agreed terms in benefit-sharing, capacity building, etc. Suggested elements that include material transfer agreements, monetary and non-monetary benefits. The monetary benefits can be access fees, fee per sample, up-front payment, milestone payment, license fees, salaries, research funding, joint ventures, joint ownership of IPR, etc. The non-monetary benefits can be collaboration in research and development programs and sharing results, product development, education and training, knowledge and technology transfer, strengthening capacities for technology transfer, institutional capacity-building, administrative capacity-building, access to scientific information, contribution to the economy, etc.

11.2 Intellectual Property Rights

Intellectual property rights (IPRs) are schemes to protect the investments of plant breeders/owners to breed new cultivars so as to promote further investments. All IPRs are implemented under a sovereign state legislation system and are, therefore, legally binding. However, in some crops such as seeded petunias,

begonias and impatiens where F_1 hybrid cultivars are solely used and are rapidly being replaced by new F_1s in a short market cycle, most breeders prefer to protect their inbred parental lines from theft rather than by a legislative mechanism. This approach is implemented in-house by controlling and securing the biological materials and is referred to as 'biological control'. It is a trade-secrets approach.

The International Union for the Protection of New Varieties of Plants (UPOV) Convention is the most commonly used IPR system to protect plant breeder's rights (PBR) in the world (UPOV, 1991). UPOV mission is "to provide and promote an effective system of plant variety protection, with the aim of encouraging the development of new varieties of plants, for the benefit of society". In 2005, 58 countries ratified the convention and enacted their own PBR Law based on regulations and requirements of UPOV to establish standardization globally. The details of the protection procedures and conditions are described in the official documentation (UPOV, 1991).

In the U.S.A., patent laws are commonly used. In fact, the U.S. Plant Patent (PP) system was the first to be used in the world since 1930 to protect novel cultivars of asexual crops other than tuber-propagated crops (The Townsend-Purnell Plant Patent Act of 1930, 2000). Seed- and tuber propagated crops were not protected until 1970 when the Plant Variety Protection (PVP) Law was enacted (PVPO, 2003). In recent years, utility patents (UP) have been increasingly used for the protection of specific genes and their expressions; isolation, construct and transformation techniques; and breeding with specified traits (USPTO, 2003). Consequently, unlike PVP and PP, a well-prepared UP can have multiple claims including naturally occurring genes. Trademarks (^{TM}) have been widely used for the marketing of ornamental plants. The claim is the rights to use a specific name and logo to brand and market a product. It has no claim on the genetics of the cultivar; different but related cultivars may be marketed under one trademark. However, the ability of the breeder to control the marketing of a cultivar allows ^{TM} to be successfully used to manage new cultivar releases. A complete discussion of plant protection throughout the globe is thoroughly discussed by Penny Aguirre's chapter in this monograph (see Chapter 4 herein).

11.3 Germplasm Conservation in Relation to IPR & CBD

The geographical distribution of a plant species and crop germplasm in its natural habitats has no political boundary. No country is self-sufficient in plant genetic resources. In the pre-CBD era, germplasm was collected, shared between countries and used in recipient countries for breeding new cultivars with resultant large-scale exploitation, e.g. corn and soybean in the U.S., and *Hevea* rubber and oil palm in SE Asia. Many new and improved germplasm from national public breeding programs and international centers of Consultative Group for International

Agricultural Research (CGIAR) were distributed *gratis* to interested countries for production. In recent years, new and improved germplasm from national public sector breeding programs is licensed for production and protected using PBR/PVP and PP. The licensing provides a line of funding for public breeders to do additional breeding. In the private sector, when F_1 hybrid cultivars began to be adopted globally, seed companies mainly protected their cultivars as trade secrets by physically preventing their inbred lines from being used by others. In the last two decades, IPR has been increasingly used for all species of crop plants. In ornamental crops, vegetatively propagated cultivars (clonal materials) represent an important segment of the industry and are similarly protected.

In fact, PBR/PVP and PP actually aid and sustain plant germplasm conservation and use. First, IPR royalties provide investment returns for plant breeders to conduct additional germplasm exploration, conservation, and further breeding of useful genes from wild species and heirlooms into modern cultivars. Second, PBR/PVP and PP allow newly bred cultivars with the useful transferred genes to be immediately used for further breeding into even better cultivars. This means that germplasm donor countries, many of which are developing countries and do not have advanced breeding programs, can immediately use the new PBR/PP cultivars bred by germplasm recipient countries for further breeding. The PBR/PVP and PP schemes thus have merit and should be encouraged. In contrast, the recent on-going trend in publicly traded seed company mergers allows significant research investment in specific genes and their transfer to create new cultivars such as the *Bt* and herbicide resistant genes. To protect this investment and to ensure returns on the investment, UP are increasingly used to protect the genes, the novel processes involved from their identification to transfer and selection, and finally the end products, i.e. the cultivars. A single well prepared UP can have as many as 30 individual claims. Unlike PBR and PP, UP blocks the patented genes from being use by other breeders during the period of the protection. If the protected gene is from a plant it should identify a country of origin. In this case, even the country where the gene (germplasm) originated is not allowed to use the gene if the country is not a party of the claims. In the CBD era, if germplasm of a targeted species has a wide distribution across different countries and when a breeding company entered into an agreement only with one of the countries of origin to exploit the germplasm, this could mean excluding neighboring countries with the same germplasm of the species from using their own germplasm. This has created on-going debates between germplasm rich nations and those with strong breeding companies.

In the last decade, plant genetic exploration and conservation activities in many countries have been deferred due to the lack of national policies to accommodate CBD and IPR requirements. The centers of origin for many herbaceous ornamentals are in the endangered and threatened 'hot spots' of the WWF Global 200 terrestrial ecoregions (Figure 5-1) facing severe environmental damage from a variety of causes, including habitat loss due to urbanization, deforestation, changing land-use

practices, and the expansion of industrialized agriculture. Views on the friendly international exchange of germplasm have changed. This affects plant germplasm conservation and genetic erosion escalates. In 2003, the World Conservation Union reported a rapid increase in the number of threatened plants in Ecuador, Malaysia, Indonesia, Brazil and Sri Lanka (IUCN, 2003). A good *ex situ* plant germplasm program is costly to maintain and difficult to justify immediate returns. In many countries, genebank maintenance programs are often of lower national priority. Similarly, in the private sector, low priority is given to the long-term conservation of unknown germplasm. As a result, germplasm is deteriorating in many genebanks and required regeneration to prevent genebank genetic erosion (Bowers, 2002).

12. CURRENT DILEMMAS & DEVELOPMENTS

In this transitional period of CBD implementation, the planning of germplasm exploration and collection missions has to concur with many legal requirements of a targeted country (Williams, 2003). For species on CITES, additional permits have to be obtained (CITES, 1979). This has caused delays or even suspension of missions. Both public scientific institutions and private seed companies are experiencing the same difficulty.

Some private sector seed companies and plant nurseries have been proactive in establishing formal legal collaborations with establishments in germplasm 'rich' countries to use germplasm, e.g. the agreement between Ball Horticultural Company and the National Botanical Institute of South Africa, based at the world-famous Kirstenbosch Botanic Garden (Corr, 2003). Some companies establish businesses in a country in order to collect, evaluate, and select new local germplasm. Local businesses also offer plants for sale worldwide by mail order, e.g. Silverhill Seeds of South Africa, Chen Yi Nursery of China. The globalization of seed and plant businesses has created additional considerations in the formulation of national laws relating to CBD. For example, Silverhill Seeds requires a contract to be signed on seed to be used for breeding such that a royalty can be returned to S. Africa upon commercializing a new cultivar (Silverhill Seeds, 2004).

From a technical point of view, it is practical to identify a pure line or a clone using DNA markers, but for an unselected population with a mixture of genotypes it is difficult to ascertain a defined marker profile. In this case, once an accession has been exported from a country, it is difficult to re-track the progenies especially after several generations of hybridization to other germplasm. It will be even more difficult to determine whether an accession was, in fact, collected pre- or post-CBD. Detailed breeder records are required to track pollination and derived progenies. We do not have the technology to accomplish this type of complex genetic analysis, especially in minor species. Finally, the implementation of a CBD-related

agreement will depend on how fair is the agreement and how respectful are the concerned parties to faithfully abide to the agreement. However, no matter how many CBD agreements are in use, one cannot prevent unscrupulous parties from illegal collection and export.

The ITPGR is the first endeavor to create a common multilateral agreement to ensure the conservation of 64 food and feed crops and in harmony with CBD for sustainable agriculture and food security, (FAO, 2003). It covers approaches in germplasm exploration and conservation, measures to induce sustainable germplasm uses, recommendations on international cooperation, measures to uphold Farmers' Rights, sovereign rights of contracting states, a standard material transfer agreement, benefit-sharing, etc. A permanent global trust fund (US$260 million), the Global Crop Diversity Trust for crop diversity conservation has been created specifically for *ex situ* conservation of IT crops. It provides a model for consideration for other groups of plants. The International Seed Federation (ISF), the world federation of seed trade associations, stated its view on IPR, "…. a commercially available variety protected only by Breeder's Right and containing patented elements should remain freely available for further breeding" (ISF, 2003). As emphasized above, PBR and PP will promote plant germplasm collection and conservation. The seed industry, international agencies and scientific community are increasingly working together to seek a common solution to the two opposite forces of IPR and CBD (benefit sharing).

13. THE ORNAMENTAL PLANT GERMPLASM CENTER (OPGC)

The OPGC was the first specialized herbaceous ornamental plant genebank in the world with a mission "to conserve the world wealth of herbaceous ornamental plant diversity by systematically save, assess, and use it to bring happiness and health to humankind" (Tay et al., 2004). The goal is to build a leading herbaceous ornamental plant genebank that is currently lacking and a "center of excellence" in developing new techniques for conserving seed and clonally propagated germplasm. Strategic global networks and partnerships with the horticulture industry, universities, botanic gardens, arboreta, crop specific societies and associations, scientists, and individuals are and will be established to achieve this goal. This will enrich research and teaching in practical plant germplasm conservation and use in ornamental crops and to fill the need for international educational programs in herbaceous ornamental genebanks.

The primary OPGC function is service-oriented, i.e. the conservation and free exchange of germplasm, and its benefit to the world. Available, unique germplasm can be accessed which are essential for the improvement of present and future flower crops that are resistant to pests and diseases, require fewer economic inputs,

promote consumer-product appeal through expansion of crop diversity, and may exhibit biological activity valuable to pharmaceutical, nutraceutical and medical researchers. The OPGC's activities include collecting, documenting and conserving genetic variation present in ornamentals and their wild relatives; exchange of germplasm domestically and internationally to broaden the genetic base; identifying and evaluating useful genetic traits desired by the industry and consumers; providing germplasm to industry for developing improved ornamentals; developing genetic maps of desirable traits for transfer into ornamental plants; identifying methods for successful long term storage of ornamental germplasm as seed, tissue culture, and bulbs; etc.

The OPGC has a floor space of 550 m^2 with a 20-30,000-accession capacity medium-term seed storage room for safe-keeping of seed for 10-15 years and a 1,060 m^2 computerized greenhouse facility for year round seed regeneration and research work. The specialized flower seed research laboratory is equipped with a thermo-gradient germinator, two accelerated aging water-jacket incubators, four germinators, a custom-built robotic single-seed weight sorting machine, a Faxitron MX-20 digital image cabinet X-ray unit, a Toyo-Living Super Dry 02 Series drying cabinet, a STS-MACS four-channel aspirator, a KA-K gravity separator, a LA-H Laboratory Brush Machine, two single deck vibratory seed separators, three single column seed blowers, a Clipper Office Tester and Cleaner, a belt thresher, a seed scarifier, a STS-1B-30C Cabinet system dryer and a STS temperature and time controlled precision dryer. For clonal germplasm, OPGC has a tissue culture laboratory for *in vitro* collection, two cryopreservation tanks, and a 2.74 x 3.05 m bulb and tuber storage room operating at 8°C. This room is also used for seed stratification and plant vernalization.

As an NPGS, all accessions are duplicated in long-term storage as base collection at the U.S.D.A. National Center for Genetic Resources Preservation in Fort Collins, Colorado. All the activities are implemented in accordance with the NPGS policies including germplasm acquisition in recognition of CBD, CITES, IPPC and other international agreements, distribution with respect to national quarantine and import regulation. Seed samples are available for distribution to all public and private researchers. The OPGC uses the GRIN germplasm database system to manage its passport, characterization, evaluation, store inventory and distribution information. OPGC has two curator teams working on seed and *in vitro* collections. It receives recommendations from the U.S.D.A. Herbaceous Ornamental Crop Germplasm Committee on germplasm and policy issues, e.g. the OPGC priority genera list. However, the diverse genera and species handled by OPGC requires that Technical Working Groups (TWGs) be established with experts on a crop. A TWG provides advises and recommends germplasm gaps, taxonomic treatments, seed regeneration methodologies, descriptor formulations, conservation methodologies, etc. At this point in its development, OPGC is focusing on the

assembly of genetically diverse crops. The needed research to improve OPGC operational efficiency is carried out with graduate students in collaboration with universities.

14. CURRENT GLOBAL STATUS & FUTURE DEVELOPMENT IN CONSERVATION

Globally, plant germplasm conservation effort continues to focus on food and industrial crops. FAO and IPGRI have no mandate in ornamental plant germplasm conservation. The establishment of the targeted US$260 million Global Crop Diversity Trust is earmarked for ITPGR crops (IPGRI, 2004). The few national genebanks that conserve some herbaceous ornamental plants in their collections include the Kew Royal Botanic Gardens genebank (Kew), the Institute of Plant Genetics and Crop Plant Research (IPK) genebank (Gatersleben), the Ministry of Agriculture, Forestry and Fisheries Genebank (Japan) and OPGC.

The US$50 billion (w) per annum global floriculture industry has no specialized genebank network in the world as that for food crops. The establishment of OPGC serves to fill this gap. In three and half years, OPGC has assembled 2,710 accessions plus another 270 accessions collected in their natural habitats in Ohio, U.S.A. Until the founding of the OPGC, herbaceous germplasm collections were/are maintained as breeder collections by public and private sector flower breeders on crops that they are breeding; as private collections by plant collectors, members of crop specific societies and specialized plant nurseries; or as collections in botanical gardens and arboreta. In these cases, there is limited information on how much and what genetic diversity has been collected and conserved in each species. Collected germplasm that did not possess any potential traits or of immediate uses were either discarded or left to deteriorate because of lack of proper conservation program.

Global networking and collaboration are urgently needed to create awareness on the value of *ex situ* conservation as an essential part of the present *in situ* conservation activities in nature preserves. Research can be applied from food crop genetic resources conservation programs. Public appeals and political supports have to be built and cooperative efforts should be explored to promote collaboration with the 1,500 national food crop genebanks. At the same time, a basic seed storage facility that can be economically built at key nature preserve laboratories and botanic gardens has to be experimented. Otherwise, domestic refrigerators and freezers are equally effective and proven for storing seeds (IBPGR, 2004). Close collaborations between *in situ* groups and botanic gardens, public research institutions, the floriculture industry, universities and researchers in other disciplinary areas such as pharmacy and public health have to be fostered to take on this enormous task. The timing is appropriate for this to happen. The creation of the

Botanic Gardens Conservation International (BGCI) with the mission "to build and maintain a world network of Botanic Gardens for plant conservation" and "to educate and promote conservation awareness and sustainability by providing technical guidance, data and support for Botanic Gardens worldwide" manifested the commitment in organized conservation of ornamental plants (BGCI, 2004). A worldwide Botanic Gardens Conservation Strategy for plant conservation has been adopted to promote exchange and sharing of information and experience among its over 500 member institutions in 112 countries. The BGCI International Agenda for Botanic Gardens in Conservation is the first global policy framework for the conservation ornamental plants (Wyse Jackson and Sutherland, 2000). Through a Memorandum of Understanding with the Convention on Biological Diversity (CBD), BGCI aligned its implementation policies and procedures to CBD requirements in international germplasm collecting, transfer, conservation, and use (see CBD, 2005).

Based on this framework, regional and national networks of botanic gardens have been organized to conserve plant germplasm, and regional and national plant collections of specific species/genera have been established. For example, the N. American Plant Collections Consortium (NAPCC) of the American Association of Botanic Gardens and Arboreta has accredited 28 collections of specific plant groups in 22 participating gardens and arboreta (AABGA, 2005). Three of the collections include herbaceous *Hosta*, *Trillium,* and *Asarum*. Similarly, in the United Kingdom, the National Council for the Conservation of Plants and Gardens manages 651 collections of both herbaceous and woody plants (NCCPG, 2005). In Australia, the Ornamental Plant Conservation Association of Australia aims to discover, identify and propagate plants once available in the horticultural trade are lost or hidden, unknown, in old gardens and to establish and preserve specific plant collections for future use (OPCAA, 2005). All these regional and national collections are maintained as living collections. The next step is to build a permanent base collection in long-term safe storage in a genebank, e.g. NPGS is developing a policy on associate collections essentially to set up a system to connect with the NAPCC collections so that a collection will serve both as NAPCC and NPGS collection. With this concept in place, a coordinated global network in the conservation of ornamental plants is coming into reality.

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Chapter 6

PREVENTION OF INVASIVENESS IN FLORICULTURAL CROPS

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Abstract: The greatest quantity of invasive crops arises from the floriculture sector of the horticulture industry. While some floriculture invasives are 'old' crops, e.g. purple loosestrife (*Lythrum salicaria*), a higher frequency are 'new' crops. This is due to the sheer number of new crops, as well as the vast quantities of cultivars and product series distributed to the floriculture global economy. Invasive species thus constitute a new and major challenge to the flower industry in the 21st century. If deliberate efforts are not taken by all parties in the distribution channel, particularly public and private flower breeding programs, restrictive legislation by countries across the globe may severely curtail the ability to collect and import new or 'exotic' germplasm for continued crop development, domestication, and distribution. The origination of invasive ornamentals is examined with a critical analysis of the floriculture distribution channel. Important factors, such as Monitoring and Control are and Economic Solutions are provided. A variety of solutions, encompassing each party in the distribution channel, are proposed to create a 'chain of non-invasiveness'. Flower breeders can implement many important plant traits in both old and new crops, prior to product release, using a new 'non-invasive crop ideotype'. Future research and education is required at all levels of the distribution channel before the continued introduction of invasive floriculture crops can be curtailed or potentially prevented.

Key words: Branding, chain of non-invasiveness, marketing, non-invasive crop ideotype, plant traits, sterility.

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1. INTRODUCTION

Horticulture is the sector of modern agriculture responsible for domesticating and commercializing exotic or native ornamental/edible landscape and indoor plants, many of which have the greatest risk of becoming invasive (Anderson, 2004a; Reichard, 1997). Within horticulture, the nursery and floriculture commodity groups have the highest number of new crop introductions. Over 60,000 plant species and varieties are available from nurseries and seed producers in North America alone (Isaacson, 1996); even more are commercially available worldwide. More than 100 new crops (primarily herbaceous) have been domesticated and introduced within the past decade and this rate is unlikely to decline (Anderson, 2001). The recent resurgence in the popularity of herbaceous perennials—a 12% increase in 2001 (compared with 2000) to US$448 million (w) in sales within the United States (USDA, 2002)—has renewed interest in new and old ornamental crops, particularly those with heat/drought tolerance and continuous flowering (Nau, 1996). Flower or nursery, seed & vegetative breeder/producer companies are scouring the globe for new taxa to domesticate and introduce. As a function of a competitive market, the floriculture industry thrives on novelty products with proven performance over a wide range of environments. New crops that were not in cultivation 20 years ago (although they may have been in cultivation a century or more earlier; Hottes, 1937) include a wide range of vegetatively propagated annual bedding plants (*Diascia, Dimorphotheca, Gaura, Scaveola, Sutera*). Innovative products continue to expand the use and market value of existing crops (Anderson, 2004a).

Horticulture trade is a global phenomenon throughout the distribution channel. The worldwide market and international competition must be kept in sharp focus for any company, product, or market to succeed. Failure to innovate and adapt as the market changes can have devastating results. This has been the historical precedent in the cut flower market, where growers in Central and South America capitalized on their climate to maximize production and flooded the United States market with less expensive, quality products which, in turn, caused many commercial cut flower greenhouse growers to go bankrupt (Widmer, 1997). Major national or international, horticulture brands or trademarks (e.g. Proven Winners®, Flower Fields®, Blooms of Bressingham®, Wave™ petunias, Flower Carpet®, My Favorite™) result in a continuous supply of products, most of which were developed elsewhere. Twenty or thirty years ago, no one in the industry would have conceived of selling non-hardy, tropical plants for summer use in northern temperate climates or that the internet/*e*-commerce would enable anyone, anywhere to purchase materials on the global market.

The horticulture market is experiencing a homogenization of products worldwide, with increasingly smaller fractions of products available for location-specific needs (Anderson, 2004a). Our modern industry is characterized by quickly

moving large quantities of products everywhere. Live plant imports into the European Union (EU) come from a wide range of non-EU countries, e.g. Israel, Thailand, Kenya, or South Africa. A similar trend is occurring in North America where potted flowering plants in soilless media are shipped into the U.S. from Canada. A few years ago this was not routine commerce, since the U.S. Department of Agriculture (USDA) banned importation of living plants growing in soil. The advent of tissue culture and a widespread increase in the number of vegetatively propagated herbaceous crops have resulted in most propagules being produced offshore in more favorable climates with lower-cost wages. This has raised the bar to ensure that stock plants are certified for the crop's important diseases. Containerized shipping and airfreight play an important role in moving hard good products and live plants from overseas. Importation of bonsai into the United States has increased from 600 to 55,000 specimens from 1995 to 1998, respectively (van Dreische, 2002).

A global economy and the continuous shipment of plant material around the world may have significant, undesirable side effects. Hardgoods or plants may contain unwanted hitchhikers, e.g. shipments of bamboo stakes received in St. Paul, MN (U.S.A.) from China also contained bamboo beetles (http://www.mda.state.mn.us/invasives/bamboobeetle.pdf) and aquatic plant shipments carried federal or state noxious weeds or exotic pests (Maki and Galatowitsch, 2004). The American Nursery & Landscape Association recently reported that the USDA will levy fines (up to US$250,000) for selling noxious aquatic weeds, such as water hyacinth (*Eichhornia azurea*) or pickerel weed (*Monochoria hastata*) (ANLA, 2003). The World Trade Organization projects a continued increase in ecological globalization due to the dramatic increase in trade volume and consumer-oriented policies, which have taken precedence over ecosystem protection (wto.org). Such policies assume that the risk of any adverse affect, such as the introduction of an invasive species, is low and controllable on a case-by-case basis. Can we deal with these problems without seriously jeopardizing the market? Shipments of small amounts of invasive species, if immediately contained and controlled, are less problematic than those that arrive at our doorstep unannounced and undetectable until they are a problem and it is too late. Then, a shared taxpayer burden on control ensues until adequate or complete control has been realized.

Figure 6-1. Purple loosestrife, *Lythrum salicaria*, is a horticultural noxious weed and invasive species throughout most of the United States and Canada. Photo credit: Luke Skinner, Minnesota Department of Natural Resources.

2. TERMINOLOGY

Weeds are defined as undesirable plants in agricultural lands, a legal term with land management implications (U.S. Congress, Office of Technology Assessment, 1993). They often severely impact natural ecosystems (Ruessink et al., 1995), by replacing diverse communities with dense monotypic stands, displacing less competitive indigenous species and endangered or threatened species (Wilcove et al., 1998). Weed eradication and control in both agricultural fields and protected lands is costly and, frequently, ineffective (Pimentel et al., 2000). Many countries and their associated provinces or states have created listings of prohibited, restricted noxious weeds. Counties or local municipalities may also have their own secondary noxious weed lists. Most prohibited noxious weed listings include exotic or native parasitic, terrestrial plants, most of which are agricultural, rather than horticultural, weeds. Several exceptions to this include purple loosestrife, *Lythrum salicaria* (Figure 6-1), glossy (*Rhamnus frangula*) and European or common buckthorn (*R. cathartica*), Jerusalem artichoke (*Helianthus tuberosus*), tall buttercup (*Ranunculus acris*), Bracken fern (*Pteridium aquilinum*), oxeye daisy (*Chrysanthemum leucanthemum*), kochia (*Kochia scoparia*), common milkweed (*Asclepias syriaca*), wild sunflower (*Helianthus annuus*), and absinthe wormwood (*Artemisia absinthium*) (Anderson, 2004b). Invasive plant species may become fully established or 'naturalized' in new areas away from horticultural cultivation, establish self-sustaining populations, spread outside their native range, and have a detrimental effect on the new environment (Davis and Thompson, 2000). Many invasive species are also noxious weeds. Invasive plant species can also cause changes in community dynamics by modifying fires regimes, erosion, water levels, and the availability of food and shelter for native fauna (D'Antonio and Vitousek 1992). Eradication of invasive species in managed lands is expensive and often ineffective (Pimentel, et al., 2000). Example ornamental invasives (inherently invasive or that have become so through adaptation or hybridization) include some maples (*Acer ginnala, A. platanoides, A. pseudoplatanus, A.* negundo—only in cultivated settings), Mexican mint *(Agastache rupestris),* Hottentot fig

(Carprobrotus edulis), oxeye daisy (*Chrysanthemum leucanthemum*), cleome *(Cleome hassleriana),* pampas grass *(Cortaderia selloana),* Scotch broom *(Cytisus scoparius)*, jimson weed (*Datura stramonium*), teasel *(Dipsacus laciniatus*), sunflower *(Helianthus annuus), g*iant hogweed *(Heracleum mantegazzianum),* kochia (*Kochia scoparia*), perennial sweet pea (*Lathyrus latifolius*), honeysuckle (*Lonicera maackii, L. morrowii, L. tatarica, L. japonica*), purple loosestrife *(Lythrum salicaria),* fountain grasses (*Pennisetum purpureum, P. polystachyon, P. pedicellatum, P. setaceum*), buckthorn (*Rhamnus cathartica, R. frangula),* roses (*Rosa multiflora*), tamarix (*Tamarix ramosissima, T. chinensis, T. parviflora*), *Verbena bonariensis* (Amrine and Stasny, 1993, Anderson and Ascher 1993, 1994, Archibold, et al., 1997, Deering and Vankat, 1999, Galatowitsch et al., 1999, Pysek et al., 1995, Randall and Marinelli 1996, Sheppard, et al., 2002, Westbrooks 1998).

Most horticultural crops are not native to the many countries, provinces, or states where global floriculture commerce operates and would be termed exotic species in those areas (Anderson, 2004b). Likewise, most, but not all, invasives or weeds are exotic. The majority (82%) of woody invasive plants in the United States (n=235 species) were introduced for landscape purposes (Reichard and White, 2001). Likewise, more than 75% of all exotic, invasive aquatic plants in northeastern United States originally escaped from cultivation (Les, 2002). A common misconception is that all exotics are "bad" and natives are "good" in the sense of their invasive or weedy potential. The difficulty arises from defining "native", which is a political boundary definition for a state, province, or country. Early American botanists often listed naturalized and invasive species as 'native' at the time they conducted plant surveys, e.g. Oxeye daisy, *Chrysanthemum leucanthemum* (Gray, 1857). Later editions of the flora revised notations to read "an abundant weed", native to Europe (Gray, 1887).

Weeds and invasives must be distinguished from 'aggressives'—plants in cultivation which quickly grow to fill an area; which are kept in check by surrounding physical barriers, e.g. sidewalks, walls, etc. (Anderson, 2001; 2004b). In most instances, aggressive crops are inherently neither weedy nor invasive. Example ornamental aggressive species include ajuga (*Ajuga genegensis, A. reptans*), bishop's cap (*Aegopodium podagraria*) (Figure 6-2), Japanese bamboo (*Fallopia japonica; =Polygonatum cuspidatum*), and creeping Jenny (*Lysimachia nummularia*). Since these spread predominantly by vegetative means, rather than seed, they remain confined to small areas even after decades on abandoned farms. This is not to say, however, that aggressive horticultural crops cannot be implicated in invasiveness. Given the correct environmental conditions for a self-incompatible species, the self incompatibility system could break down and allow for the creation of hybrid seedlings, which could backcross to the non-invasive parent and become invasive. Anderson (unpublished data, 2000-2004) observed an aggressive clone of variegated *Aegopodium podagraria* to set seed. Subsequent non-variegated seedlings established outside of the physical barriers that kept the variegated clone

Figure 6-2. Aggressive crops such as bishop's cap or snow-on-the-mountain (*Aegopodium podagraria*) spread vegetatively and completely fill in an area until they reach physical barriers.

in place. This population rapidly expanded via seed to cover ~1 hectare within the course of two years.

Most cultivated horticultural species lack the ability to compete with native taxa and their continued existence in the landscape depends on remaining in cultivated sites (Anderson, 2004b). However, exceptional exotic, ornamental introductions occasionally become invasive and severely impact natural ecosystems (Anderson and Ascher, 1993). Cohen and Carlton (1998) noted a significant increase in the rate of invasion by exotics in the San Francisco Bay (California, U.S.A.) region due, in part, to horticulture. On average, one exotic species established every 55 weeks from 1851-1960, whereas the rate of establishment had increased to one species every 14 weeks from 1961 to 1995. The introduction of such taxa (derived from either conventional breeding or molecular biology) with an inherent tendency to be invasive or a proclivity to interbreed with related, sympatric native flora, weeds, or crops is a common concern (Anderson and Ascher, 1994).

Many of the domesticated horticultural crops have had sufficient time (several hundred or thousands of years) to develop into noxious weeds or invasive species (Anderson, 2004b). However, most important horticultural crops have never been reported to develop into noxious weeds, despite worldwide cultivation for centuries or millennia (chrysanthemum--*Dendranthema x grandiflora,* azaleas--*Rhododendron spp.*, viburnum--*Viburnum spp.*) or decades (impatiens--*Impatiens wallerana,* petunia--*Petunia hybrida*). A few floricultural crops have become weedy or invasive due to their importation and cultivation in non-native geographical regions (where insect predation does not keep them in check), as well as hybridization with related species: Hottentot fig (*Carpobrotus edulis*), pampas grass (*Cortaderia jubata, C. selloana*), Japanese bamboo (*Fallopia japonica*), lantana (*Lantana camara*), butter and eggs (*Linaria vulgaris*), purple loosestrife (*Lythrum salicaria*), fountain grass (*Pennisetum purpureum, P. polystachyon, P. pedicellatum*), and wild rose (*Rosa multiflora*) (Amrine and Stasny, 1993; Anderson

and Ascher, 1993, 1994; Galatowitsch, et al., 1999; Pysek, et al., 1995; Randall and Marinelli, 1996; Westbrooks, 1998). Several new crops commercialized for the worldwide market (*Agastache rugosa, Anagallis arvensis, Asclepias incarnata*, *Cotula spp.*, *Cymbalaria muralis, Gaura lindheimeri*, *and Verbena bonariensis*) are already becoming invasive (Anderson 2001, Carr and Crisci 1988, Goldblatt and Raven 1997, Hickman 1993, Randall and Marinelli 1996, Raven and Gregory 1972a, Westbrooks 1998). The introduction of ornamentals with the potential to be invasive is a common concern (Ellstrand and Hoffman, 1990; Anderson and Ascher, 1994; Clark, 1999; Mooney and Cleland, 2001; Pooler, et al., 2002).

Since most horticultural crops have not become weedy or invasive, why be concerned? Surely, the few horticultural crops that have become invasive are the exception to the rule. There's no need to change the way the industry does business, is there? These are important questions for the new millennium (Anderson, 2004b). However, the few "poster-child" horticultural invasive crops have caused the enactment of restrictive legislation and negative publicity for the industry. The issue is not going to disappear. Customers may ask whether a product they purchase will become invasive or return should it appear to become so. Given the diverse nature of the horticulture industry and the large number of crops and cultivars in cultivation, it's a big issue that is not easily resolved. What can be done to proactively prevent future horticultural, invasive species from being released? In this chapter, we'll examine the major issues and possible solutions to minimizing or eliminating the invasive nature of some horticultural crops.

3. WHERE DO INVASIVE SPECIES ORIGINATE?

The horticulture industry encompasses a wide variety of plant products, hard-goods, and services (Anderson, 2004c). Every annual industry tradeshow, pack trial, fair, and exhibition around the globe carries a wide diversity of vendors and products. An examination of the international distribution system, from an operational standpoint, as well as which player(s) are the most likely sources of invasive species demonstrates the complexity of determining which party is the source of an invasive species (Figure 6-3).

Domestication of new crops has recently become a major emphasis within the floriculture industry (Janick 1999, Janick and Simon 1993). While other horticulture sectors are also involved in new crop development, the floriculture market is the best example sector to illustrate the potential flow of invasive *or potentially invasive* crops through the trade. More than 100 new crop species have been introduced in the past decade (Anderson, 2001) and this rate is unlikely to decline (Reichard and Hamilton, 1997). The sources of these new crops are amateur/professional plant explorers or breeding companies (flower breeders), which continuously scour the globe for new taxa to introduce and/or domesticate

(Figure 6-3). Before embarking on plant collection trips, published flora, records from public herbaria, and/or consultations with native plant experts provide information for the country being visited and the potential collection sites (Anderson, 2004b, c). In addition, plant explorers may choose to examine manuals of weedy plants for each continent and select any with ornamental traits for possible introduction (Anderson, 2004b, c). Weedy or invasive species are easily adaptable to cultivation and require little domestication, since traits conferring invasiveness may be similar to those that enable domestication as horticultural crops (Table 6-1). Abundant fruit production, high germination rates, intrinsic growth rate, and tolerance to a wide range of environmental conditions are traits associated with invasive potential (Elton 1958, Dozier 1999). Initial introduction size may also contribute to invasive ability—the more widespread an invasive product is marketed, the higher the likelihood of facilitating establishment (Reichard and Hamilton, 1997; Reichard and White, 2001).

Plant explorers are people well acquainted with the market and have an 'eye' for wild species with ornamental value that can fit into existing product classes (Anderson, 2004c). Several collection trips—at considerable cost—may also be needed before the desired plant material is found and at the proper physiological state for collection (mature seed or vegetative propagules). Funding for collection trips include commercial firms (breeder, producer, propagation, grower or retailer companies), granting agencies, the Germplasm Banks (e.g. the United States Dept. of Agric. Germplasm Resources Information Network; http://www.ars-grin.gov/) or plant resource centers (USDA Ornamental Plant Germplasm Center, Columbus, Ohio; http://www.ars-grin.gov/npgs/rephomepgs.html), botanical gardens or arboreta, plant breeder consortiums, amateur societies, and private individuals (Figure 6-3).

In some instances, plants collected from the wild may not require product development or enhancement (breeding, selection, trialing, propagation) and become direct-selected products, provided they can be propagated and perform as well as comparisons in evaluative trials (Figure 6-3). Plant patents cannot protect products collected directly from the wild. These are rare, but profitable exceptions, whereas most collected specimens require breeding and domestication to become viable products. Additionally, these may also be used as parents to cross with related crop species, providing new trait(s) with perceived economic value (disease/insect resistance, drought tolerance).

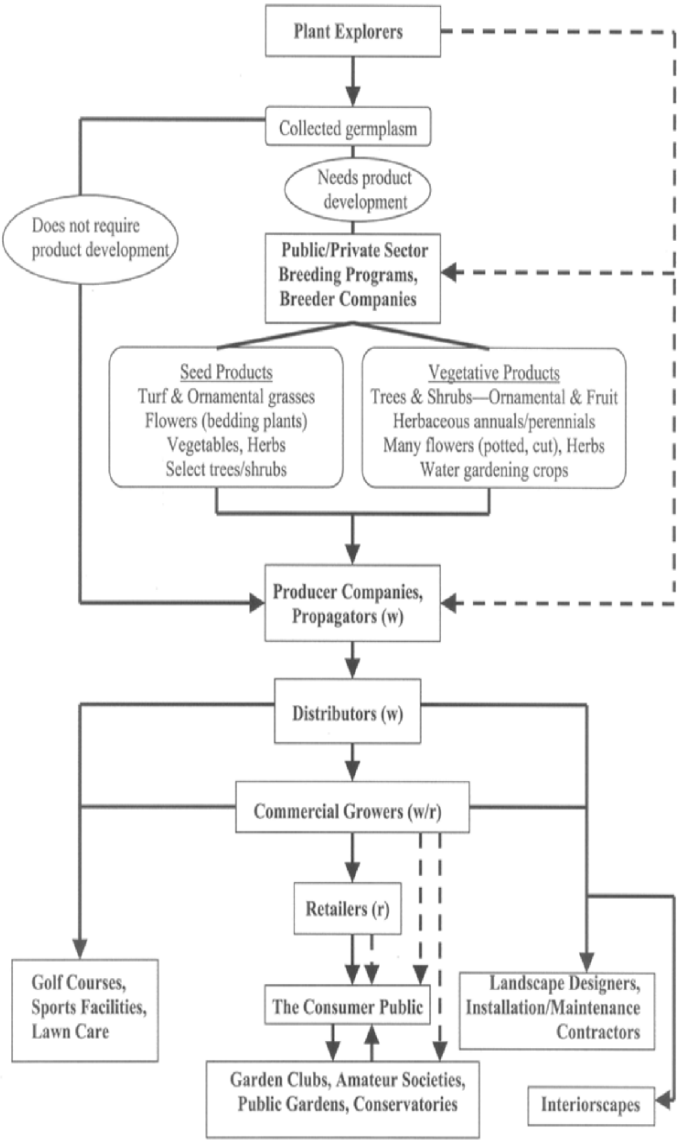

Figure 6-3. The international distribution system of horticultural plant products (wholesale, retail), including classical (solid arrows) and *e*-commerce/web-based (dashed arrows) marketing conduits (Anderson, 2004b; Anderson, et al., 2005a). Potential sources or dispersal agents of invasive species may distribute downstream in the system beginning with the plant explorers.

Plant exploration provides cultural and commercial relationships, which continue the flow of future new plant material. While plant exploration seems

relatively straightforward, but venturesome, plant collectors must adhere to the collection, domestication, and distribution requirements of the source country and the Convention on Biological Diversity (CBD; http://www.biodiv.org/)—both of which may severely restrict the flow of plants out of the country of origin. While some countries, such as the U.S., are not signatories on the CBD, most other countries are and require adherence to CBD standards. Many countries, e.g. Brazil (a primary source of wild *Petunia*), have already closed their borders to restrict the flow of wild germplasm into commercial products by foreign companies. Genetic resources are viewed as potential revenue streams, particularly if an endemic plant species has valuable medicinal properties (cancer cures). Fortunately, some private companies have responded with legal agreements with the respective governments and sources of plant germplasm. An excellent example of this new venture is the agreement signed between Ball Horticultural Company (W. Chicago, IL, U.S.A.), which owns distributor/breeder companies, and the National Botanical Institute (NBI) under the authority of the South African government (http://www.nbi.ac.za/research/ball.htm). This Ball/NBI agreement provides remuneration (royalties) to the S. African people when products from collection trips have been commercialized. Further elaboration on plant collecting and adherence to international treaties are presented by David Tay in Chapter 5 (entitled 'Herbaceous ornamental plant germplasm collection and use') of this monograph.

Many choose to avoid negotiating the CBD requirements directly and opt for easier access to germplasm. Computers and *e*-commerce have made accessibility to plant germplasm worldwide relatively easy and may circumvent the need for direct plant exploration (Figure 6-3). Commercial interests (breeder or producer / propagator companies) or private individuals can search available plant collection lists from plant explorers (e.g. Siberia & the Russian far East, http://www.meconopsis.st/Berkutenko.html), packet seed companies (Seed Hunt, http://seedhunt.com/), seed lists of botanical gardens (annual *Index Seminum*, cf. Nacionālais Botāņiskais Dārzs, 2004), or amateur plant societies (North American Rock Garden Society, 2004) to obtain propagules of plant material collected directly in the wild or open-pollinated seed from collected plants. Germplasm obtained in this manner is still subject to CBD adherence, as per the country of origin.

Table 6-1. Example traits in ornamental horticulture or food crops, which may be correlated with invasiveness, the respective alterations (with crop examples), made by horticultural plant breeders during crop domestication (Anderson, et al., 2005b).

Trait	Breeding alteration	Crop example(s)	Citation
Life history	Annualized to flower in first year from seed	*Verbascum x hybridum* 'Southern Charm'	PanAmerican Seed Co., 1999
Winter hardiness	Extend hardiness zones (Z) for northern growers	*Alstroemeria x hybrida* 'Jazze' series (Z5), *Dendranthema x grandiflora* (Z3-Z4), *Monarda didyma* (Z5)	Chicago Botanic Garden, 1998; PanAmerican Seed Co., 1999; Widmer, 1958
Light preference	Selected for sun-tolerance in shade-loving taxa	*Hosta plantaginea* 'Gold Standard'	Still, 1994
Seed dormancy	Removal of stratification requirements	*Aquilegia x hybrida* 'Songbird' series	Ball Seed Co., 1999-2000; Beattie and German, 1985
Seed size	Increase seed size and uniformity	*Petunia x hybrida*	Craig and Laughner, 1985; Weddle, 1965
Reproductive barriers	Removal of self incompatibility to create inbred parents	*Nemesia strumosa, Petunia x hybrida*	Flaschenriem and Ascher, 1979; Robacker and Ascher, 1982
	Apomixis superseding sexual reproduction	*Alchemilla vulgaris, Ranunculus auricomus*	Briggs and Walters, 1997
Flowering season	Extend normal flowering period by changing flower bud initiation / development	*Dendranthema x grandiflora* (from short-day to day-neutral plant)	Anderson, 1991
Seed:ovule ratios	Increase ratio to mimic annual species	*Lythrum salicaria*	Anderson and Ascher, 1993; Cutright, 1986
Ploidy	Create gigantic plants, using interspecific hybridization (allopolyploids created *via 2n* gametes)	*Alstroemeria x hybrida, Campanula persicifolia* 'Telham Beauty'	Crane and Lawrence, 1934; Ramanna, 1992
Genetic stability	Aneuploidy and mutagenesis used to create clonal families	*Lathyrus odoratus* 'Spencer' types	Crane and Lawrence, 1934
Genetic variation	Increase levels to widen the	*Impatiens hawkeri*	Arisumi, 1978; Arisumi and Cathey,

Trait	Breeding alteration	Crop example(s)	Citation
	germplasm base		1976; Lerch and Dudzinski, 1992
Branching	Increased to enhance flower number for commercial seed production and garden performance	*Antirrhinum majus* (bedding plant types)	Craig and Laughner, 1985; Goldsmith Seed Co., 1999
Disease tolerance / resistance	Create powdery mildew resistant or tolerant hybrids	*Monarda didyma* 'Marshall's Delight', 'Petite Delight', *Zinnia x hybrida* 'Profusion' series	Collicut, 1989; Collicut and Davidson, 1999; Sakata Seed Co., 1999-2000
Clonal perpetuation	Change rhizomatous species from vegetative to seed propagation	*Alstroemeria x hybrida* 'Jazze' series	PanAmerican Seed Co., 1999
	Change vegetatively-propagated (cuttings) crop to seed propagation	*Pelargonium x hortorum* 'Nittany Lion'	Craig, 1968; Craig and Laughner, 1985

Once new products have successfully gone through the plant exploration, breeding, and propagation sectors of the distribution channels, the marketing and sales forces of the distributors begin creating the demand among growers, retailers, landscape designers, turf specialists, and consumers (publicity and the press; Figure 6-3). Clearly, *if* an invasive species makes it into the distribution channel due to its superior performance as a horticultural crop then all parties (from the plant explorer to the retailer) aid in its spread. The more successful a new product is on the world market (number of units produced and sold), the more likely that multiple introduction sites may enhance the likelihood of its escape from cultivation as an invasive species. Thus, while one party may be initially responsible for the collection or introduction of an invasive species to the market, all subsequent components in the distribution channel are "aiding and abetting" in its dissemination. Further discussion of the many complex issues connected with this process, consult Anderson, et al. (2005a, b) and the special issue of the journal *Euphytica* entitled "Plant breeding and crop domestication as sources of new invasive species" published in 2005 (co-edited by N. Anderson and S. Galatowitsch; consult www.wkap.nl).

Early breeder or producer trials may indicate the proclivity of a new product towards invasiveness, but this may be an inaccurate assessment since not all possible environmental conditions worldwide are tested. Research has demonstrated that, in some instances, a species may not be invasive in its native habitat. Yet, when this species is introduced into a new country or environment it is "suddenly" invasive. Examples of horticultural woody shrubs, trees, grasses, and herbaceous plants that have become invasive when grown in a new environment include *Acacia* (native to

Australia; invasive in S. Africa; Musil, 1993), *Rhamnus cathartica* (native to Europe; invasive in Minnesota, U.S.A. and elsewhere; Archibold, et al., 1997), *Cortaderia jubata* (pampas grass, invasive in California, U.S.A.; Lambrinos, 2000), and *Dipsacus* (teasel, widespread invasive in the U.S.A.; Solecki and McKnight, 1993). Many detrimental effects have been documented with such invasive horticultural crops. For instance, *Acacia* in S. Africa are depleting the water supply at a faster rate than native species and a 'Working for Water' program has been instituted to remove these Australian species (http://www-dwaf.pwv.gov.za/wfw/). Likewise, *Cortaderia selloana* grows widely on hillsides in the coastal California mountain ranges. Its large crown diameter and evergreen nature cause accumulation of a large mass (weight), which causes mudslides during the winter rainy season (Figure 6-4).

Figure 6-4. Invasive horticultural crops such as *Cortaderia selloana* can cause significant loss to habitats, resulting in costly road repairs on Highway 1 of coastal California, U.S.A.

If a new horticultural crop or cultivar enters the market and is not invasive does that mean it is environmentally 'safe' for the life of the product and beyond? Not necessarily! The scientific evidence would suggest otherwise in a fraction of cases. For instance, purple loosestrife (*Lythrum salicaria*) was not an invasive horticultural crop when it was introduced into the North America in 1814 (Pursh, 1814). The initial major seed source was probably moist sand from tidal flats used as ship ballast in Europe, later emptied on American shores (Wilcox, 1989; Thompson, et al., 1987). The seeds were likely dispersed inland by air, water and animal transport. *L. salicaria* spread further as it was domesticated as an ornamental crop (Cutright, 1986; Woehler and Henderson, 1986). It was planted and grown widely throughout N. America by European immigrants, beekeepers, and horticulturists for more than a century. Then, in the 1930's—more than a century later, it 'suddenly' became an invasive species in Quebec pastures (Louis-Marie, 1944; Barabe, 1951; Woehler and Henderson 1986). Due to multiple dispersal mechanisms, *L. salicaria* had many separate, independent opportunities to form colonizing populations

(Stuckey 1980). No plant explorer, flower breeder, propagator or distributor could have postulated this change in 1814, the time of its introduction to the N. America, particularly since it was not an invasive species in its native habitat (throughout most of Eurasia). We now know that purple loosestrife is kept 'in check' by predatory insects in European populations, but these insects were not present in N. America (Nechols, et al., 1996). However, this is not the primary reason for its invasiveness in N. America, as it should have been instantaneously invasive once it was brought to the New World. That process took more than a century. What happened in the interim? It may have been accumulating the appropriate gene(s) conferring widespread adaptability in N. America by adapting (survival of the fittest) and/or hybridization with native *Lythrum* relatives (Anderson and Ascher, 2000; Galatowitsch, et al., 1999). Nonetheless, the eventual invasiveness of purple loosestrife would not have been identified nor predicted in 1814.

As one examines the distribution channels of horticultural products (Figure 6-3), those sectors at the beginning of the process might be perceived to shoulder a larger portion of the burden in preventing release of invasive species. However, all sectors downstream in the distribution channel have shared responsibility. One might wisely conclude that all sectors share responsibility, since all sectors loose when invasive crops with market value or the initial germplasm from plant collectors are removed from commerce. What can be done to preserve the integrity and long-term viability of all market sectors? There are no easy answers to resolving the dilemma of invasive species, but there are prudent business practices for each sector to follow until invasive species research can offer viable alternatives.

In commercial floriculture breeding programs, a wide variety of traits are selected for adaptation to cultivation (Table 6-1). Traits such as disease and pest resistance, drought/heat stress tolerance, high male/female fertility, lack of seed dormancy, rapid germination, high yield potential, short generation cycles, hybrid vigor or large plant sizes would be advantageous in the wild and could enable the evolution of cultivated horticultural crops into invasive weeds (Van Gaal et al., 1998). Additional selected traits would not be expected to have an adaptive advantage in the wild, e.g. non-shattering seed heads, reduced internode lengths (conferring dwarfism), variegated foliage (reduced growth rate and competitive ability), or extra petalage (double flowers) (Ellstrand and Hoffman 1990, Klinger et al., 1992, Raybould and Gray 1994).

Plant explorers, flower breeders and/or propagators implement selection procedures whereby the release of some invasives may be prevented. Generally, if a species is determined to be 'too aggressive' (it reseeds easily) with the potential to develop into an invasive crop or cultivar in breeder trials or subsequent comparison evaluations, most breeding companies will not introduce it. Those species, which become invasive after introduction, however, would be missed with this screening procedure. Additionally, if the environments in which the trials are not conducive for a plant to suddenly express its hidden, invasive nature it will only be a matter of

time before an environment is found for such expression. However, there are no objective methods to screen species for invasive potential that can be applied throughout the commercial decision-making process for new crop development and release. Such a screening program would be unique, allowing for *a priori* decisions to be made in the breeding/developmental process before releasing taxa that are inherently, or have the tendency to become, invasive.

Failure to implement evaluation procedures or create cultivars with reduced invasive potential will have devastating effects on the horticulture industry in the near future, should national or state restrictive legislation be implemented preventing the importation, domestication, and product development of exotic plant species. Plant breeders and molecular geneticists may implement selection against invasive potential or create sterile hybrid cultivars during the developmental phase. If this is done before a new cultivar or species is released, the environmental risks would be significantly minimized or completely eliminated. Until that time, however, shared responsibility must come from all sectors of the distribution chain—breeders, producers, distributors, retailers, landscape designers, educators—as all are part of the horticulture industry fabric.

4. MONITORING & CONTROL

Agriculture, forestry, and fisheries losses, as well as maintenance of open waterways due to invasive species in the United States, for example, cost an estimated US$138 billion per year. Restrictive legislation is currently the most powerful tool available for preventing the introduction of invasive species in the U.S. (by restricting weed contaminants in crop seed and ballast discharge from ships) or interstate commerce (preventing interstate trade between states with laws restricting invasives). This curtails the ability of the ornamental plant industry to either import or offer such plants for sale on the market (Anderson, 2004d).

At the Federal level, for example, the U.S. Dept. of Agriculture-Animal Plant Health Inspection Service / Plant Protection & Quarantine Office (USDA-APHIS/PPQ), Invasive Species and Pest Management (ISPM) has responsibility for preventing the introduction of endangered species, weeds, or organisms on the Bioterrorism List (http://www.aphis.usda.gov/). The amended Federal Noxious Weed Act of 1974 provided the authority for restricting the entry of agricultural "weeds" (currently >100 species) into the U.S. (U.S. Congress Office of Technology Assessment 1993). This act has not been entirely effective, since it has been applied primarily to agricultural pest plants, but excludes >700 "noxious" species. Additionally, the Act of 1974 only restricts the importation and movement of species shown to pose a significant risk. It does not address the evaluation of new species introductions that are weedy, invasive, or that might have a tendency to develop into noxious taxa. Example species that are horticultural crops or relatives

of crops on the USDA-APHIS/PPQ Regulated Plant Pest List, include *Rubus* (two species), aquatic or water-gardening plants (*Eichhornia azurea, Hydrilla verticillata, Sagittaria sagittifolia*), ornamental grasses (*Pennisetum*--four species), herbaceous plants (*Alternanthera sessilis, Commelina benghalensis, Opuntia aurantiaca*), and woody trees (*Melaleuca quinquenervia, Mimosa diplotricha, M. pigra var. pigra)* (http://www.aphis.usda.gov/). There are ~110 parasitic, terrestrial species on the Federal Noxious Weed List (Title 7, Sec. 360.200; http://plants.usda.gov/cgi_bin/topics.cgi?earl=noxious.cgi). Fortunately, however, the U.S. borders are less porous than in recent years with the USDA-APHIS/PPQ inspectors and dogs detecting and/or destroying plant material (seeds, plant parts--especially bulbs and fruit) from citizens who do not possess importation permits. Similar scenarios are present in other countries worldwide.

The U.S. National Invasive Species Council (NISC) coordinates oversight of the Federal Government's activities on invasive species and was established on February 3, 1999, by President Clinton's Executive Order No. 13112. Council members include the Secretaries of the Interior, Agriculture, Commerce, State, Defense, Treasury, Transportation, Health and Human Services, as well as the Administrators of the Environmental Protection Agency and the U.S. Agency for International Development. The Council co-chairs are the Secretaries of the Interior, Agriculture, and Commerce. A National Invasive Species Management Plan has been created and task teams and subcommittees are implementing action items of the plan (http://www.invasivespecies.gov/council/mpfinal.pdf). NISC also works with the Invasive Species Advisory Committee (ISAC), which represents stakeholders and advises the federal government on invasive species.

Control of invasive species and crops within states/province and interstate commerce between states/provinces is dictated by state legislation, depending on the country. For example, in the U.S., each state or province may create a listing of crops, in addition to the U.S. Federal Noxious Weed Listing, which prohibits the sale, distribution, or cultivation of invasive species. This makes distribution of invasive species with restrictions in some, but not all, of the 50 U.S. states and territories challenging. Distributors must list the states where an invasive crop is illegal. For instance, many, but not all, states have listed purple loosestrife (*Lythrum salicaria*) as a noxious weed. Propagators would be prohibited from shipping this plant from West Virginia, U.S.A. (where it is not illegal) to any other state (where it is illegal). No agency checks travelers arriving into the state who may have obtained illegal plants in another state, even though it is illegal to bring them here. For instance, it would be possible to purchase seedlings or cuttings of purple loosestrife in West Virginia and bring them to the state by any means of transportation without detection (Figure 6-5). Likewise, purchasing material on the web can also be accomplished. National borders are porous at this level.

Figure 6-5. Photograph of purple loosestrife (*Lythrum salicaria*) seedlings available for sale at the Charlestown, West Virginia (U.S.A.) farmer's market in May, 2004. While illegal, no other U.S. state agency would prevent their purchase and importation by private citizens into each respective U.S. state or territory.

States or provinces within each country may also have their own regulatory programs to control the distribution of invasive flower crops. For instance, at the U.S. state level, the Minnesota Department of Natural Resources (DNR), Harmful Exotic Species Program prevents the introduction and/or spread of harmful exotic species in Minnesota (http://www.dnr.state.mn.us/ecological_services/exotics.html), as well as reducing their impact to the state. The Minnesota Department of Agriculture (MDA), Plant Protection Program (http://www.mda.state.mn.us/) staff work with invasive species and survey the state for early detection of invasive species. This method of prevention is a cost-effective means of early detection. The state PPQ program also has goals of educating the public on invasive species, evaluating the risks of introducing invasive species, identify/monitor/exclude invasive species pathways, developing a state-wide invasive species location database with the DNR, and evaluating the impact of invasives on the state economy and human health. Plants currently on the state's prohibited noxious weed list (injurious to public health, roads, crops, livestock, and other property) include those on the Federal Noxious Weed List plus 11 species (MN Rules 1505.0730). Minnesota Restricted Noxious Weeds (MN Rule 1505.0732) include glossy and European buckthorn (*Rhamnus cathartica, R. frangula*). The state of Minnesota Secondary Noxious weeds (MN Rule 1505.0740), may be placed on a county noxious weed list, and include 51 species, 14 of which are native to Minnesota. The Minnesota Invasive Species Advisory Council (MISAC), which includes DNR, MDA, industry, and academic members, has developed lists of the invasive species most threatening to Minnesota (http://www.mda.state.mn.us/invasives/default.htm) and a potential checklist for use in assessing whether new crops may become risks. Other examples, too numerous to delineate, are present in other states and provinces throughout many world countries.

Counties or parishes within a state or province may also petition for prohibited noxious weeds. Horticultural crops prohibited within specific counties in the state of Minnesota (U.S.) include: tall buttercup (*Ranunculus acris)*, Jimson weed (*Datura stramonium*), Jerusalem artichoke (*Helianthus tuberosus*), kochia (*Kochia scoparia*), milkweed (*Asclepias syriaca*), oxeye daisy (*Chrysanthemum leucanthemum*), wild sunflower (*Helianthus annuus*), tansy (*Tanacetum vulgare*), and wormwood (*Artemisia absinthium*) (Anderson, 2004c).

Due to the rising costs associated with invasive species control, early detection systems are necessary to identify invasions quickly and eradicate them. Not doing anything can have disastrous results, as we know from purple loosestrife, Eurasian watermilfoil, and other invasive plant species. Industry cooperation with federal and state/provincial agencies will become increasingly important as the number of invasive horticultural species continues to rise. Failure to adhere to national, state/province, or local statutes can have disastrous consequences, as the recent fining of Mr. Stuart Lee (Milford, Michigan, U.S.A.) for shipping ash trees infested with Emerald Ash borer into Prince Georges Co., Maryland, U.S.A., illustrates (http://www.detnews.com/2003/metro/0312/07/metro-342964.htm).

5. ECONOMIC SOLUTIONS

Proposing possible economic solutions, which one could implement to mitigate or eliminate the spread of invasive species through the horticulture industry pathway, is not an easy task. However, as each component of the distribution channel (Figure 6-3) has multiple global connections, prioritizing invasive species control as an international, global "public good" (Perrings, et al., 2002) is essential. Various countries become interlinked with the flow of invasive species. The country wherein an invasive species is native becomes linked to countries invaded by the invasive crop by the movement of humans or products. However, because there is no international agency coordinating the control efforts of all affected countries, the independent efforts of some countries will complicate the risks of others (Perrings, et al., 2002). Likewise, protection against invasive species spread is limited by economic resources of the poorest affected country, making invasive species control the "weakest-link public good" (Sandler 1997).

In capitalistic economies, monetary interests (product viability and profitability) drive the market and floriculture is no exception (Anderson, 2004d). Typically, the economics of invasive species is considered in terms of damage inflicted or the cost(s) of control. However, invasive species economics is an important 'framework' to consider the interaction between human actions (i.e. making a living selling plants) and natural processes (the spread of invasive crops into natural ecosystems) in order to find solutions (Perrings et al., 2002). Human behavior formulates the breeding, domestication, introduction, establishment, and spread of

invasive horticultural crops. Intentionally introduced crops were/are bred and selected for superior performance and survival in the target environment(s) (Lonsdale, 1994; Smith, et al., 1999). Thus, introduced crops that are invasive will have a greater probability of invasion than non-crop species that are unintentionally introduced. Likewise, horticultural crops, which are marketed repeatedly on a global scale, have a greater chance of establishment as invasive species than those which may be marketed once or whose market life is short-lived due to economic inviability (Enserink, 1999).

As one might expect, market prices of potentially invasive horticultural products rarely reflect any subsequent costs imposed on society (Perrings, et al., 2002). Thus, the market is primarily external to any potential harm invasive species inflict. Governmental policies may prevent agricultural markets from being cost-effective due to agricultural policies. These situations enable the increasing risk of horticultural crop invasions. For instance, consider the risk of invasive disease organisms and their impact on horticulture, particularly when a disease may be on a country's bioterrorism list. For the past two years, the accidental introduction of *Ralstonia solanacearum race 3 biovar 2* in geraniums (*Pelargonium x hortorum* 'Americana' series) into the United States resulted in serious economic losses and crop destruction in the infected greenhouse rooting stations and finishers (http://www.aphis.usda.gov/lpa/pubs/fsheet_faq_notice/faq_phralstonia.pdf). The *Ralstonia* case illustrates how the 'weakest link' has a serious impact on all parties connected with the geranium crop or those growing crops in the Nightshade family (Solanaceae), particularly potatoes (the reason this organism is on the U.S. bioterrorism list in the first place). The 'weakest link' invasive species would have a similar effect on the floriculture market, particularly if a major crop were implicated as being invasive (Anderson, 2004d).

Invasions are a problem rooted in human causes and consequences that encompass and surpass any market. Providing incentives to the stakeholders, whose market products are invasive, is the challenge we now face. Until an international organization is formed by the United Nations to coordinate worldwide responses to invasive species, we need to create and effectively implement our own solutions. Failure to do so may seriously jeopardize the long-term viability of the industry.

Examination of a market case in the floriculture industry's history emphasizes our vulnerability and provides direction for constructive approaches to invasives. The 'Chain of Life' program was instituted by Roses, Inc. (a U.S. rose-grower organization; now the International Cut Flower Growers Association) to promote post-harvest health and longevity of cut roses (http://www.rosesinc.org, http://www.chainoflifenetwork.org). Prior to the advent of the 'chain of life' process, growers, wholesalers, and retailers acted independently without regard to the longevity of cut roses for customers downstream in the distribution channel. This meant that poor quality roses entered the market, evoking customer dissatisfaction and returns/credits upward in the chain. Now the cut flower industry realizes that

market viability relies on 100% participation in proper care and handling of cut products. The same challenge with invasive species faces the floriculture industry and flower breeding programs must respond accordingly. Otherwise, the ramifications will implicate all previous participants in the distribution channel, as well as those downstream. Thus, a **'Chain of Non-invasiveness'** is necessary for invasive species control and prevention (Anderson, 2004d). Otherwise, the weakest link in the chain will jeopardize the control of invasives.

An effective, but negative, incentive to stop the sale of invasive species is to charge the persons or companies that are knowingly responsible for importation, domestication, and introduction with the full economic cost of their behavior (Perrings, et al., 2002). The fining of Stuart Lee (Milford, Michigan, U.S.A.) for shipping ash trees infested with Emerald Ash borer into Maryland, U.S.A. (http://www.detnews.com/2003/metro/0312/07/metro-342964.htm), is a prime example of this incentive at work. A more positive approach might be for importers of new crops (that may potentially be invasive) to acquire insurance or post environmental bonds, if insurance is not available. This has been proposed in the state of Florida, U.S.A. for the horticulture industry (http://www.floridafarmbureau.org/issues/farmpol.html) and is already in effect in South Africa for protection against brush fires caused by invasive species (Perrings, et al., 2002). Whether this type of insurance is economically viable is unknown.

How can the industry balance economic viability with environmentally responsible business practices? Perhaps other solutions that are more economically sound can be implemented by all stakeholders in the horticulture distribution channel from breeder/producer companies to the end consumer. Some ideas that have been proposed include the following (Anderson, 2004d; Anderson, et al., 2005a, b).

5.1 What Can Be Done

5.1.1 Industry Self-Regulation

Adherence to the voluntary code of conduct developed by the Missouri Botanical Garden (St. Louis, Missouri, U.S.A.) for floriculturists, nursery professionals, botanical gardens, the gardening public, or landscape architects (http://www.centerforplantconservation.org/invasives/codesN.html) may offer some solutions. However, such a code of conduct is strictly voluntary. Anderson, et al., (2005b) proposed that mandatory codes of conduct would ensure that all parties in the distribution channel (Figure 5-3) participate.

Each of these codes of conduct encourages working with all interested stakeholders to prevent continued dissemination of invasive species in the marketplace. These may entail pulling specific species from the market which have

a high likelihood of becoming invasive in your climate conditions, promoting alternative plants with a similar growth form to the invasive crop, following the laws and quarantines governing importing new plants, and encouraging customers to purchase non-invasive plants. This may involve balancing the risks and rewards of non-invasive species within a corporation and between local, national, or international competitors. The Chain of Non-invasiveness will involve effort of all stakeholders to resolve the challenges of a fractionated industry in a competitive marketplace.

5.1.2 Product Types

Until research provides data on the specific traits conferring invasiveness, the continued spread of invasive crops which are seed-propagated can be prevented by selling sterile cultivars (male/female sterility or at least male-sterile double-flowered types) or those that rarely produce seeds (which may be the result of growing a single clone alone when it cannot set any self-pollinated seed or is self-incompatible). For instance, *Verbascum x hybridum* 'Southern Charm' is a completely sterile triploid alternative to other seed-propagated mulleins (PanAmerican Seed Co., 1999). Double-flowered or completely sterile crops have added benefits to home gardeners by reducing pollen-induced allergies (Ogren, 2003).

5.1.3 Marketing

There are trademarks and branding opportunities that could provide new series of flower products. A company could promote sterile cultivars under a new brand name, such as *Problem-free*™*,* that guarantees they will not become invasive, seed-propagated problems. Consumers will pay extra money for products with value-added traits. Offering a lifetime guarantee that this *Problem-free*™ sterile product will not be invasive. Consumers in northern temperate climates are accustomed to asking for and receiving a one-year, money-back guarantee on woody and herbaceous perennials for winter hardiness in temperate North America. A more extensive guarantee might promote additional sales.

5.1.4 Signage

The commitment of any party in the distribution channel, particularly the private and public sector flower breeding programs, is an important step in crop domestication. Breeder, producer, distributor, grower, and retailer companies committed to preventing the creation and release of invasive floricultural crops would instill confidence in the consumer public. Posting statements on websites or

in the place of business regarding a corporate firm's commitment to selling non-invasive crops would aid in instilling such confidence.

5.1.5 Breeder/Producers

Exercising visionary leadership at the breeder/producer level (Figure 5-3) would aid to prevent the continued breeding and release of potentially invasive crops. Avoiding new products that reseed easily in breeder and cultivar trials could curtail the invasive development of new crops. Introduction of crops with double flowers (male-sterile), complete sterility (male/female due to triploidy), non-shattering seed pods, non-flowering mutants, and avoidance of domesticating crops that are already listed as weeds would all aid in this process.

5.1.6 Distributors, Mail-Order Firms, Growers, Wholesalers, Retailers, Landscapers

The voluntarily withdrawal of invasive species from sales listings by all marketing parties (distributors, mail-order firms, growers, wholesalers, retailers, or landscapers) would prevent the continued flow of invasive products into the marketplace. Such actions would overcome the oversight by or avoidance of selection against invasiveness by breeder/producer companies. For those products that may be high-risk species, clear delineation of the potential danger should be presented in all marketing efforts, i.e. a *caveat emptor* or "buyer beware" notification. High-risk species could have a higher price point, the profits from which could be designated into invasive species insurance. Replacing popular invasive species with 'look-alike', non-invasive products is another effective means of preventing the transfer of invasive species into the gardening public's hands (Burrell, 2000, pers. comm.).

5.1.7 Garden clubs, Horticultural Societies, Public Gardens, Conservatories

Possible ideas to implement by garden clubs, horticultural societies, public gardens or conservatories include banning the listing/exchange of invasive or high-risk species in seed/plant exchanges (*Index Seminum*) and the promotion of gardener awareness for the need to remove invasive species from the landscape. A classic example of this is the publication of an invasive species awareness and identification compact disc or CD, entitled "Woody invasive plants", by the St. Anthony Park Garden Club (St. Paul, Minnesota, U.S.A.; http://www.justaddwater.ws/). This garden club actively promoted the identification and removal of invasives (http://www.nextstep.state.mn.us/res_detail.cfm?id=848), particularly buckthorn (*Rhamnus frangula, R. cathartica*) within the Saint Anthony Park garden club's

neighborhoods. Buckthorn removal teams (members of the International Brotherhood of Buckthorn Busters) from the club printed tee-shirts which were worn by the participants (from 50 households), reading 'Die Buckthorn Scum', to highlight the importance of their efforts. Such invasive species removal efforts were funded by a grant from the Minnesota Environment and Natural Resources Trust Fund and promoted this garden club as being 'on the cutting edge'. The efforts became a case study for the January 26, 2002, Twin Cities Neighborhood Conference (http://www.nextstep.state.mn.us/res_detail.cfm?id=848).

5.1.8 Industry Advocacy Groups

Local or national lobbying groups, such as commercial flower growers or nursery associations, can create and implement functional invasive species policies or risk assessment strategies for their membership to follow. Funding research and educational initiatives by these association will also promote floricultural stewardship. The promotion of responsible horticulture and lobbying for effective legislation based on scientific research, rather than emotional responses, will further aid in preventing invasive flower species dissemination.

5.1.9 Industry Marketing & Promotional Organizations

Promotional organizations, e.g. All America Selections, Fleuroselect, Perennial Plant Association, Cut Flower Growers, etc. could institute selection criteria against invasive tendencies in their respective yearly evaluation trials. This would heighten the awareness of all stakeholders to invasive species. The marketing and promotion of environmentally friendly or 'safe' products and promoting education would enhance the industry's perception that floriculturists are proactively working with the invasive species issue.

5.1.10 Consumers

Proper education of the gardening public is critical to the success of any major change in the flower industry. Marketing campaigns promoting consumers to be locally responsible, to ask for non-invasive products, to purchase from responsible companies, practice good horticultural practices by instituting proper weed control to prevent reseeding, and removing plants if they start to spread vigorously will all enable to gardening public to act locally and prevent the spread of any invasive species out of their growing environments.

5.1.11 Education

Educating the next generation of students regarding invasive crops and promoting their awareness of the problem will create a new cadre of intellectuals who can offer additional solutions to the invasive challenge. Educators can incorporate case studies and decision cases into their classroom activities to stimulate interest and dedication to researching and resolving the many challenges facing the floriculture industry. For example, Anderson (2005) developed a decision case in a bedding plant production class at the University of Minnesota to promote student discussions and higher order thinking on the domestication of new crops which could become invasive.

It is possible to circumvent the invasive species challenge; but this will require concerted, deliberative efforts and thoughtful / persistent research and actions. Balancing the economic viability with sensitivity to preventing international commerce of invasive products requires dedication and strategic thinking.

5.2 Research Can Offer Solutions

Since the advent of invasive species on the horticulture radar screen early in the 20th century (e.g. purple loosestrife first became invasive in the 1930's) research has been devoted to characterizing the invasive species phenomenon from many different perspectives (Anderson and Gomez, 2004). The horticulture industry has traditionally focused on the control of weeds under cultivation, but the participation of the industry in the creation and distribution of invasive species makes it relevant for us to research the connections between crops grown under cultivation and their potential invasiveness in non-cultivated settings.

Research comparing invasive and non-invasive species has determined that some plant traits are associated with the likelihood of invasion. Some of these traits include germination rates, seed production, growth rates, ploidy levels, and DNA levels in plant cells (Bennett, et al., 1998; Dozier, 1999; Wheeler and Starrett, 2001). Research has also focused on the characteristics of the environment that are conducive to invasions. Davis et al. (2000) found that an excess of unused resources either by addition of nutrients or removal of competitors results in more plant invasions. Levine (2000) demonstrated that plant density and community diversity are important factors determining the invasibility of a habitat. Even atmospheric CO_2 and soil water have been implicated in the process of invasion (Polley, et al., 2003). It has also been determined that crops which are most likely to be invasive are those that have a history of weediness elsewhere (Reichard and Hamilton, 1997), possess broad environmental tolerances (Roy, et al., 1991), and lack natural enemies in their new environment (Cousens and Mortimer, 1995). Therefore, it shouldn't come as a surprise, that most weed risk assessments consider geographical and ecological traits to predict invasiveness (Virtue, et al., 1999).

Due to the complexity of the invasive phenomenon, however, there are no silver bullets (Anderson and Gomez, 2004). It is highly unlikely that a gene conferring invasiveness will ever be identified and that a single test would allow us to screen invasive from non-invasive crops. Though numerous correlations between traits and invasive potential exist, there are many exceptions to the rules. For example, within a genus, weedy taxa tend to have broader native distributions than those that are not (Forcella and Wood, 1984), but *Pinus contorta* is highly invasive in New Zealand even though it has a limited native range (Virtue, et al., 1999). In some cases, certain traits lose predictive ability in the presence of overriding factors. Even though high genetic variation has been associated with invasiveness (Mulvaney, 1991), it may not be predictive depending on whether the species is annual, biennial, or perennial (Barrett and Richardson, 1986). Finally, there are limits to the conclusions derived from research. For example, certain families have been considered prone to weediness (e.g. Asteraceae, Brassicaceae), but it is not clear whether the proportion of invaders is actually higher in these families than in others (Scott and Panetta, 1993). In addition, the ability to predict invasive potential may be limited to comparisons among related species within a region with a similar disturbance regime (Richardson, et al., 1990). Therefore, research is required in each species and cultivars to determine their particular propensity to invasion (Anderson and Gomez, 2004; Anderson, et al., 2005a, b).

Since evaluating invasive potential for every single ornamental crop is impractical, flower breeders may need to focus on the effect horticulturally important traits have on invasive potential. Horticulturally important traits may be selected in order to produce less invasive crops. Some of these traits, such as non-shattering seed heads, sterility (non-functional gametes), reduced internode lengths (dwarfism), variegated foliage, or extra petalage (double flowers) would not be expected to confer invasiveness (Ellstrand and Hoffman, 1999; Klinger, et al., 1992; Raybould and Gray, 1994). Other traits such as disease / pest resistance, drought / heat stress tolerance, high male / female fertility, seed dormancy, rapid germination, high yield potential (stand establishment), short generation cycles, and hybrid vigor could increase invasive potential (van Gaal, et al., 1998).

Flower breeders may select for non-invasive traits, sometimes assuming these cultivars will have a value-added benefit. Example crops bred and selected for non-invasive traits include common buckthorn (*Rhamnus frangula* 'Fine Line™'), burning bush (*Euonymous alatus* 'Rudy Hang'), *Berberis thunbergii* 'Concorde', and *Verbascum x hybridum* 'Southern Charm' (PanAmerican Seed Company 1999; Wood 2004), all of which reduce or eliminate seed set. Flower breeders also assume, often with a lack of rigorous evaluations, that sterility or reduced fertility will guarantee non-invasiveness and that such traits are stable in multiple environments over years. The validity of such assumptions is sometimes questionable. It is known that, in some cases, high temperatures may cause male fertility restoration and that male sterility can be linked with disease susceptibility (Estrada et al., 1984;

Levings, 1990). One must not forget the well-known case of purple loosestrife in which the cultivars 'Morden Pink', 'Morden Gleam', and 'Robert' were considered ‘safe’ by the nursery and floriculture industry because they were presumed to be sterile (Anderson and Ascher 1993).

Theoretical and empirical data suggest that plant sterility may reduce invasiveness, but there are many other traits that, depending on the crop, may be more relevant (Anderson and Gomez, 2004). For example, the lack of sexual reproduction in a clonally spreading plant, may be of less relevance to its invasive potential than its growth rate or longevity. Focusing on a suite of traits that account for reduced invasiveness and high ornamental value may be more promising than focusing on a single one. The goals of reduced invasive potential and enhanced ornamental value in future crops can be woven into the idea of a single crop objective or 'ideotype'. Scientific research, specifically in horticulture, has ample ground to contribute to the development of crops that uphold this ideotype. Some examples of the traits that might be included in a non-invasive crop ideotype were proposed by Anderson and Gomez (2004) and Anderson, et al. (2005a, b):

5.2.1 Non-Flowering

This trait would completely eliminate seed production and dispersal, but would be useful only for foliage plants or landscape trees where flowers are undesirable. Cultivars bred for this trait might require vegetative propagation, unless flowering for seed production could be induced at the production level with very specific promoters. Non-flowering plants could be created through interspecific hybridization (Mickelson, 1992) or by selecting mutants that either lack the ability to detect environmental cues for flowering (temperature, photoperiod, etc.) or that are never released from juvenility. The required environmental stability of this trait would necessitate extensive trialing over years and locations. In addition, there may be marketing difficulties due to low consumer demand for some non-flowering plants (Strauss, 2003).

5.2.2 Lack of Pollinator Rewards

Deterring pollinator visitation of flowers, in pollinator-dependent species, would reduce seed set. Abundance of pollinator rewards, such as pollen and nectar, increase pollinator visitation. Other pollinator attractants such as flower color cues and fragrance could also be manipulated to reduce pollinator visits. For example, a crop may be bred to produce flowers that always display the floral pigments and cues characteristic of flowers already pollinated (Weiss, 1991). For this strategy to work, the species to use must not be self-compatible and have an absolute requirement of pollinator visitation for seed production.

5.2.3 Non-Shattering Seed

The seed-bearing structure (capsule, berry, silique, etc.) would be resistant to degradation and the seeds would not be released, preventing them from reaching a suitable environment in which to germinate. An example crop with this trait is sweet corn (*Zea mays*) which has been bred and selected for the seeds (kernels) to remain on the cob, surrounded by the husk. Even when the husk degrades in the soil, most seeds will never germinate. As a result, corn requires human intervention to continue as a crop. Many conifers (pines) have a portion of this trait whereby the cones do not open to release their seeds immediately. They may, however, do so after a fire. This trait would delay the time period before invasive seeds would germinate and, when combined with short viability, might significantly reduce invasive potential of seed-propagated crops.

5.2.4 Non-Fleshy Fruits

Fleshy fruits are usually attractive to wildlife which tend to increase dispersal distance of invasive species. In ornamental species and crops where fleshy fruits are messy and undesirable ('nuisance litter'), breeders could select against this trait making them less palatable for animal dispersers. As an example, buckthorn's fleshy fruits are eaten by birds and spread widely (Archibold, et al., 1997). Non-fleshy buckthorn fruits would prevent long-distance dispersal by birds. This would provide only partial control as seeds are still produced.

5.2.5 Slower Growth Rate

This trait could help in preventing asexual (vegetative) spread of aggressive and/or invasive crops, such as poplars (*Populus spp.*), Japanese knotweed (*Fallopia japonica*), grasses (*Phalaris, Cortaderia, Pennisetum*), or Bishop's cap (*Aegopodium podagraria*). Slower growth rates may reduce or inhibit new propagules (cuttings, rhizomes, etc.) from establishing, although this would be undesirable for commercial propagation/production phases unless a specific trigger to rapid growth rate can be identified and employed when necessary. Additionally, this trait will not prevent flowering and seed set although it may reduce the total number of flowers (seeds) or lengthen the time period before flowering.

5.2.6 Prevention of Seed Germination

Dormancy and seed abortion could be manipulated to prevent seed production and germination. Alternatively, a 'kill' gene can deactivate germination protocols normally present within the seed resulting in seed abortion or lack of germination**.** If open-pollinated seeds produced after retail sale could be bred to never germinate,

it would effectively prevent buildup of seeds in the seed bank. Most likely, however, for this trait to be effective, seeds would be alive but incapable of germination until some very specific trigger is applied. If seed germination were prevented by the imposition of dormancy, this would only delay germination over a period of months or years until the necessary environmental cue(s) arrive to cause germination.

5.2.7 Sterility

This "utilitarian feature" would aid both society and the environment (Warren, 2003). As previously mentioned, in many cases seed set is the greatest determinant for invasiveness (Hutchinson and Vankat, 1997). Thus, sterility is regarded as the penultimate trait to prevent invasiveness of seed-propagated crops. Both male and female sterility are necessary to prevent gene flow and seed set. Traditionally, breeders have bred cultivars that are either male- or female-sterile, but not both. Male sterility systems are routinely used in seed production of many vegetable or oil crops (onions, canola oil) to avoid costly emasculation of the female (seed) parent during hybrid seed production (Peterson and Foskett, 1953). Male sterility is also useful in preventing pollen production to reduce allergies (Ogren, 2003). In landscaping, male plants of dioecious crops are used to prevent messy fruit production in gingko (*Ginkgo biloba*), kiwi (*Actinidia*), etc. Conventional breeding can create sterility in only a fraction of all horticultural crops, sometimes by crossing parents with different ploidy levels (tetraploid x diploid, *4x* x *2x)* to create sterile triploids (*3x*). Though conventional breeding methods could result in the production of sterile cultivars, sterility genes may not be available or, if they are, these genes may be linked with other deleterious genes that would hamper the production of marketable ornamental cultivars. The greatest advantage of developing a protocol to produce sterile cultivars using genetic engineering is that a research lab with minimal laboratory equipment (for tissue culture and transformation) could potentially transform all crop species in a relatively short time-period. On the other hand, one of the most significant drawbacks of this technology is the sensitivity and resistance of some cultivars to tissue culture propagation and transformation.

5.2.8 Programmed Death Prior to Seed Production

The ultimate non-invasive trait, apoptosis, would allow a plant breeder to ensure plant death before seeds are produced (Gray, 2003). In systems in which apoptosis has been studied, some environmental cue(s) or self-recognition system is required to induce programmed cell death (Thomas and Franklin-Tong, 2004; Yamada and Wataru, 2003). Since one of the mechanisms of invasiveness concerns the ability to disperse seeds, the use of apoptosis to halt the life cycle of a species when seeds are produced might have significant benefit. Unlike annuals which complete their life

cycle in ≤1 year and then senesce, apoptotic cultivars would die earlier--prior to the production of viable seeds. In castor bean (*Ricinus communis*) apoptosis stopped seed production after fertilization by impairing endosperm development (Than, et al., 2004). This could be a mechanism to adopt as a fail-safe trait of 'self-destruction', in addition to the other characteristics listed for this ideotype.

6. CONCLUSIONS

The non-invasive crop ideotype ensures that non-invasive traits would be accompanied by traits conferring ornamental value (Anderson and Gomez, 2004; Anderson, et al., 2005a, b). There are examples supporting that ornamental quality does not have to be sacrificed in order to achieve non-invasiveness. Sterile aspen, cottonwood, and pine trees, for example, often have increased growth rates (Eis, et al., 1965). If increased growth rates in these species are not associated with greater invasive potential, then this might be a desirable trait of horticultural value. Another example comes from floriculture where the male sterile tiger lily, *Lilium lancifolium* 'Flore-Pleno', has higher market value from having double flowers. Sterile ornamental crops can still produce acceptable flowers and maintain their 'flower power', e.g. *Verbascum* 'Southern Charm', and sterile edible fruits or vegetable crops, e.g. seedless watermelons ('Orange Sunshine') or parthenocarpic grapes ('Thompson Seedless'), can also have greater market value if fruit quality is preserved.

Programmatic examples of breeding programs actively involved in *a priori* prevention of invasive crop creation include that of the University of Minnesota (St. Paul, Minnesota, U.S.A.), Department of Horticultural Science Herbaceous Perennial Breeding Program (Anderson and Gomez, 2004). In this program, Dr. Neil Anderson's research team is researching various ways that flower breeders may select against invasive potential during crop domestication and breeding. *Cleome* species are being studied to determine whether germination, establishment, and performance in cultivated settings (gardens) are predictive of this crop's behavior in non-cultivated (roadsides, prairies) sites in Minnesota (Anderson and Gomez, 2004). Thus far, it has determined that germination in cultivated habitats differed from that in non-cultivated environments. The lack of correlation between germination in cultivated and non-cultivated environments suggests that studying plants in standard plant breeder cultivar trials might be insufficient to predict invasive potential in non-cultivated habitats. However, differences among cultivars have been observed suggesting that some may be less invasive relative to others in terms of germination percentage and seedling survival. This offers the opportunity of exploring cultivar-specific differences and selecting those with reduced invasive potential. Such research will benefit the ornamental plant industry by beginning the identification of invasive tendencies of a flower crop, allowing for the development of breeding

program methodologies to proactively select against invasiveness prior to the release of crops and cultivars. The long-term outcome encompasses researching which floriculture crops are potentially invasive and how plant breeding programs may breed / select alternative cultivars with high horticultural performance that do not pose a threat to ecosystems.

Another aspect under development to reduce crop invasiveness focuses on sterility. Dr. Alan Smith's lab (Dept. of Horticultural Science, U of Minnesota) has successfully used genetic engineering to create male- and female-sterile tobacco, petunia, and other crops (Bucciaglia et al., 2003; Dotson, et al., 1996). Cooperative research efforts with Dr. Smith's laboratory and the herbaceous perennial breeding program (Dr. Anderson) is underway to create sterile, non-invasive ornamental plants. There are many herbaceous and woody crop candidates for transformation into sterile cultivars, e.g. urban tree crops include hedge maple (*Acer campestre*), Freeman maple (*A. x freemanii*), Amur maple (*A. grinnala*), Norway maple (*A. platanoides*), red maple (*A. rubrum*), Russian olive (*Eleagnus angustifolia*), sweetgum (*Liquidambar styraciflua*), some crabapples (*Malus*), flowering pear (*Pyrus calleryana*), and elms (*Ulmus spp.*) (Strauss, et al., 2003). Flowering herbaceous crops include purple loosestrife (*Lythrum salicaria*), butter and eggs (*Linaria vulgaris*), Dame's Rocket (*Hesperis matronalis*), gaura (*Gaura lindheimeri, G. coccinea*), shasta daisy (*Leucanthemum x hybridum*), verbena (*Verbena bonariensis*), fountain grass (*Pennisetum setaceum*), pampas grass (*Cortaderia selloana, C. jubata*), Hottentot fig (*Carprobrotus edulis*), etc. Significant amounts of research must be conducted before sterile, non-invasive cultivars can be released, particularly in the efficiency of transformation, stability of the sterility systems, and actual prevention of invasiveness.

Despite its controversial nature, genetic engineering may be the most useful tool to create non-invasive crops. The prevention of seed formation with sterility in new cultivars makes sense from an environmental perspective. However, financial investments to create sterile cultivars with unknown market potential create a paradox, since new cultivars are sold in small numbers until the demand increases. Until the costs of creating sterile hybrids via classical breeding or genetic engineering can be reduced we cannot move beyond this dilemma. The primary obstacles to the widespread use of genetically-modified sterile plants include economic (investment costs, risk), biological (the need for development of crop-specific tissue culture regeneration / transformation systems), and societal (risk of GMO escape, public acceptance) challenges. All of these obstacles are significant and must be surmounted before this becomes a viable and widespread technique and it may be possible to use marketing (branding) to create interest in sterile cultivars that are 'environmentally safe'.

The University of Minnesota is also engaging in constructive ways to educate graduate and undergraduate students by offering courses related to invasive species and encouraging participation in discussions and research via the Invasion Biology

Research Consortium (http://www.mnibrc.umn.edu/). Having students problem-solving invasive species issues and being involved in civic engagement—where citizens interact to make decisions and act collectively on a common problem—would catapult them beyond the intellectual boundaries of a curriculum. This would transcend simplistic answers to complex problems and students would be enabled to reflect the issues' complexities. New graduates entering the marketplace need to be educated and trained in problem-solving methodology to promote creative thinking and problem-solving for the invasive challenge. A vast array of researchers and educators in all relevant fields (horticulture, economics, ecology, business, law, public ethics, political science, etc.) are needed to conduct research and educate such students (Anderson and Gomez, 2004).

The advancement of any science relies on rigorous, repeatable, and realistic research for testing theories. Broad generalities do not hold across the plant kingdom and there are exceptions to every rule since invasiveness is a complex phenomenon. From experimental results we can extrapolate and speculate about the invasive potential of a certain crop under specific conditions. There is still no way, however, to predict what genetic changes might happen in a cultivar years after its initial introduction or what precise combination of genetic and environmental conditions will lead to an invasion in a specific crop. Since we cannot predict which characteristics will become available to natural selection, we can only speculate about the changes in population dynamics that can result over time. While research may help us understand what traits are more likely correlated with invasiveness and which characteristics make certain habitats more invasive than others, there is still a chance that we may introduce something that may become invasive in the future. Whether flower breeders are willing to accept the risk is a question of risk management and ethics rather than one of biological nature.

We are now faced with a serious issue that, if allowed to proceed unchecked, may threaten the quality of urban and regional life worldwide and create costly cleanup bills for regulatory agencies. Everyone needs to be well-informed of the issues concerning invasive horticultural crops and, most importantly, how they may be eliminated from our industry. Home gardeners may unknowingly cultivate invasive ornamentals. Greenhouse growers and industry retailers only vaguely understand invasive species complexity. This is why there is a profound need for research and education on invasiveness & its prevention in all sectors of the horticulture distribution channel from the moment a new species is collected in the wild, bred, domesticated, propagated as a new cultivar, distributed, sold, and grown by customers. Most importantly, this responsibility lies with flower breeders who can aid in the prevention and spread of invasive floricultural crops.

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PART II

CROP-SPECIFIC BREEDING & GENETICS

BEDDING PLANTS

Chapter 7

AGERATUM

Ageratum houstonianum

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Abstract: *Ageratum houstonianum* is native to Mexico and Central America, with many escapes becoming established as weeds in all parts of the world. Early cultivation in Europe led to the establishment of ageratum as a garden ornamental in Europe and the United States by the 19th century. Seed companies successfully introduced F1 hybrid ageratum cultivars in the mid-20th century by using either self-incompatibility or genetic male sterility as a pollination control. Uses of ageratum range from dwarf cultivars used for containers, borders, or edging to tall cultivars used for cut flowers. Improvements in ageratum as cut flowers, dried flowers, larger flowers, more intense flower colour, an expanded flower colour range, more uniform plant habit, earlier flowering, and an extended bloom time are future breeding objectives.

Key words: ageratum, *Ageratum houstonianum*, F_1 hybrid, self-incompatibility, genetic male sterility.

1. HISTORY AND DOMESTICATION

Although native to the Americas, *Ageratum houstonianum*, floss flower, was known in Europe as early as the late 1600s or early 1700s, being routinely cultivated in European gardens by the 1800s (Johnson, 1971). Presumably, the plant was used much as it came from the wild, but because of its fairly long history in 19th-century gardens, some selection for traits such as uniformity and short stature must have been practised by gardeners. Currently, ageratum is an important seed-propagated, annual bedding plant. In addition to its use as an ornamental, *A. houstonianum* and the closely related *A. conyzoides* have been used in folk medicine (Johnson, 1971),

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and more recently as the source of a potential insecticide that acts as an anti-juvenile hormone (Bowers et al., 1976).

2. SPECIES ORIGIN, CENTERS OF DIVERSITY, REPRODUCTIVE BARRIERS, AND TAXONOMY

A houstonianum originated in Mexico and Central America (Johnson, 1971). Specimens have also been found in the southern United States, but these are thought to be escapes that became established as weeds. The genus is closely related to *Eupatorium*, Joe-Pye Weed. Although *A. houstonianum* has many forms and related species, little is known of their crossing relationships. According to Johnson (1971), the species within the genus *Ageratum* are very distinct morphologically. "Introgression and consequent blurring of species limits, if it is occurring, is not evident in this genus" although "cytodemes of tetraploid plants do occur". Some *A. houstonianum* tetraploids have been cultivated, but not necessarily with noticeably larger leaves or inflorescences (Johnson, 1971). Although the genus contains about 30 recognised species, only *A. houstonianum* is grown commercially, while *A. conyzoides* has become a widely distributed weed in the tropics (Johnson, 1971). Thus, breeders, growers, and retailers commonly refer to *A. houstonianum* simply as ageratum. As described by Johnson (1971), *A. houstonianum* has simple or branched stems that are erect or decumbent, with leaves that are opposite or alternate, ovate to deltoid. Inflorescences are terminal, the 5-15 heads borne in tight or open cymose clusters on bracteolate peduncles. The corolla is funnelform, with a white tube and a throat of blue, lilac, lavender or white, the 5 lobes upright or spreading. Achenes are 5-angled, black when mature, 1.5-1.75 mm long, crowned with a pappus of 5 free oblong scarious scales 2-3 mm long, with a setaceous apex. At times the pappus scales are much shorter, 0.1-0.15 mm long and without setae. *A. houstonianum* is similar to *A. conyzoides* in habit, achenes, pappus, corolla, and the odor of crushed fresh leaves. "However, the combination of ovate leaves with cordate bases and narrowly lanceolate, conspicuously pilose involucral bracts with stipitate glandular pubescence on the gradually acuminate apex distinguishes *A. houstonianum* from similar species" (Johnson, 1971).

3. CROSSING MECHANISMS

Emasculation is not a practical method of controlling pollination due to the small size and structure of the composite flower (inflorescence) of ageratum. Ageratum flowers are apetalous (gynoecious ray florets are lacking), leaving only hermaphroditic disc florets for seed production. There is one ovule/floret. Since ageratum exhibits self-incompatibility (Stephens et al., 1982), a controlled cross can

be made by covering the flower just prior to the first florets opening. Then, as successive rings of florets become receptive, pollen can be applied using flowers of another cross-compatible plant by dusting the flower of the pollen donor on the flower of the prospective seed parent. After 4 to 6 weeks, or when the seed-head turns a light brown color, the seed-head can be harvested and threshed. The mature filled seeds (achenes) are nearly black, whereas unfilled seeds are light brown and empty.

4. GENETIC TRAITS AFFECTING COMMERCIAL PRODUCTION

Important genetic traits used for F_1 hybrid production are self-incompatibility and male sterility. Ageratum possesses a sporophytic self-incompatibility system (Makarem and Ascher, 1977, Reimann-Philipp, 1965, Stephens et al., 1982), although the specific type of incompatibility probably has not played an important role in how self-incompatibility is used to produce commercial cultivars. In general, breeders can produce inbred lines by sib crossing or by selfing lines that are pseudo-self-compatible (Stephens et al., 1982). When two uniform lines have been inbred so that they are self-incompatible but cross-compatible, they are interplanted in a production greenhouse. Seeds are harvested from all plants, cleaned and packaged as the final F_1 hybrid cultivar. Since the self-incompatibility system in ageratum is normally quite stable, these F_1 hybrid cultivars are very uniform for flower color and plant height. In F_1 hybrid seed lots, no more than 1-2% selfed seeds are acceptable (Denis Flaschenriem, Pan American Seed Co., pers. commun., 2002).

Genetic male sterility has also been found in ageratum and has been used to produce F_1 hybrids (Reimann-Philipp and Fuchs, 1971). In this method, the progeny of a female parent of a line segregating 1:1 for male sterility is planted and, at flowering, the approximately 50% male-fertile plants are rogued. F_1 seeds are matured and collected on the remaining male-sterile plants.

Seeds are readily produced during the length of a normal growing season. Historically, seed crops have been grown in coastal California, where conditions are conducive to maturing and drying of the seed crop. As labour costs have escalated, most seed production has moved to Central America and Africa (Boodley, 1996).

Data on yield potential are lacking, but the structure of the mature seed lends itself to fairly easy cleaning and packaging. Seeds are not particularly difficult to store and will hold their viability for at least one year without expensive storage. *A. houstonianum* has no apparent seed dormancy, with seeds germinating soon after sowing the freshly harvested seed under normal sowing conditions of light, temperature, and moisture.

5. TRAITS AND GENES IDENTIFIED

Aside from pollination control, the flower color of ageratum is probably its most important and popular trait, because of the general lack of blue flower color in many flowering annuals. White flowers also occur in natural populations of *A. houstonianum* (Johnson, 1971), and white-flowered cultivars are available in limited numbers (Nau, 1998). Some breeders have selected plants with purple or pink flowers, but blue is still the most popular flower color. Blue (B) is dominant to white (b) (Stephens et al., 1982), but genetic information on other flower colours is lacking or unavailable. Long (P) is dominant to short (p) pappus scales (Stephens et al., 1982) and genetically dwarf cultivars produce short-statured plant habits that are used for border plantings, but the genetic control of dwarfness is still considered proprietary information by flower seed companies. Nevertheless, both short and tall cultivars have been bred, and with the recent popularity of cut and dried flowers, taller long-stemmed cultivars have become increasingly popular (Nau, 1998). Spider mites, whiteflies and fall army worm can be serious pests, and all cultivars are extremely susceptible to frost. The latter is an unstudied trait and one that might result in important genetic improvements to extend the growing season.

6. COMMERCIAL PRODUCT EXAMPLES, MARKETING, AND CROP IDEOTYPES

Short-statured F_1 cultivars used for containers, borders, or edging include 'Blue Hawaii', 'Blue Pearl', 'Blue Puffs', and 'Blue Blazer', which are currently the most popular (Nau 1998). The purple 'Royal Hawaii' and whites, including 'White Hawaii' and 'Silver Pearl' are also available. 'Blue Horizon' produces a plant 1 m tall that reportedly makes a good cut flower, used either fresh or dried in arrangements (Nau 1998). 'Leilani Blue', a slightly shorter cultivar, reaches 14 to 16 inches (36 to 41 cm) in the garden, producing masses of fluffy, self-cleaning flowers (Onofrey, 2000). Less popular, but also available commercially from flower-seed companies, are open-pollinated and tetraploid ageratum cultivars.

7. BREEDING AND GENETIC DIRECTIVES FOR FUTURE RESEARCH

Improvements in cut flowers, dried flowers, larger flowers, and deeper blue color are some that should continue to be future breeding objectives. In addition, breeders are working on expanding the flower color range, improving the uniformity of plant habit, and selecting for earlier flowering and an extended bloom time (Denis Flaschenriem, Pan American Seed Co., pers. comm., 2002). The widespread

implementation and use of molecular technologies in ageratum breeding is doubtful, in large part because the crop is a minor one in the bedding plant industry. However, because of rapid advances in molecular biology, particularly genomics, it is difficult to forecast a genetic trait that may not be available for genetic transformation of ageratum. Still, obstacles to the use of molecular technologies remain. Some tissue culture research has been done on ageratum (Al-Atabee and Power, 1990) but not enough to produce a regeneration method or transformation protocol. If and when genetic engineering is successful with ageratum, caution would be warranted because *A. houstonianum* readily establishes itself as a weed and transgenic races could easily escape cultivation, especially in the tropics.

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Chapter 8

ANAGALLIS

Anagallis monellii

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Abstract: The blue pimpernel, *Anagallis monellii,* is a new bedding, container, and hanging basket plant due to its blue flower coloration. Historically, this has been a seed crop, although newer cultivars are vegetatively propagated. A gametophytic self incompatibility is found within this species, although at least two related species at self-compatible. Current breeding objectives include a compact growth habit and shortened internodes. Other flower colors can be found in the species, which are gaining in popularity.

Key words: *Anagallis monelli*, Pimpernel, breeding, ornamental plants.

1. SPECIES ORIGIN

Anagallis monelli (Blue Pimpernel) is used as an annual bedding plant and for hanging baskets. Most plants available in the floriculture market have a beautiful deep blue flower color and profuse flowering. Blue is the most sought-after flower color and since there are few true blue flowering plants available, *Anagallis* has excellent marketing potential.

The genus *Anagallis* is in the Primulaceae, although recent phylogenetic studies based on DNA sequence data from three chloroplast genes and morphology have placed it in the Myrsinaceae (Källersjö et al., 2000). There are about 28 species in the genus *Anagallis*, mostly native to Europe, Asia, Africa, and America (Claphan et al., 1987). The genus name comes from the Greek word "to make laugh" and refers to a use of the plant to relieve sadness (Geneve 2000).

Anagallis monelli L. (= *A. collina* Schousboe, *A. linifolia* L.) is a short-lived perennial with blue or blue-pink flowers. It is found in dry, open habitats in the western Mediterranean region. In some cases it is found near the coast in permanent

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dune formations, far from the tide line and protected from the wind by moving dunes that are closer to the shore. These permanent dunes have sandy soils with high salt content, but there is some organic matter from decay of plant material and the vegetation is more diversified. In other locations *A. monelli* has been found in rock fissures in cliffs near the coast.

Other European species of *Anagallis* include *A. minima* (Chaffweed), an erect annual with small white or pink flowers found throughout Europe in damp, open habitats, especially on sandy soils. *A. crassifolia* is a creeping perennial with small white or cream flowers, found in wet places in south western Europe. *A. tenella* (Bog Pimpernel), a creeping perennial with small pink flowers, is found in damp turf and bogs in west Europe and the British Isles. *A. arvensis* (Scarlet Pimpernel, Poor man's weatherglass, Common Pimpernel), is an annual plant with small orange or blue flowers, found in cultivated ground, waste places and maritime sands throughout Europe. Two species, *A. parviflora* and *A. foemina* (= *A. caerulea*), are both very similar to *A. arvensis* (Tutin et al., 1972).

2. HISTORY

Information is scarce regarding history and domestication of *Anagallis monelli* and very few cultivars are available. Hortus Third (The Staff of the L.H. Bailey Hortorium 1976) mentions *A. monelli* 'Philipsii' with deep gentian-blue flowers, but this cultivar is no longer available. Several commercial seed companies carry *A. monelli* (commonly labeled as *A. linifolia* or *A. monelli* ssp. *linifolia*) individually or in wildflower seed mixes. When I initiated research on *Anagallis* in 1998, cultivars 'Blue Light' and 'Gentian Blue' were found through Park Seed (Greenwood, SC) and Thompson & Morgan Seedsmen, Inc. (Jackson, NJ) respectively, but these are no longer available. Currently, none of the seed available from commercial seed companies has a cultivar name.

In the vegetatively-propagated market, *A. monelli* 'Pacific Blue' was developed at the University of British Columbia (Macdonald 1993). This form was selected from open-pollinated seed collected from plants growing in the Alpine Garden Society of England in 1980. 'Pacific Blue' was registered with the Canadian Ornamental Plant Foundation and the International Authority for Registration of Herbaceous Perennials. 'Pacific Blue' is no longer commercially available. Since the mid-1990's, the only vegetative cultivars available have been *A. monelli* 'Skylover Blue' with deep blue flowers and 'Sunrise' with small salmon-orange flowers, small leaves, and compact growth. This cultivar is labeled by different sources as *A. tenella* 'Sunrise', *A. monelli* 'Sunrise', and *Anagallis* 'Red'. Taxonomically, a Primulaceae specialist (A.F. Cholewa 2001, personal communication) has identified 'Sunrise' as A. monelli. 'Sunrise' has profuse flowering and performs very well as a hanging basket, but has very poor vigor as a

bedding plant. Neither 'Skylover Blue' nor 'Sunrise' are patented cultivars. Their origin is unknown, but they may have been introduced in Germany. Both cultivars can be found through several plant propagators in the U.S.A, Europe, and Australia.

3. REPRODUCTIVE BIOLOGY

Anagallis species have perfect flowers. *A. monellii* has five bright yellow anthers and a style exserted at an angle from the top of the ovary, thus the stigma is situated away from the anthers. Research on natural populations of *A. monelli* from South Western Spain, found that automatic self-pollination did not occur, in contrast with *A. arvensis* and *A. parviflora* (Gibbs and Talavera 2001). Further studies identified gametophytic self-incompatibility in the natural, diploid populations of *A. monelli* (Talavera et al., 2001).

In populations derived from commercial seed sources of *A. monelli*, formation of fruits from self-pollination has been observed in greenhouse conditions and on isolated plants (Freyre, unpublished data). These plant lines may have been purposely selected by seed companies for their profuse fruiting. In *A. monelli* flowers, anthers dehisce pollen and self-pollination may occur before buds open. Therefore, for the purpose of controlled hand-pollinations, emasculation of the anthers in unopened buds is recommended. Anthers from the plant selected as male parent are then rubbed on the stigma of the emasculated flower and its pedicel is tagged for identification.

In pollinated or fertilized flowers the pedicels take on a characteristic curvature, while unfertilized flowers retain a straight pedicel. The fruit is a small pyxis or capsule with circumscissle dehiscence, with 20-30 small dark brown seeds, of size similar to poppy seeds. *A. arvensis* plants senesce after fruiting. Fruits dry and split transversally, the top half comes off like a lid and seeds are dispersed. In *A. monelli* the fruits and seeds remain green for a long period of time. Harvesting green fruits and drying them in an oven results in low or no seed germination. After completing hand pollinations and allowing approximately three weeks for fruit formation, it is best to stop watering the plants and allow them to become totally dry (Freyre, unpublished data). Even when fruits appear to be brown and brittle on the outside, the placenta may still be green and seeds are not mature, so it is preferable to check well before harvesting the fruits. In greenhouse conditions fruits rarely dehisce and disperse the seeds.

4. BREEDING OBJECTIVES

A breeding program was initiated at the University of New Hampshire (UNH) on *Anagallis monelli* in 1998. The main objectives were to develop new and

improved cultivars for the vegetatively-propagated market. Starting with germplasm available from commercial sources (from seed or clonal material), the breeding approach has been a combination of multi-trait selection (Harding et al., 1991), use of interspecific hybridizations, and induced polyploidization. Plants selected for specific characteristics have been maintained through vegetative propagation and used to develop new generations.

4.1 *Anagallis* Breeding Cycles

Typically, *Anagallis* plants selected as parents because they have desirable characteristics are kept in a greenhouse with night-interruption lighting (46 $\mu mol.m^{-2}.s$ between 10 p.m. and 2 a.m.) during the fall to induce flowering and perform hybridizations. In late fall, seed of the new generation are harvested and sown. Germination is usually uneven, ranging from ten days to five weeks after sowing. As seedlings emerge they are transplanted into 6-cell-pack containers and later on into 15 cm. diameter pots and grown under light interruption lighting during the winter for a first cycle of selection. Traits considered during this initial selection cycle are early flowering, bloom color and size, and compact growth habit. Selected plants are then pinched and maintained at natural daylength (at 43^{o}N in Durham, NH) to select for early flowering in the spring. Final plant selections are propagated by cuttings and trialed during the summer in the greenhouse in 25 cm. hanging baskets, as well as replicated trials as bedding plants in the field.

4.2 Characteristics Used During the Selection process

4.2.1 Plant Growth Habit

One of the main disadvantages of *A. monelli* 'Skylover Blue' is that it has stems with very long internodes, which give it a "leggy" appearance (Jeff and Henry Huntington, Pleasant View Gardens Inc., Loudon, NH, pers. communication). Therefore, one of the main breeding objectives at UNH has been to develop plants with short internodes and a compact growth habit. Figure 8-1 shows a comparison of 'Skylover Blue' and a UNH hybrid grown in a replicated experiment in spring 2001. Both plants were grown in 15 cm pots under the same treatment, with night-interruption lighting starting 28 days after propagation from cuttings, and a soft manual pinch on day 49. The photo was taken on day 120. On average, the length of the longest stem of the UNH hybrid was 12 cm. shorter than the longest stem in 'Skylover Blue', and it flowered nine days earlier. Although these differences were not significant, overall rating based on plant appearance and performance was significantly higher for the UNH hybrid.

Figure 8-1. Comparison of 'Skylover Blue' (left) and a selected UNH hybrid (right).

Some of the compact plant material developed at UNH is derived from interspecific hybridizations with *A. arvensis*. Although this interspecific cross was hard to obtain, the F_1 was fertile and a number of F_2 progeny were generated. These were characterised by very compact growth but also late flowering, so the earlier flowering individuals were selected for further use in the breeding program.

In populations of *A. monelli*, considerable genetic variation in growth habit has been observed. In hanging baskets, plants that have vigorous growth and fill the pot upwards followed by a trailing habit are more desirable than plants that initially trail. For outdoor use, plants with an upright growth look more attractive than those which form a mat close to the ground. Ideally, we can select plants that have good performance and appearance under both growing conditions.

4.2.2 Early Flowering

Studies on the induction of flowering in *A. arvensis* were reviewed by Ballard (1969). *A. arvensis* has an absolute long day requirement for flowering and the critical photoperiod for induction is 12 – 12.5 hrs. In contrast, *A. tenella* requires vernalization, followed by long days for flower induction (Thomas and Vince-Prue 1997). In the case of *A. monelli*, we have observed that long days are also required for flowering. During the Fall and Winter when plants are grown in greenhouse conditions they remain vegetative and branch profusely. As daylength increases in the Spring, plants become reproductive. They form buds at the nodes and bloom acropetally, proceeding from the base or proximal end of the stems toward the distal end. In northern U.S.A, when 'Skylover' is propagated vegetatively and grown under a natural photoperiod, it starts to flower at the end of May when the daylength is longer than 14 hours. For commercial purposes, it is desirable to have plants in flower by early or mid-May, since the largest sales for annual plants are at Mother's Day in the U.S. Few customers will buy plants if they are vegetative and not in

bloom, although this is not the consumer preference in all countries. Therefore, another important selection criteria in the *Anagallis* breeding program at UNH has been early flowering.

There can be two factors affecting flower earliness (Fig. 8-2), the critical photoperiod and the response time, which is the period of time from when the plant has the necessary daylength to induce bloom, until flowering actually occurs.

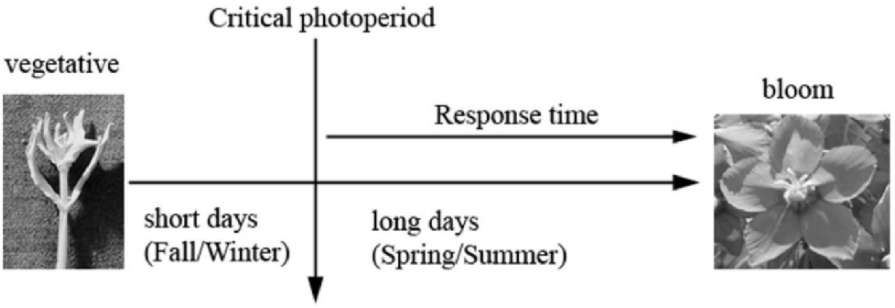

Figure 8-2. Critical photoperiod and response time affecting flower induction in *Anagallis*.

We have observed variation in flower initiation in *Anagallis* populations, which allows selection for earlier-flowering plant types. In greenhouse conditions at UNH, we can run two types of experiments to select for flowering earliness. Typically, for a first cycle of selection, *Anagallis* seedlings are grown during the Fall and Winter in a greenhouse with night-interruption lighting (from 10 p.m. to 2 a.m.). This is physiological equivalent of a long-day photoperiod. Therefore, the timing of induction is controlled, i.e. when the artificial lighting is initiated. This allows to test for differences in response time only and select for plants that bloom within the shortest period from when lighting is started. Another set of experiments can be run by growing plants at the end of the Winter under natural (short days) photoperiod. In the Spring as natural daylength increases, there is genetic variation for flowering, and selection is for a combination of both critical photoperiod and response time.

For example, Figure 8-3 shows the results of flowering in a population of blue-flowered *Anagallis* plants (n=328) grown at the UNH greenhouses in 1999. Seedlings were transplanted into 15 cm. diameter pots. For every family, half the progeny were grown under night-interruption lighting while the other half was kept under natural daylength. In plants grown under night-interruption lighting, flowering response time was from 7 to 18 weeks with an average of 9 weeks. For plants grown under natural daylength, the average time to bloom was 22 weeks. The earliest flowering plants bloomed after 14 weeks on week 13 (March 20th), when

daylength was approximately 12.5 hours, while the latest flowering bloomed after 29 weeks on week 25 (June 19 th) when daylength was approximately 15.5 hours long. This variation in flowering time allows for selection of the earliest flowering lines. We have confirmed in replicated trials under long days that some of the UNH selections bloom 2 weeks earlier than 'Skylover Blue'.

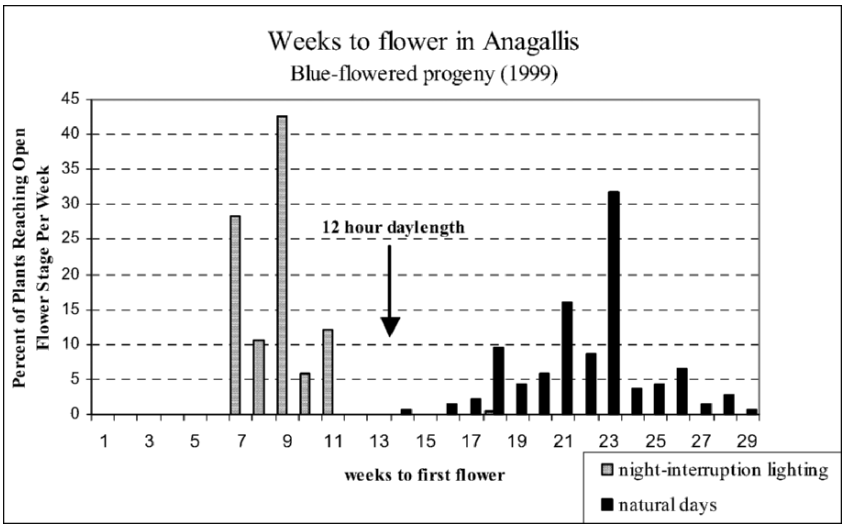

Figure 8-3. Number of weeks to flower in a population of blue-flowered *Anagallis monelli* grown in the UNH greenhouses in 1999.

4.2.3 Flower Size and Shape

While *A. monelli* 'Skylover Blue' has flowers 3 cm. in diameter, the flowers of 'Sunrise' are only half this size, 1.5 cm. Clearly, selection for large flower size in both colors is desirable. Plants with larger flowers have been created by induced polyploidization and also from hybrid vigor. For example, in orange-flowered plants, Figure 8-4 shows a flower of 'Sunrise', a polyploid, and a hybrid. Size of flowers has been increased from 1.5 cm. to over 3 cm.

It appears that there is correlation between large flower size and long internodes, particularly in the blue-flowered types. Therefore, multi-trait selection must be applied.

With respect to flower shape, many seed-propagated plants obtained from commercial sources exhibit petals that curl inwards. This trait is not attractive and is a negative selection criterion during the screening process.

Figure 8-4. Flower size of *Anagallis* orange-flowered 'Sunrise' (left), a polyploid (center) and a hybrid (right).

4.2.4 Profuse Flowering

Another selection criterion in *Anagallis* breeding is profuse blooming for a long period of time. Plants that are self-incompatible or sterile and do not form fruit tend to flower for a longer period, e.g. 'Sunrise'. Fruit formation also detracts from a plant's attractiveness. Although capsules are small, as a plant's flowering period decreases, the curved pedicels and fruits become quite evident. In contrast, if a plant is self-incompatible or has low female fertility, when the corolla dehisces the pedicels are not very noticeable. Therefore, for purposes of plants intended for the vegetatively-propagated market, either self-incompatibility or sterility is a desirable trait.

'Skylover Blue' has low female fertility forming few fruits, and abundant blooms. On average, it has three to four blooms per node. Some new hybrids (unnumbered selections at UNH) have been selected with up to six blooms per node, lack of fruit formation, and longer flowering period than 'Skylover Blue'.

4.2.5 Flowers That Remain Open Under Low Light Levels

Gibbs and Talavera (2001) report that flowers of *Anagallis* species are characterized by nyctinastic movements of the petals, closing at dusk and reopening the following day. Thus, some of the common names for *A. arvensis* are "Poor Man's Weather Glass" or "Shepherd's Clock" because flowers close in the late afternoon or when it is overcast. Clearly, it is more desirable to have *A. monelli* plants that have open flowers even with cloudy weather or low light levels.

For example, Figure 8-5 shows two plants with different lineage, growing side-by-side in the same environment. The plant shown on the right has flowers that are fully open, while those on the plant in the left are still closed. It has yet to be confirmed whether the plant with open flowers lacks the nyctinastic response altogether, or has a lower light level requirement to maintain open blooms.

Figure 8-5. Flowers on two different *Anagallis monelli* plants grown under the same conditions in the greenhouse, at 0900 HRS in winter, with low light levels.

4.2.6 Range of Colors

While 'Skylover Blue' has a deep blue flower color (RHS 99B), some commercial seed-propagated *A. monelli* plants have a powdery blue color (RHS 98A). Natural populations have been reported to have blue or blue-pink color. Initially the breeding objective at UNH was to develop improved deep-blue-flowered plants. Since 1999, we have also developed lines with large salmon-orange blooms (RHS 33B) that are superior to 'Sunrise'. Recently, red- (RHS 58B) and violet-flowered (RHS 82B) lines have been selected (Fig. 8-6), to create a series of *Anagallis* with different flower colors.

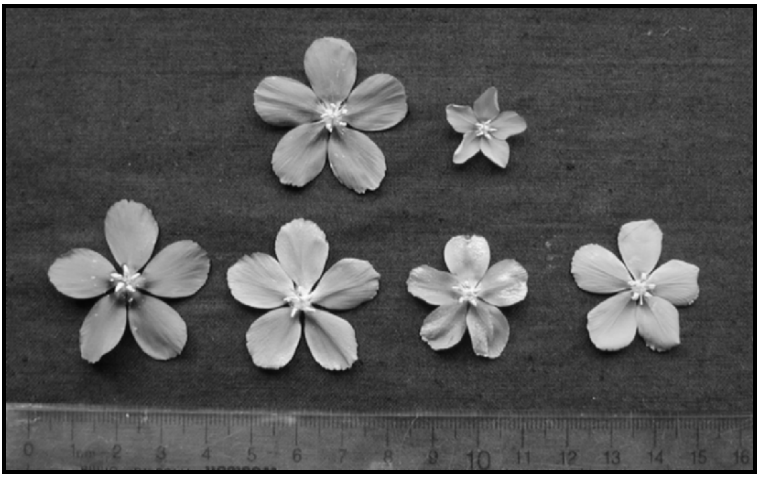

Figure 8-6. Flowers of 'Skylover' and 'Sunrise' (top left and right, respectively) and blue, violet, red and orange flowers in *Anagallis* hybrids (bottom, from left to right).

6. MARKETING

Two of the best UNH hybrids grown in 25 cm. diameter hanging baskets in greenhouse Summer trials in 2001 are shown in Figure 8-7. In Spring 2002, the first two *Anagallis* cultivars developed at UNH, 'Wildcat Blue' and 'Wildcat Orange' will be released by Proven Winners™, which specialize in vegetatively-propagated new and innovative plants. After trial of the recently-developed red and violet lines in several locations, we hope to add more cultivars to this series.

Figure 8-7. Blue and orange-flowered UNH *Anagallis* hybrids grown in 25 cm. hanging baskets in greenhouse Summer trials in 2001.

7. INVASIVENESS

Before releasing new genera or species, it is important to test for potential invasiveness. There are many documented cases where ornamental cultivars have escaped cultivation and are now weeds and a threat to natural environments. Throughout the world, *A. arvensis* is a weed dispersed by seed. It is found primarily in turfgrass, landscapes, roadsides and waste areas, particularly in sandy soils. It is less common in fields of cultivated crops (Uva et al., 1997). In Spain, which is one of the centers of origin of *Anagallis*, *A. arvensis*, *A. foemina* and *A. monelli* have been reported as weeds in maize fields and vineyards. They are described as a "botanical curiosity" in cornfields and they rarely cause serious damage to the crop because their development is prevented by the taller-growing maize (Villarías 2000). Similarly, the three species can be found in vineyards but they rarely cause important damage because they are easily controlled by cultivation (Villarías and Alvarez 2000). For control of *A. arvensis* and other broadleaf weeds, the pre-emergence herbicide Isoxaban and a combination of Trifuralin and Isoxaban are registered for use in non croplands, nut trees, ornamentals and woody plants, small fruits and tree fruits (Curran et al., 2002).

Reiter (1983) reported *A. arvensis* as a robust weed that cannot be eradicated easily in USDA hardiness zones 9 – 11 (Cathey 1990). However, he described *A. monelli* to be a "benign weed" that during cool weather months "sits patiently" and then grows and flowers with warm summer weather. He considers it a poor perennial, best treated as an annual. Clearly, *A. monelli* does not represent a potential threat as an aggressive weed, even in mild weather areas. The breeding objective of selecting for sterile or self-incompatible plants eliminates the risk of plants reseeding. Plants pulled out of the ground by mid-September never reseeded with volunteer seedlings the following year, based on data from three years of field trials (Freyre, unpublished data).

8. FUTURE RESEARCH

The genetics of flower color have been studied in different subspecies of *A. arvensis* from Britain (Marsden-Jones and Weiss 1959). Harborne (1968) reported the presence of flavonoid pigments pelargonidin and malvidin glucosides in petals of *A. arvensis*. Malvidin 3-rhamnoside, luteolin, luteolin 7-glucoside and quercitin 3-rhamnoside were found in petals of the blue *A. arvensis* f. *coerulea* (Ishikura 1981). *A. monelli* 'Skylover Blue' was shown to have almost exclusively malvidin derivatives while 'Sunrise' had primarily pelargonidin derivatives (Elsherif 2000). One of the aims of present research at UNH, with collaboration from other institutions, is to analyse the pigment contents and elucidate the genetics of flower color *in A. monelli*. Ultimately this will also include studies on the molecular

genetics of flower color using the candidate gene approach, which is a means of isolating genes known only by phenotype (Deng and Davis 2001).

Another research area at UNH involves cytogenetic studies. Chromosome numbers have been reported as $2n=2x=22$ for *A. monelli* and *A. tenella*, and $2n=2x=40$ for *A. arvensis* (Darlington and Wylie 1956; Kollman and Feinbrun 1968; Kliphuis and Wieffering 1972; Tutin et al., 1972). Other reports indicate $2n=2x=20$ for *A. monelli* (Kress 1969; Valdés 1970; Šveřepová 1972; Talavera et al. 1997). Many of the hybrids developed at UNH may have different ploidy levels. Ploidy information is crucial to determine segregation ratios for flower color.

From a horticultural perspective, and due to the fact that *A. monelli* is not a major crop, we have conducted experiments at UNH to develop production blueprints for this crop. These are aimed at optimizing conditions of daylength, pruning or use of growth regulators so that when our new cultivars are released, we will be able to provide to growers the "recipe" they need to follow to be able to grow the best-looking potted plants.

Additionally, the phenomenon flowering reversion observed in *A. arvensis,* is of scientific interest. In reverted or proliferous flowers, the meristem resumes leaf production. This is due to a change in the balance between vegetative and floral stimuli during the development of the affected flowers (Brulfert and Chouard 1961; Brulfert 1965; Battey and Lyndon 1990). Occassionally, proliferous flowers terminating in a leafy shoot can be seen in *A. monelli*, either in the Fall when daylength is decreasing or when a period of flower induction with supplemental lighting is terminated (unpublished data). Reversion can occur at any stage of flower formation. Figure 8-8 shows a case of reversion at the latest stage, when the terminal leafy shoot is formed from the placenta of the ovary, the last organ formed by the floral meristem. Some breeding lines seem to be more prone to this reversion than others. Further studies could be addressed in regards to environmental conditions that are conducive to the reversion, including scanning electron microscopy studies of the affected flowers.

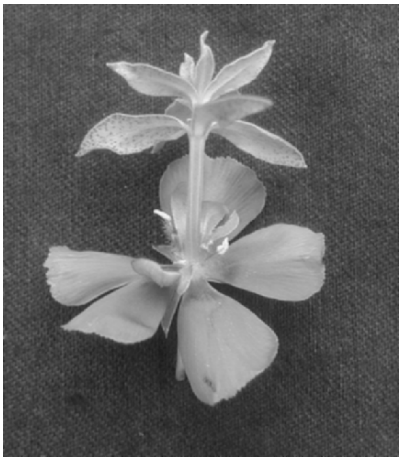

Figure 8-8. Proliferous flower type in *A. monelli*, where the terminal shoot originates from the placenta.

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Chapter 9

BEGONIA

History & breeding

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Abstract: There are many forms of begonias which are popular flowering potted plants and annual bedding plants for containers and hanging baskets, including tuberous, Elatior, Loraine, gracilis, semperflorens, and Rex. Begonias readily hybridise and many natural hybrids exist; new species are still being discovered around the globe. This exciting group of plants is well known for the colourful foliage, thick and succulent stems, and large showy flowers.

Key words: Tuberous begonia, Elatior begonia, Loraine begonia, Semperflorens begonia, Rex begonia, interspecific hybrids, shade plants, monoecy, polyploidy.

1. INTRODUCTION

'The rich colours and beautiful form of the flowers of Begonia, their striking foliage, free-growth and free-flowering habit have long ensured a place for them among favourite garden flowers. There is no week throughout the year but some species and varieties are in flower in the cool and warm greenhouse, while the many beautiful tuberous- and fibrous-rooted varieties are very popular and useful for summer-bedding' (Chittend, 1951).

Easy propagation of all begonia species might have helped to gain their popularity. Begonia can be grown from seeds, propagated vegetatively and some species form a tuber that can be stored for the winter season under frost-free conditions. For vegetative propagation cuttings, leaves or leaf parts can be used, these root easily under humid conditions. In addition to being easy to propagate, they are usually very prolific in flowering and flowers in all colours through reds, scarlets, and pinks, yellow and white and make a spectacular show all through the seasons.

N.O. Anderson (ed.), Flower Breeding and Genetics, 241–275.
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1.1 Begoniaceae

The family to which *Begonia* belongs has three other genera, *Haplophragma, Hillebrandia* and *Symbegonia* (Fig. 9-1); these have very few species each. Most of them are very hard to cultivate unlike most begonia species (Chittend, 1951). Literature contains different estimations on the number of begonia species. The most recent publication estimated the number of botanical begonias to be 1,550 and an additional 1,500 hybrids (Wagner, 1999). Some of these species and subspecies are under threat of extinction and one way of preserving them is through clonal collections and tissue culture in botanical gardens. Bowes & Curtis (1991) have described how they were able to propagate 49 lines of rare and endangered begonias.

The enormous number of *Begonia* hybrids is probably due to the fact that hybrids have been made since the day begonias were introduced in Europe. Indeed, they so readily form hybrids that they do so in nature: *B. taipieiensis* is a newly described natural hybrid from Taiwan between *B. formosana* and *B. aptera* (Peng & Sue, 2000: Chiang et al., 2001). Another begonia, *B. buimontana* Yamamoto, was considered an endemic species with a restricted distribution at elevations between 1,000 and 1,600 m in southern Taiwan. Studies on morphology, flowering habit, pollen stainability, and meiotic chromosome behaviour now suggest a hybrid origin (Peng & Chen, 1991). Morphological comparisons, distribution patterns, chromosome cytology and experimental hybridisation show that *B. buimontana* consists of F_1-hybrids between *B. palamata* and *B. taiwaniana.* The hybrid nature and meiotic abnormalities in *B. buimontana* may account for its sterility and explain in part its rarity in nature.

The genera *Begonia* is therefore a complex one to classify solely on morphological features. Modern methods have been very useful when it comes to distinguishing between different species and forming new classifications. Oginuma & Peng (2002) investigated the karyomorphology of all 14 species of Taiwanese *Begonia* to elucidate their chromosome features and chromosomal evolution. Among all species investigated, differences in chromosome features were found in: (1) chromosome number and (2) frequencies of chromosomes with secondary, tertiary, and /or small constrictions of polyploids, ranging from 23% to 63%, which was much higher than the expected value of 9%. The variation in chromosomal features was more complex than the variation in floral and fruit morphologies. Karyomorphological data also supports the recognition of five new species in Taiwan: *B. bouffordii, B. chuyunshanensis, B. pingliensis, B. tengchiana* and *B. wutaiana* (Oginuma & Peng, 2002). Based on molecular analysis, it was possible to reveal biased inheritance of the internal transcribed spacer of the nuclear ribosomal DNA in the newly discovered natural hybrid *B. taipieiensis* (Peng & Chiang, 2000; Chiang et al., 2001) and describe the polymorphisms in the hybrids.

Figure 9-1. Close up of the flowers, *Symbegonia arfakiensis.*

1.2 Discovery of Begonia

The first publication found on begonia was made by Hernandez (1651). He lived in Mexico from 1570 to 1577, and his publication was dedicated to the time he spent there. The work contained a drawing of a plant called 'Tononcaxoxo coyollin', which at later date was recognised as *Begonia gracilis* (Fig. 9-2).

The name *Begonia* is given by the Franciscan monk Charles Plumier to plants he found on his journeys to Haiti and the island Martinique. He dedicated them to Michel Begon, the man who had the direct mandate over the expeditions. Prior known Asian species were not described until Plumiers publications in 1700 (Haegeman, 1979). Michel Begon was administrator of Rochefort from 1688 until his death in 1710. This has become the home of the main *Begonia* collection in France (Wagner, 1999).

In the wild, begonias are found in all subtropical climates except Australia. Some have an extremely local distribution. Even today, new begonia species continue to be discovered and described; *B. lyman-smithii* from Oaxaca, Mexico (Burtutley & Utley, 1987), B. *ravenii* from Taiwan (Peng et al., 1988), B. *austrotaiwanensis* a new species from southern Taiwan (Peng & Chen, 1990), B. *mariannensis* is a new species from Trinidad (Wasshausen & McLellan, 1995), *B. siccacaudata a* new species from Sulawesi (Doorenbos, 2000), two new species, *B. salesoplonsis* and *B. jureiensis* are from the Atlantic coastal forest in Sao Paulo, Brazil (da Silva & Maende, 2000) and finally: *B. sillensis* subsp. *mengyangensis* was recently discovered in Yunnan, China (Tebbitt & Guan, 2002).

Today's commercially cultivated begonias are mostly hybrids between wild types from all over the world. The best reference for a botanical species, is *Begoniaceae* Part I illustrated key (Smith et al., 1986). The seeds can also help when differentiating between botanical begonia species (Lange & Bouman, 1999).

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alia fellis purgationes: Idem videmus in Viorna, cuius folia vehementer astringunt, flos tamen, & semen vrunt. Quod quidem non aliunde oritur, quam ex diuersa salis cum terra mixtione. nam si minus sit salis quam terra, sapor oritur austerus; si plus, actis. Crescit autem copia salis ex humidiore & pingui terra, in qua magnam nitri quantitatem inesse conspicimus. Vide non mirum Millefolium Americanum esse amarum & actiuz quam nostrum, cum non tam calens ibi humidus & salsius, solique cultura similis à multitudus adhibeatur.

De TOTONCAXOXO COYOLLIN, seu Riuorum Medicina. Cap. XXII.

TOTONCAXOXO COYOLLIN quam nonnulli *Atrhuapatli* vocant, seu medicinam iuxta rines prouenientem, alij *Tetzaxo coyollin*, herba est vnicum caulem proferens, coccineum, cauum, rarisq; foliis rubrescentibus, ac vitigineis refertum, nec palmitibus quibusdam rubrescentibus dissimilem. flores rubeos, & orbiculares, è pediculis longis dependentes. semen verò exile, & quod è luteo colore in coccineum vergat, inclusum vasculis trigonis, papilionum ferè forma, in eaq; figuram compositis, vt si rubrescenti ac orbiculari membranæ, circulum alterum concolorem eiusdem materiei, ita vt angulos rectos efficiant, inseruerit. radicem verò rotundam, candidam, ac totam ferè capillis inuolutam. Prouenit montanis, glareosisq; locis, regionum temperatarum, qualis est Mexicana, & *Tetzatquensis*. Amarum saporem radix præsefert, & nonnullum acorem, tertij ferè ordinis calorem nacta. eadem tusa, & infusa è liquore aliquo eidem rei vtili, expurgat intestina, semen retentum pellit, inflammatis oculis confert, & vrinam elicit. deuorata verò sesquidrachmæ mensura, humores omnes per inferna deerahit.

Inter Lapathi species hanc reponendam esse, cuius manifestum est. rumicis vires etiam respondent, quæ similes sunt ipsi Rhabarbaro. quod etiam & amarum, & acidum est.

Figure 9-2. Probably the earliest record of a begonia (Hernandez, 1651): illustration and description of '*Totoncaxoxo coyollin'*, probably *B. gracialis.*

There are very few references with respect to molecular genetic background of the different species found worldwide. Chromosome counts of some hybrids have been made, but most studies on commercially cultivated hybrids were done before molecular genetics was a common method to study plants.

However, genetic diversity and gene flow studies have been performed for B. *dregei* and B. *homonyma* (Matolweni et al., 2000).

2. CLASSIFICATION

The classification of the genus *Begonia* is divided into six groups (Haegeman, 1979).

(1) Tuberous hybrid begonias (hybrids of different tuberous begonia species from the Andes) botanical name *Begonia x tuberhybrida* Voss are commonly known as summer-flowering begonias (Table 9-1; Fig. 9-3).

(2) Loraine begonia (*B. socotrana x B. dregei*), botanical name *B.* x *cheimantha* Everett are commonly known as Scandinavian- Norwegian- or Christmas- begonias, a typical winter flowering begonia.

(3) Elatior begonias (*B. socotrana* x tuberous hybrids), botanical name *B*. x *hiemalis* (Fotsch, 1933) are commonly known as autumn or winter-flowering begonias and hiemalis begonias.

(4) Semperflorens begonias (*B. semperflorens* x *B. schmidtiana*) Botanical name: *B.* semperflorens cultorum. This type is called 'Semperflorens gracilis' in Europe and sometimes Wax begonias in the USA.

(5) Begonias with ornamental foliage are mainly *B. rex* cultivars (*B. rex cultorum* (Bailey, 1919)), but also *B. masoniana* (*B*. 'Iron Cross') and hybrids of other Mexican species.

(6) Other – those that do not fall into any of the categories above.

In Europe a specific pattern for the localisation of begonia nurseries has developed, depending on the type of begonia. Tuberous begonias are mainly produced in Belgium. Elatior begonia producers usually come from either Germany or The Netherlands, while Christmas begonia has become a speciality for the Scandinavian countries. Semperflorens begonias have the largest market share in the United Kingdom and southern Europe. Not surprisingly, the same distribution is found for breeding traditions of the different begonias.

Figure 9-3. Begonia x tuberhybrida Voss selections, illustrating the various plant habits, uses, and flower coloration.

Table 9-1. Features of the Andean tuberous species found in modern hybrids (Haegeman, 1979).

FEATURE	SPECIES	GROUP OR CULTIVAR
Tuber:	All species Largest: *B. boliviensis* Smallest: *B. dreigei*	All groups Largest: *B.* 'Bertinii' Smallest: large-flowered white cultivars
Stem: Long, glabrous Strong, erect	 *B. boliviensis* *B. veitchii* *B. pearcei* *B. davisii* *B. cinnabarina*	 Pendula group All other groups and cultivars
Flower stalk: Slender, pendulous Strong, erect	 *B. boliviensis* *B. veitchii* *B. pearcei* *B. cinnabarina*	 Pendula group All other groups and cultivars
Perianth: Acute Rounded	 *B. boliviensis* *B. veitchii* *B. boliviensis* *B. cinnabarina*	 Pendula group All other groups and cultivars
Flower colour: Red Orange Pink Yellow White	 *B. boliviensis* *B. veitchii, B. cinnabarina* *B. roseaflora* (or a colour variant of *B. veitchii*) *B. pearcei* *B. dreigei*, a white mutant of *B. roseaflora* (or *B. veitchii*)	 Small-flowered pendula group, B. 'Bertinii' All other groups
Stamens numerous:	All species	Double flowers in certain groups
Dwarf habit:	*B. davisii*	Certain *multifloras*
Leaves: Blotched with brown Large Small	 *B. boliviensis* *B. cinnabarina* *B. boliviensis*	Some yellow cultivars All large-flowered cultivars Small-flowered *pendula* group

2.1 Tuberous Begonias

B. tuberhybrida is a large and heterogeneous group of begonias compared to the other groups (Fig. 9-3). Crossing different tuberous botanical begonia species made the first varieties. Subsequent crossing and backcrossing makes the group very diversified and complex. The most complete background reference is 'Tuberous Begonias' by Haegeman (1979) (Table9-1). Tuberous begonias are found in all colours from lilac to yellow and from 0.5 m high and erect plants, to plants with equally long branches hanging down. They display the full range from totally filled to single flowers, from relatively small and up to 5 cm in diameter. Plants with single coloured or bicoloured flowers with different patterns are also found. Tubers can have different shapes and sizes, but are possibly the only feature necessary to be classified as a tuberous begonia.

2.1.1 Botanical Parents

The botanical parents, used for breeding all tuberous begonias, are a colourful choice among the botanical species. Their specific features can often be recognised in commercially bred material. There are no scientific articles on molecular classification or certification of parenthood in tuberous begonias, since most of these crosses have been done in commercial breeding companies and the origin may be a well-kept secret. However, a good look on a variety gives away many clues for tracing its botanical parents.

B. boliviensis (Fig. 9-4) is a slender growing species; its branches attain a maximum 60 cm in length. The flowers are drooping and have a dark brown-red colour. It was introduced to England in 1864.

B. cinnabarina has downy growth and cinnamon red flowers, introduced in 1849.

B. clarkei is a 60 cm high plant, with an erect growth habit and abundant large bright red flowers hanging down in racemes, introduced in 1868.

B. davisii is a natural dwarf of approximately 15 cm high. It has bluish green leaves and abundant bright red (orange) flowers with yellow stamens growing erect stalks above the leaves, introduced in 1879.

B. dregei has an erect growth of maximum 90 cm and has small white flowers, introduced to England in 1836.

B. pearcei (Fig. 9-5) grows to 20 cm high, has dark coloured leaves with pale venation and bright yellow flowers in clusters, introduced in 1865.

B. roseaflora is maximum 30 cm high. It has rose-red coloured flowers on erect stalks high above the leaves, introduced in 1867.

B. socotrana (Fig. 9-6) can be 30 cm high and has bright rose flowers. This is a winter flowering species, introduced in 1880. It is an important parent species for many commercially grown begonias (e.g. *B. x cheimantha* and *B. x hiemalis*).

B. sutherlandii (Fig. 9-7) is a slender growing species of maximum 60 cm. The flowers are copper to salmon red in colour, introduced in 1867. Distributed to the rest of Europe since 1890.

B. veichii is only 20 cm high with one single cinnamon-red flower per stalk, introduced in 1867 (Bailey, 1919).

Figure 9-4. B. boliviensis 'Bonfire' displays fiery-red flowers resembling *Fuchsia* flowers and has a cascading growth habit.

Figure 9-5. B. pearcei has yellow flowers and shiny, deeply veined leaves.

Figure 9-6. B. socotrana displays its rounded leaves outwards with shy pink flowers.

Figure 9-7. B. sutherlandii has brilliantly coloured stems, petioles, and flowers.

2.2 Christmas Begonias

'Gloire de Lorraine' (*B. socotrana* x *B. dreigei*) is the mother of all Christmas begonias. 'Gloire de Lorraine' is often referred to as 'one of the finest hybrid begonia ever raised' (Bailey, 1919). The plant is about 20 cm high with abundant pink flowers on erect stalks above the green leaves. Lemoine introduced this hybrid in 1882. There were white ('Turnford Hall' and 'Caledonia') and darker types ('Rothschilds') found. The darker flowers seem to be more popular with the growers in Norway, but sadly the darker shades have been linked to poor keeping quality of the flowers (Hvoslef-Eide et al., 1994).

The first Christmas begonia 'Glory of Cincinnati' (*B. socotrana* x Gloire de Lorraine 'Lonsdale light pink') was introduced on the market in USA in 1910 by J. A. Petterson. It was known for its very long lasting satiny pink flowers (Bailey, 1919). In Germany G. Kettenbeil made a similar cross with *B*. socotrana x Gloire de

Lorraine 'Superba', this was given the name 'Konkurrent' (Sandved, 1969). To this day, we are not certain which of these are the origin of today's different varieties of Christmas begonia (Fig. 9-8). The Gloire de Lorraine varieties used as parents for 'Glory of Cincinnati' and 'Konkurrent' both had a double chromosome number. This gave triploid varieties with backcrossing to the diploid *B. socotrana*. Triploids are naturally sterile. The varieties grown at the moment in Scandinavia are triploid mutants of either 'Glory of Cincinnati' or 'Konkurrent'. The 25 varieties tested by Sandved (1969) were all different shades of pink.

Figure 9-8. B.x hiemalis 'Schwaberland' series of Christmas begonia.

2.3 Elatior (Hiemalis) Begonias

The first Elatior begonia 'John Heal' was introduced in 1883. It was the result of the crossing of *B. socotrana* with a very early hybrid tuberous begonia 'Viscountess doneraile' (*B.* 'Monarch' x *B.* 'Sedenii'). 'John Heal' was probably a tetraploid plant with terracotta-red single flowers (Haegeman, 1979). This plant had the typical characteristics of Elatior begonias: a plant with the compact growth habit and the bright flower colour of the tuberous parent, but without a tuber or dormancy in autumn. This cultivar was produced in England, but is almost totally replaced by the German cultivars like 'Riegers Schwabenland' (Fig. 9-9). The German cultivars have improved flower longevity, mildew resistance and easy propagation by leaf cuttings. It is most likely that the cultivar 'Riegers Schwabenland' is a crossing of *B. socotrana* x *B.'* Bertinii compacta 'Leuchtfeuer' with terracotta coloured flowers (Fig. 9-10). The tuberous parent was a result of crossing between (*B. bertinii* x *B.* 'Le Falmboyante') x *B.* 'Single'. The chromosome numbers found in Elatior begonias were mostly triploid, consisting of 26, 27 or 28 long chromosomes from

the tuberous tetraploid parent and 14 short chromosomes of *B. socotrana*. Since they are triploid, Elatior begonias are sterile and are vegetatively reproduced (Doorenbos, 1973).

The commercial grower can choose from many cultivars. Most cultivars belong to one of these groups: Schwaberland group (from the company Rieger in 1961), Afrodite group (from Rieger in 1961), Nixe group (from Rieger in 1979), Ilona group (from the company Man, 1980), Barkos group (from Man in 1995), Azoltus group (from Man in 1995) (Pettersen & Aa, 1998).

Figure 9-9. B. x cheimanta Christmas begonia comes in a wide variety of flower colors.

Figure 9-10. B. 'Bertinii' possess terracotta-coloured, semi-double and double flowers.

2.4 Semperflorens Begonias

*Begonia semperflorens (*synon. *B. cucullata* var. *hookeri* (Horn, 2002)) came from south Brazil to Germany in 1821 and to Great Britain in 1828. It is a botanical species of maximum 45 cm with pale rose-to-rose flowers. *B. semperflorens* is more or less indifferent to weather and prefers relatively cooler weather (Bailey, 1919).

The semperflorens begonia, an important bedding plant species is derived from this species.

2.4.1 Semperflorens Group

The early varieties were mutations found in among the seedlings. The first new variety was called *B. semperflorens rosea* (1879), which had changed flower colour from light to deep rose. Many variations of this followed, for example the variety *B. semperflorens rubra* 'Vernon' (Fig. 9-11), which has red flowers and dark bronze green leaves. Many dwarf mutations appeared also. Those might also be the result of crossings between *B. semperflorens* x *B. schmidtiana* (Fig. 9-12). *B. schmidtiana* arrived from south Brazil to Germany in 1880 (Horn, 2002) and these two parents created many varieties made before 1900 (Zeilinga, 1992). *B. schmidtiana* has white or pale rose flowers, which grows to a maximum of 30 cm (Bailey, 1919).

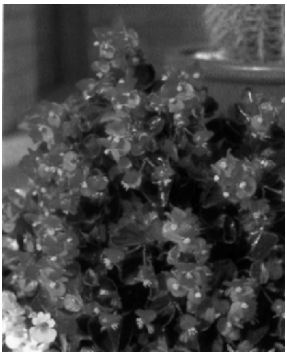

Figure 9-11. B. semperflorens rubra 'Vernon' is an example of a naturally-arising sport or mutation.

Figure 9-12. B. schmidtiana is a dwarf mutation.

2.4.2 Gracilis Group

B. semperflorens gracilis was a result from *B versaliensis* Hort. (*B. semperflorens* 'Vernon' x *B. schmidtiana*) x *B. semperflorens* 'Vernon' (Zeilinga, 1992). Their speciality is the dark bronze green foliage. This semperflorens variety should not be confused with the botanical species *B. gracilis*, which was found in Mexico, introduced on the market in 1829 and is also known as *B. diversifolia* or *B. bicolor*. This species spell out. 160 cm high with pink flowers (Bailey, 1919).

In 1903 a catalogue was printed with 117 different semperflorens begonia names and in the next 30 years breeding (1903-1933) about 100 varieties were on the market (Fotsch, 1933).

2.5 Ornamental Foliage Begonias

The botanical species *B. rex* is grown especially for its ornamental leaves. It is a rhizobious plant, the leaves are large (up to 30 x 20 cm), and the flowers tiny and pale rose. *Begonia rex* cultivars have been made by crossing the botanical species and other begonia species. Many ornamental leaf begonia varieties have been grown from the inbred lines called *B. rex cultorum* (Fig, 9-13) (Chittend, 1951).

Later other botanical species and hybrids grown for their ornamental leafs are collected under the name Begonia Rex. For example B. *masoniana* is better known as B. 'Iron cross', this plant is grown for its red brown patterned leaves.

Other rhizomatous begonias are a large botanical group. Usually they grow just below or above the ground, but some erect growing species are also found. They are grown for their interesting foliage and are flowering in winter. Recently the popularity of Rex begonias increased in the sub stream of the increasing popularity of green plants and increased attention for indoor environment.

Figure 9-13. B. rex cultorum and *B. mansoniana* collection, illustrating various leaf patterns.

2.5.1 Other Commercially Grown Begonias

Some botanical species and their hybrids, can make very interesting plants for a small market. Some examples are listed below.

B. partitia is a 30 cm high plant with lobed leaves and small single white flowers (Reimherr, 1991).

B. x *hybrida* 'Dragon wing' grows max. 40 cm high, has shiny dark green leaves and clusters of scarlet red flowers (Panamseed, 2002)

B. listada (Fig. 9-14) a small-flowered compact growing begonia, with single upright flower stalks and small flowers above the foliage.

B. *venosa* 'Velvet' is grown for its round hairy leaves that have a greyish colour and a velvety appearance.

B. *dregei* 'Minibaobab' (Fig. 9-15) is a tree-shape trimmed version of the botanical species. It has green leaves with a red venation, small white flowers and a bushy growth habit on a thick fleshy stem (Flora Dania, 2002).

Cane-like begonias can grow very tall up to 2 meters. They have cane like stems with asymmetrical sharp pointed leaves usually with silvery spots (Angelwing). The hanging inflorescences have rose coloured flowers. Those plants are seldom found in shops, but are widespread on windowsills, because they are easy to grow and to propagate (Canadian Begonia Society, 2002).

B. baumanii grows to 45 cm high and has rose-red flowers with a rose fragrance. It is a pity that all lovely coloured begonias do not have a noticeable fragrance although the genes are present in the species. But commercial breeders have not emphasised this character so far.

Organised begonia enthusiasts make their own interesting hybrids. Today, the Internet makes it easy to make contact with begonia societies worldwide.

Figure 9-14. B. listada has a very distinct leaf orientation and midvein coloration.

Figure 9-15. B. dregei 'mini Baobab' trimmed to form a topiary tree form.

3. PESTS AND DISEASES

Remarkable little breeding is done to obtain more pest and disease resistant plants. In Elatior begonia, some differences in disease resistance were found between different groups (Horn, 2002). Vegetatively propagated crops like many of the begonias accumulate fungal and bacterial diseases. Many countries have disease-free plant procedures to clean the stock plants through tissue culture and subsequent testing for diseases. In Norway there has been such a Disease-free Plant scheme since 1976. Elatior begonias were the first to enter the scheme because bacterial leaf spot and blight (*Xanthomonas begoniae*) threatened to wipe out an important ornamental production plant in Norway (Appelgren, 1984).

3.1 Insect Pests

According to literature there are five different kind pests on begonia cultures:

(1) Mealy bugs and their white cottony mass will reduce plant quality. The pest can be controlled with pesticides.

(2) Aphids damage the begonia leaves and make them more susceptible to fungi. The pest can be controlled with pesticide but biological remedies to control the population are also found.

(3) Whitefly is a pest in begonia culture. Effective chemicals should be used several times because of the mobility of the fly. The pest can be biologically controlled as well with help of nematodes. Of all chemicals evaluated by Walker (et al., 1997) only Oxamyl 10 G was found to be effective in suppressing the population.

(4) Cyclamen- and spider mite damage begonia leaves as the population grows into a pest. Resistance to miticides is large, this makes them difficult to control.

(5) Foliar nematodes (*Aphelenchiodes fragariae*) are a particular pest for Rieger and Elatior begonias. It spreads easy with water. Several remedies can be used to control the problem. Differences in susceptibility are found in different groups of Elatior begonia.

3.2 Fungi

Powdery mildew is a major disease in begonia, especially Elatior begonias. The fungus grows on the leaves and can eventually kill the plant. Different chemicals are available for control. These might damage the flower and leave white residue on the leaves. More resistant cultivars have been bred, but immunity lasted only for a short period. Vaporising sulphur is still used as an effective method.

Botrytis blight and stem rot (*Botrytis cincerea*) is a typical Elatior begonia problem. It causes brown leaves and in a severe state it kills the plant. It is very contagious because of sporulation. The disease can be controlled with chemicals. There are no reports on resistant cultivars.

Pythium crown and stem rot affect the basal portion of begonia plants. Clean soil and sanitary practices are the method to avoid this disease (Larson, 1980).

A new fungal infection was recently found in nurseries growing Elatior begonia. This is fungal infection is called *Fusarium sacchari*. It is closely related to *Fusarium oxysporum* and is very contagious. There is no remedy yet, so prevention is the only method to control this disease (Kamminga, 2002).

3.3 Bacteria

Bacterial leaf spot and blight (*Xanthomonas begoniae*) causes translucent spots on the foliage, which become blister-like dead areas. There are effective bactericides, but some damage the plants. Differences in susceptibility between cultivars have been found. Clean starting material and good sanitary methods are the best method to avoid the bacteria (Larson, 1980). Since the introduction of the disease-free plant scheme in Norway, there have only been sporadic outbreaks of bacterial blight. These outbreaks were largely due to some growers not having the patience to wait for new cultivars to go through the meristem culture system and testing for cultivar true-to-type afterwards. Hence they took some shortcuts and ended up importing both novel cultivars and the disease.

4. MODIFYING GROWTH HABITS

Many articles have been published about changing growth habit. Traditionally this has been obtained by using chemicals. Chemicals such as ancymidol and chlormequat have been used as growth reducers, and are very effective in reducing the plant height (Bævre & Moe, 1992). The growth reducing chemical Paclobutrazol was also very effective in begonia (Million et al., 1999). Chemicals can also change the male:female ratio of flowers, but this could not be changed with external application of the plant hormones gibberellins or cytokinins. In case of double flowers male flowers have more petals than the female and a ratio change in favour of the male flowers is therefore desired (Bessler, 1996). To increase the longevity of flowers in Christmas begonia, they are usually sprayed with the ethylene-trapping chemical: silver thiosulphate, STS (Fjeld, 1991).

Modifying growth habits using climate control, such as altering the day and night temperatures and the difference between them has become the modern way of ornamental production to reduce the use of chemicals. Temperature drop gave a growth reduction in Elatior begonia cultures, but had also a negative influence on the number and the size of the flowers (Grindal & Moe, 1994). The irradiation amount and quality also influences plant quality; red light gives growth reduction and increase in the number of flowers (Fjeld et al., 1993).

5. BREEDING – CLASSICAL AND MODERN METHODS

Breeding of begonias is usually done the classical way by placing the pollen of a male flower onto the stamen of a female flower. If the chromosome number is not too different this results in seed. Seeds can grow out in a new begonia plant, which can be used for breeding again, if it is fertile. Almost all varieties sold today are obtained this way. Mutation breeding of seedlings or established cultivars is also a very common way of creating new variation in the begonias. Genetic engineering has been tried with some begonias, but has not resulted in any GM cultivar on the market yet.

5.1 Classical Breeding

Begonias are easily grown from seed and they cross-hybridise quite easily. The seeds are extremely small, and they will benefit from red light during germination (Kari Boger, 2004, pers. com). This may be because the red light prevents growth of moss and algae while the tiny seeds are germinating. Because they hybridise so easily, there is almost no limitation to the potential variation in begonia hybrids that can be obtained. Spontaneous self-pollination can easily be prevented, as the

begonias are monoecieous; the female and male flowers are in separate flowers, but on the same plant. The flowers are therefore easily emasculated and large numbers of seed can be obtained from each hand-pollinated flower. In large breeding operations, the male and female parents are kept in separate greenhouses. In small operations this is not necessary because the wing-based female flowers are so easily recognisable, that emasculation of the male flowers to prevent self-pollination is really easy even before bud opening.

5.1.1 Breeding *B. x semperflorens*

Begonia x semperflorens-cultorum can serve as an excellent example of the evolution of a bedding plant species, and the significance of polyploidy and F_1-hybrids (Horn, 2002). Begonia became the first species in which F_1-hybrids cultivars were developed. Crosses between *B. semperflorens* and *B. schmidtiana* were made as soon as *B. schmidtiana* was introduced in 1880, and resulted in 1894 in 'Erforida' (syn. 'Blütenmeer', 'Rosamunde'), a uniform but nearly sterile hybrid which had to be produced anew yearly from the original parents. It showed hybrid vigour with regard to flower number and tolerance to adverse weather conditions and quite remarkably was on the market until 1964 (Horn, 2002). Early breeders then tried to select a fertile and true-breeding F_1 generation, as well as B_1F_1 progenies from backcrossing the F_1 to both parental species (Skiebe, 1966). In each case, polyploids emerged in single triploid plants originating via the occurrence of non-reducing gametes, and from further inter-pollination of virtually fertile tetraploids in F_3 and B_1F_2, respectively (Horn, 2002). In 1909 a tetraploids F_1-hybrid cultivar ('Primadonna') was introduced which surpassed the true breeding cultivars in hybrid vigour, and was produced from parent clones until 1961 (Skiebe, 1966).

Horn (2002) reported of another milestone in *B x semperflorens* breeding with the introduction of a triploid hybrid cultivar ('Rosa Tausenschön', syn. 'Rosalinde') in 1934. Due to its sterility, it had exceptionally long performance season as a bedding plant. At present, triploid cultivars are produced from crossing diploid and tetraploid populations, carefully chosen on the basis of their recombination ability and performance as F_1-hybrids. Double flowers in *B x semperflorens* is due to homozygous recessive at two loci (Reimann-Philipp & Seidel, 1963; Reimann-Philipp & Lorenz, 1978; Preil, 1974), although environmental conditions and other modifying genes can alter the expression of double flowers (Preil, 1974).

Several attempts have been made to cross *B. x semperflorens* with other begonia species. Most of these attempts have been made in private breeding companies, which is often the case with ornamentals and hence it is difficult to get precise knowledge of the heritage of many begonias. One of the examples published is the reciprocal cross made between *B. socotrana* and *B. x semperflorens* (Preil & Lorenz, 1983). They were hoping to create a bedding plant with larger flowers, as *B. socotrana* has spectacular large, pink flowers. Unfortunately, the cross was not a

commercial success; the plants could not withstand the weather conditions and summer suns (a delicate feature inherited from *B. socotrana*). We even tried it under Norwegian conditions, hoping that maybe the summer conditions were not as harsh as in northern Germany, but saw the same failure (Hvoslef-Eide, unpublished data).

Sometimes reciprocal crosses between begonia species, reveal differences between the hybrids. One such example is in the newly described hybrid from Taiwan; *B. x taipieiensis* (Peng & Chiang, 2000). They describe a unidirectional hybridisation between *B. formosa* and *B. aptera* where the only viable seeds and healthy F_1 plants only when *B. formosa* is used as the female parent. Molecular data confirmed the unidirectional hybridisation. No natural hybrid populations with a maternal origin from *B. aptera* have been detected. Abortion caused by a post-pollination barrier occurs when *B. aptera* was used as the female parent (Peng & Chiang, 2000).

5.1.2 Recreation of the Christmas Begonia (Lorraine Begonia)

The recreation of the Christmas begonia/ Lorraine begonia (*B. x cheimantha)* by crossing the original parents (*B. socotrana* and *B. dregei)* has been performed both in Germany (Zimmer, 1975; Horn et al., 1976) and in Norway (Hvoslef-Eide et al., 1992; 1994; 1995; 1997; 2000). The aim in Germany was to investigate the possibilities of creating a seed propagated Lorraine begonia. They created a triploid F_1-hybrids by (1) colchicine treatment of *B. socotrana* (*SSSS*), *B. dregei* (*DDDD*) and the Lorraine begonia hybrid (*SSDD*). Allotetraploids were then created by (2) backcrossing the autotetraploids with diploid *B. socotrana.* The reciprocal cross was made between tetraploid *B. socotrana* and diploid *B. dregei.* These backcrosses resulted in 28 different triploid progenies (*SSD*) with very different seed set and seed production (Horn et al., 1976). The method has not been commercially viable but revealed many interesting characteristics of the begonias.

In Norway the aim of the recreation of the crosses of the original parents were driven by the necessity to create novel variation in the interspecific vegetatively propagated hybrid. The breeding goal was to increase keeping quality. The Christmas begonia tends to shed their flowers after any stress induction that enhances the autocatalytic endogenous ethylene production (Fjeld, 1991). Spraying with Silver Thiosulphate (STS) two weeks prior to sale blocks the action of ethylene and prevents senescence. To comply with the overall aim of reducing the use of chemicals in ornamentals, a breeding programme was set up to increase longevity without the use of STS.

We obtained the parent plants from Botanischer Garten Hamburg (*B. socotrana)* and Royal Botanic Gardens, Kew, London (*B. dregei)* because *B. socotrana* comes from the island of Sokotra outside South Yemen, and there was a Soviet military base there at the time. *B. dregei* originates from South Africa, and Norwegians were not visiting the apartheid regime for political reasons. *B. socotrana* is a short day

plant with large pink flowers and the requirements for germination of seeds and conditions leading to flowering have been thoroughly investigated by Zimmer (1972; 1975). *B. dregei* is a long day plant with much smaller flowers than *B. socotrana*. *Rünger (1968) and Zimmer (1975) have investigated B. dregei's requirements for growth and induction of flowering*. When planning our crosses, we set up the parent plants in conditions for temperature and day length, which would lead to flowering on the same date; the Wednesday after Easter in 1990. After the Easter holidays, both parent plants had their first open flower on the exact same day. We did the crosses, using *B. socotrana* as the maternal parent and *B. dregei* as the pollinator, as was done in 1891. We germinated the hybrid seeds under red light as this seemed to prevent algae growth and hence improved germination of the very small seeds. The first step in breeding is to increase variation, and we got a large variation both in flower colour, shape and size, as well as leaf shape and size (Fig. 9-16). All F_1 plants had intermediate characters compared to the parent plants, except for flower colour, because all the 887 plants were pink, none were white. We have not selfed any plants to look for segregation in F_2 to verify that a white flower is a recessive character, but we assume that it is.

Figure 9-16. Variation in flower size, shape and colour (top left) and leaves (bottom right) in the first generation after crosses between *B. socotrana* and *B. dregei. B. socotrana* have the large pink flowers (top right and bottom right), while *B. dregei* have small white flowers (middle right on both pictures). The round, light green leaves of *B. socotrana* can be seen on bottom far right, while the two leaves above this are the two leaf variations in the paternal parent.

The 887 plants were grown and induced to flowering by a two week short day period, as normal for Christmas begonia and sprayed with increasing amounts of ethephon, which releases ethylene. We used 100, 200 and up to 400 ppm ethephon selecting for ethylene tolerance. When applying 400 ppm, only the best 10% of the plants still had flowers, the rest had abscised both buds and flowers and were discarded. The interesting, but rather unfortunate observation, was that all the dark coloured pinks were amongst the discarded 90%, in fact they were the first to abscise their flowers (Fig. 9-17).

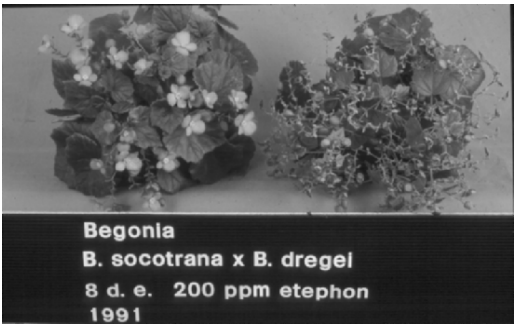

Figure 9-17. The effect of 200 ppm ethephon on abscission of flowers and buds in light pink (left) and dark pink (right) siblings of crosses between *B. socotrana* and *B. dregei,* 8 days after ethephon treatment.

We concluded that dark flowers were correlated to poor keeping quality and these characters may be coupled. It was unfortunate because the darker colour is the preferred colour by the growers. The only cultivar that came out of this work, 'Kari' (Fig. 9-18), is too pale pink to rise an interest amongst the growers, but the consumers have heard about it and it's other characteristics and have started to ask for it in the shops.

The best 10% (approx. 100 plants) were subjected to a heat treatment where the plants were given 35 ˚C for 48h and subsequently placed in individual sealed glass containers were the autocatalytic (endogenous) ethylene production was measured by withdrawing samples and subsequently analysed on a gas chromatograph. The 20 best performing were propagated *in vitro* by methods normally used for commercial propagation of Christmas begonia. Only ten clones passed this test and were grown to flowering plants and compared with the best varieties at the time, 'Hanne' and ' Emma' in a standardised keeping quality test. The comparison with the best existing cultivars revealed that none of the selected clones were improved with regards to keeping quality, but some of them equalled 'Hanne' and 'Emma'.

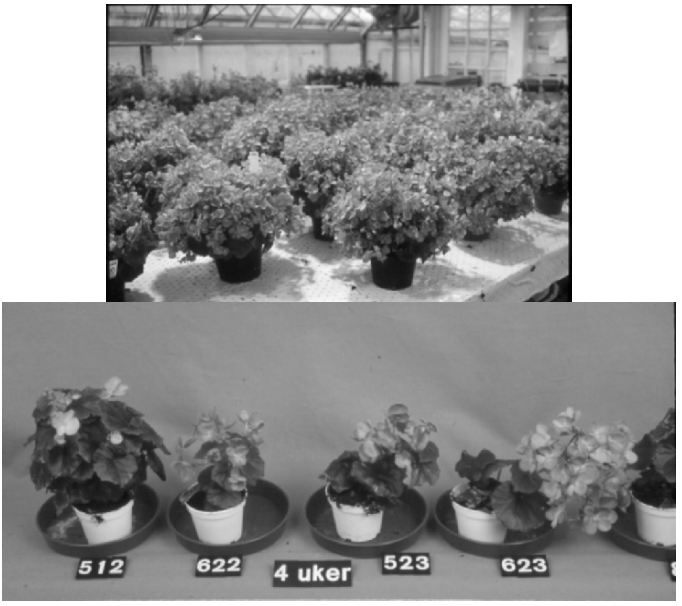

Figure 9-18. 'Kari', normal sized plants (top) and mini plants of different treatments in 6 cm pots (bottom). Mini plants can be induced to flower in 12-14 weeks after transfer to soil directly in the sale pot, with SD treatment starting 2 weeks after transfer from in vitro culture.

More than one hundred years of conscious or unconscious selection produced the best cultivars over time and our new recombination of genes had not generated anything novel that wasn't found with traditional breeding. Another explanation could be that existing cultivars are all triploid and triploidy lengthens flowering periods in begonia (Horn, 2002). The triploid cultivars have very few female flowers with the winged seed capsule, while our crosses had normal looking female flowers. We could, however, see clear differences between different parents with regards to ethylene tolerance; one of the *B. dregei* pollen-donor plants turned out to give the worst F_1 consistently. More detailed descriptions, with tables and figures are given in Hvoslef-Eide et al. (1992; 1994; 1995).

One interesting clone has come out of this work, later named 'Kari' and was registered as a new cultivar in 1997, the same year it was chosen to be the Jubilee flower for the Centennial Anniversary of the Agricultural University of Norway. 'Kari' has masses of pale pink flowers and the size of the flowers and leaves makes it suitable for mini production in 6 cm pots (Fig. 9-18). Existing cultivars would have leaves that were too large to produce a small mini plant without using masses of growth retardants, if at all possible. The variety can be grown in all sizes, flowers are abundant, the requirement for short day treatment to induce flowering is not as critical as existing cultivars and it flowers earlier.

5.2 Spontaneous Mutations

Details on the economic value of early spontaneous 'sports' or mutations are virtually absent, whereas for artificially induced cultivars of ornamentals, only few data are available (van Harten, 2002). This is explained by the large number of plants involved, the reluctance of many plant breeders to disclose the origin of their cultivars, and the fact that the local and international market for ornamentals is not easy to understand. In 1956, Wasscher reported that 70% of the wintering flowering begonias (which would be mainly Elatior and Christmas begonias) were so-called 'sports' or mutations. This is the highest figure of all the species mentioned. This is not surprising, considering the hybrid nature of these begonias and with the many colour variations possible in the Elatior hybrids, this must have been a heaven to pick new cultivars from. In Christmas begonias (*B. x cheimantha)* the cultivars on the Norwegian market today are still spontaneous (and since 1950 also induced) mutations from the backcrosses made in USA or Germany (see earlier part of this chapter) in 1910 and 1915. The cultivar 'Marina' was introduced to the Norwegian market in 1948. All the commercially grown Christmas begonias to date are descendants of 'Marina' (Sandved, 1969), with only one recent exception, 'Kari' (Hvoslef-Eide et al., 1994), made from crossing two begonia species, recreating a cross that made a species hybrid in 1891; 'Gloire de Lorraine' (see above).

5.2.1 Chimeras

Winkler (1907) introduced the term 'chimera' for graft-hybrids that arise when a scion of one plant species is grafted onto a rootstock of another species. Baur (1909) proposed to apply chimera to all situations in which the somatic tissue of a plant is not genetically homogeneous and this interpretation has been applied since (van Harten, 2002). Mutation breeders and breeders who use genetic transformations have to be well aware of the fact that mutations and transformations occur as single-cell events. When starting from multicellular plant material, in nature as well as after successful mutagenic/transformation treatment, each mutation is in principle present in one cell only. An important prerequisite for successful mutation breeding is to understand the fate of the mutated cell (van Harten, 2002). A mutated cell in the middle of the original plant is by nature a chimera.

Broertjes & van Harten (1978) have a beautiful set of illustrations on how a mutation is a single-cell event, where one cell has mutated from a light pink to a darker pink in a micrograph of a flower. Later, this cell has divided and becomes a distinct area of mutated cells, sharply divided from the original light pink background. The same figure goes on to describe how a small sector in a purple flower has mutated to yellow, the next picture show this yellow sector has produced an axillary shoot with all yellow flowers. When this shoot is used as a cutting, the new plant is a solid yellow, and no trace of purple flowers anywhere. Yellow is one

of the precursors of purple pigments and if a mutation shuts down the expression of one of the genes coding for enzymes, responsible for the conversion of yellow further down the pigment synthetic pathway, then the yellow can occur in the middle of a purple flower. Flower colour is a very visual form of chimera; plants can be chimeras for all the other characters too, but this is often difficult to recognise. Such chimeras are crypto-chimeras and may be more common than we realise. Some of the somaclonal variation described may be attributed to such crypto-chimeras, where an *in vitro* culture can reveal the concealed phenotype through the development of adventitious shoots from different sectors in the plant. This could well be the explanation of why adventitious shoots are regarded as more prone to result in somaclonal variation than axillary shoots are. Axillary shoots originate from all three-cell layers (L-I, L-II, L-III) in the plant and are the only way to successfully propagate a chimera.

The three cell layers can be described as a hand with two gloves; the L-III layer is the hand and L-I and L-II are the gloves, one on top of the other. L-I and L-II divide in one direction, while L-III divides in all directions and forms the core of the plant. Lineberger & Druckenbrod (1985) have described in detail the nature and propagation of chimeras in *Saintpaulia*; the pinwheel flowering types can only be propagated true-to-type by axillary shoots *in vitro,* or by dividing the plant. Adventitious shoots from leaf cuttings or adventitious shoots *in vitro* will lead to a break-up of the chimera, as they almost always descend from L-I.

An old Elatior begonia cultivar 'Aida' (Fig. 9-19) is a chimera with salmon pink flowers and white edges. Like all chimeras, it could only be propagated through top cuttings (axillary) and not leaf cuttings (adventitious). Preil (1994) describes the break-up of chimeral poinsettia cultivars through cell cultures and somatic embryogenesis, some of these were visual (like pink and variegated bracts), while others were crypto-chimeras and only showed up after the break-up.

Figure 9-19. 'Aida', an old Elatior begonia cultivar with salmon pink flowers and white edges (left flower). Sometimes, such a chimera will produce a shoot from just one or two of the cell layers and give rise to another flower colour, like in this case, the white to the right, most probably originating from the white L-I layer of this plant. (Photo credit: Erling Strømme).

5.3 Induced Mutations – Mutation Breeding

Nuclear radiation and chemical mutation can be used to obtain new varieties. This is a valuable tool in breeding vegetatively propagated species, especially ornamentals as they can create a whole 'family' of closely related varieties that have the same growth and production requirements, but differ only in colour variations. In this way, large greenhouses can be filled with plants with the same production scheme (to suit the growers), but still be able to offer the whole range of colours (to suit the consumers). To obtain mutants in begonia, detached leaves forming adventitious buds can be used. Adventitious shoots in begonia originate from single cells and hence the adventitious shoots will predominantly be solid mutants rather than chimeras. The detached leaves are irradiated with a dose of 1,5 to 2,5 kR (Broertjes, 1977). Hiemalis, or Elatior begonias can serve as good examples of this breeding method. As soon as a new crossing has been made to create a broad base of variation, the breeder will select the best of these based on their most desired characters, mostly plant architecture, growth rate, flowering abundance, and maybe disease resistance. Then the selected variety undergoes a series of induced mutations to initiate the variation of flower colour to create a 'family' of Elatior begonias offering the whole range of colours that can be grown under identical growth conditions. The yellow cultivar 'Tiara' was created through mutation breeding (Doorenbos & Karper, 1975).

The FAO/IAEA database contains 25 releases of begonia mutants as new varieties. Half of these have a different flower colour, other new features are: different flower architecture, differences in the leaves or the growth habit like dwarfism. All new varieties registered in the FAQ/IAEA database are dated between 1972 and 1983 and made at different places in the world (FAO/IAEA, 2002).

5.4 Somaclonal Variation

Propagation of begonia *in vitro* has been done since the early start of the use of *in vitro* techniques. Larkin and Scowcroft (1981) to describe the genetic variation in plants regenerated from tissue culture, and launched as a novel way of creating variation first used the term somaclonal variation. It has not really fulfilled the expectations in breeding of food crops, but may still be a valuable tool for the ornamental breeder. Although quantitative data are scarce it is often that plants generated from the adventitious meristems are genetically different from the mother plant. Attempts have been made to measure the genetic differences on both molecular- and phenotypic-level. Measuring the coefficient of variation of for example leaf shape gave most accurate results in Elatior begonias. This technique was found to be more accurate and less time consuming than RAPDs technique and

required less material than calculating the percent of aberrant plants (Bouman & de Klerk, 2001).

For mass propagation somaclonal variation is unwanted, but for breeding it could have some potential. When first discovered, little was known about the nature of somaclonal variation. All possible types of genetic variation have been observed, but the difference between genetic and epigenetic variation is not a black and white phenomenon, more a matter of degree. The state of methylation can be influenced and influences the plant phenotype, but this can be reversible. It is believed that methylation can be induced by stress imposed under tissue culture conditions. Results of research on begonia strongly suggest the occurrence of alterations in methylation.

Leaf variegation in combination with sectoral chimeras is often found after a callus phase. Only a small part of those show their aberrances after regeneration. Other variants with some potential for ornamental value were also found, for example dwarfs, minis, and creepers. Those aberrances remained after regeneration (Bouman & de Klerk 1997). Cassells & Morrish (1987) reported of long-term callus cultures of *B. rex* producing a higher number of polyploid plants when kept on a high dose of the artificial auxin 2,4-D. In our laboratory in Norway, novel leaf and flower colour variants arose after *in vitro* culture in a tuberous begonia; *Begonia tuberhybrida* ‘ ‘ (Sivertsen, pers. com). One of these became a commercial variety, ‘Flamme’, the other did not (Fig. 9-20).

Figure 9-20. B. tuberhybrida ‘Karelsk jomfru’, normal flower colour (left), a somaclonal variant with darker flowers (middle) and another somaclonal variant with darker flowers and darker leaves, ‘Flamme’ (right). These variants emerged after shoot tip culture in a disease-free plant scheme at the Agricultural University of Norway. (Photo credit: Astrid Sivertsen).

5.5 In Vitro Selection

In vitro selection can be a very powerful tool when breeding for characters that are expressed in culture, or that correlates with characters that are expressed. Hundreds of plantlets can be tested simultaneously in a small vessel. Repeats can be performed in a small space. During our breeding programme for increased keeping quality of Christmas begonia, we initially thought we would be doing mutation breeding and we developed a selection method for ethylene tolerance (Hvoslef-Eide et al., 1992). The idea was to see if the ethylene response of shoots *in vitro* would correlate with the ethylene response of the flowering plants in standardised keeping quality tests developed for Christmas begonia by Fjeld (1991). We used sterile filters and moisturised 0.15 ppm ethylene gas into *in vitro* cultures of Christmas begonia of three different varieties; 'Emma' and 'Hanne' as the best performing, 'Elfrid' as the representative for poor keeping quality. The *in vitro* plants were exposed for 48 h. The flowing days, we kept a close eye on the development of the *in vitro* shoots and scored them for colour every 24 h. Eight to twelve days after exposure, we observed the largest and significant difference between the cultivars and their response correlated very well with the keeping quality tests on flowering plants (Hvoslef-Eide et al., 1992). We have later done the same experiment with the clones from the crosses between *B. socotrana* and *B. dregei* and verified the correlations (Hvoslef-Eide, unpublished data).

5.6 Genetic Transformation

When our attempt to breed a better variety for keeping quality through conventional crosses failed, we started to look for collaborators who could help us with genetic modification. Hence, the first publication on a transgenic begonia was that of Einset & Kopperud (1995), in which the species was mistakenly named *Begonia x hiemalis.* It was actually *Begonia x cheimantha,* Christmas begonia. 'Hanne' was transformed with a gene from the ethylene biosynthetic pathway; coding for ACC oxidase. Although this gene did not reduce the plant's endogenous production of measurable ethylene (Einset & Kopperud, 1995; Hvoslef-Eide et al, 1995), the transgenic plants did respond positively to the foreign gene. Statistical differences could be measured in keeping quality tests (Hvoslef-Eide et al., 1995). Fig. 9-21 shows transgenic and control plant in flowering state, the control clearly showing cup-shaped flowers (the flowers never open fully, but senesce not fully opened), the first sign of ethylene influence and premature senescence. Since, we have found that the increased keeping quality is statistically comparable with plants sprayed with silver thiosulphate (STS) to increase longevity (Hvoslef-Eide & Fjeld, manuscript in prep). This GM has the potential of being introduced on the market, since it could replace varieties that need chemical sprays with the silver-containing STS with the same benefit to the consumer, provided the plants are not subjected to

exogenous ethylene, in which case autocatalytic ethylene production could get triggered (Fjeld, 1991).

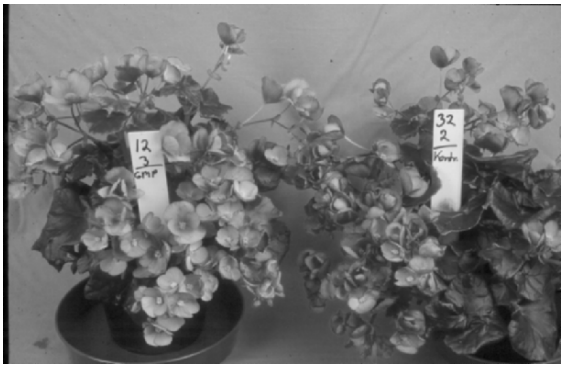

Figure 9-21. 'Hanne' (right) and transgenic 'Hanne' (left) after 8 weeks keeping quality test under simulated interior conditions. Note that the transgenic 'Hanne' has far less cup-shaped flowers than the original cultivar. Cup-shaped flowers in begonia is a clear, first sign of ethylene stress.

Genetic modification of *Begonia x tuberhybrida* was reported in 1996 by Kiyokawa et al. and then *Begonia x hiemalis* by Kishimoto et al. in 2002. Both these were using GUS as a reporter gene (Fig. 9-22), and Kiyokawa also used *rolC*. The method of transformation in all these three cases were *Agrobacterium tumefaciens,* strain LBA4404 for all three and in addition AGL0 for *B. x hiemalis.* The transformation frequencies were very variable; Einset & Kopperud (1995) obtained one plant (pers. comm.), Kishimoto et al. (2002) got between 0 (LBA4404) and 3.2 % (AGL0) and Kiyokawa et al. (1996) obtained 24% frequency.

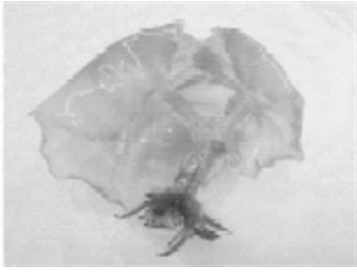

Figure 9-22. B.x hiemalis expessing the GUS gene (Kishimoto et al. 2002).

These three reported transformations show the potential of genetic engineering in three begonias species. Even if transformation frequencies can be low, the vegetatively propagated begonias are easily mass propagated to reach the market soon after the final tests of the transgenic plants have been performed. In all three cases, *Agrobacterium tumefaciens* was used as the vector of the novel genes. Begonia readily forms adventitious buds and *Agrobacterium* is a suited method of transformation (Deroles et al., 2002).

6. MASS PROPAGATION OF NOVEL BEGONIAS

Propagation of a novel cultivar is the last important step in any breeding programme. In ornamentals, much of the profits lie in getting a novel cultivar on the market as fast as possible, since novelties are especially valued. Mass propagation of ornamentals is a large industry worldwide.

6.1 Seed Propagation

Of the main commercially important begonias, the *Semperflorens* group is the only one that is seed propagated. This group on the other hand, was the first to be introduced as F_1 hybrid seeds on the market in 1894 (Skiebe, 1966). Hybrid seeds are sought after for two reasons; (1) is to take advantage of any hybrid vigour, i.e. that the progeny exceeds both its parents in certain characters, (2) to ensure an even, homogenous crop that flower at the same time and can be marketed at the same time. The hybrids are produced by inbreeding (selfing) parent lines for as long as it takes to obtain a homozygous parent line (often 6-7 generations), or as long as the species can endure it without being adversely affected by inbreed depression. When the inbred lines are obtained, these are crossed and the first progeny is the F_1 hybrid. It is relatively easy to produce F_1 hybrid seeds in begonia, as the plants are monoecious and the separation of male and female flowers is easy. Even before the petals open, the winged base of the female flower can be observed and unwanted flowers on a plant can be removed. Still, hybrid seeds do require more manual labour than open pollinated varieties and hence are more expensive. For the breeders producing F_1 hybrid and keeping the parent lines in-house is the safest way to have control of the breeding and ensuring that no one can use their own seeds to produce the same variety. See Horn (2002) for a more detailed description of a hybrid scheme.

6.2 Vegetative Propagation

Many of the important begonia species, and especially species hybrids, are vegetatively (clonally) propagated to maintain the variety. Many of the species

hybrids are sterile and can only be propagated vegetatively. Even if they were not sterile, any progeny from seeds would segregate into a whole new combination of the plant's characters and not be the variety sought. The traditional way of vegetative propagation is to take cuttings (top or leaf cuttings) or divide tubers of tuberous begonias. This has been the successful method of propagation over more than a century of begonias on the market. Occasionally mutations have occurred, and if an improvement, they have become new varieties. The variety owner would maintain a stock of true-to-type mother plants to supply propagators with the best starting material possible.

In Christmas begonias, problems in the propagation may occur when the temperatures of the greenhouses where mother plants are kept, rise in the early summer. Heide (1964; 1965a and b; 1967; summarised in 2000) have performed careful studies with mother plant treatments and the effects on regeneration ability of leaf cuttings. He found that the endogenous hormone levels were unfavourable for adventitious bud break in leaf cuttings if the mother plants were grown at higher than 20 ˚C (too high auxin and too low cytokinin). This explained the experiences growers had if they did not shade their greenhouses well enough in spring; the leaf cuttings would develop roots, but did not produce any shoots and a rooted leaf is of no use to the propagator. The advice to lower the temperature in the greenhouses for mother plants worked well for many years, until a more efficient and faster way of producing Christmas begonias was developed by Valso (198?). This method of shorter production time, had the consequence that the growers wanted their rooted cuttings delivered later in the season. The growers could produce a quality product in 12-14 weeks with supplementary lighting, which normally would take 18 weeks without supplementary light. Christmas comes at the same time every year, so the propagators had to shift their production to 4-6 weeks later in the season. This caused difficulty as the temperatures in their mother stock increased above 20°C. They tried to solve this by using top cuttings (which already have buds) instead of leaf cuttings. This method has two major drawbacks: (1) it needs much more mother plant material, as there are fewer top cuttings on a mother plant than leaf cuttings, (2) the top cuttings do not produce high quality plants, their growth is not compact enough. To resolve this, the growers then started using *in vitro* propagated material with great success. Today all Christmas begonias in Norway are produced from *in vitro* shoots, produced solely by one laboratory in Sweden (Katrinebergs Handelsträdgard, Frövi, Sweden). It is still important to consider mother plants treatment by optimising temperature and daylength. The recommendation is to give high supplementary lighting to flowering plants (induced by short days or low temperature). In addition it is important to use only half strength of Murashige & Skoog's (1962) medium (Borgen, 1983). The *in vitro* shoots can be produced either on solid medium or in liquid. A preliminary trial with the use of bioreactors to multiply shoots was successful and use of bioreactors does not seem to induce

higher degree of somaclonal variation than on solid media (Hvoslef-Eide, unpublished data).

7. FUTURE PROSPECTS

Given the enormous variation in begonia species, their ability to hybridise and the diverse uses (bedding plants and pot plants), it is hard to imagine a future without them having an important place in the world's ornamental industry. Since they are propagated both by seed and vegetatively, depending on species and hybrids, most of the breeding techniques have been and still will be applicable. Begonias are susceptible to many pests and diseases and would probably benefit from genetic modifications to introduce disease resistance/tolerance from the increasing number of available resistance sources/genes.

Judging from where the papers are coming out on genetic transformations, Norway and Japan seem to be the countries with the most focused interest on taking begonias into the genomic era. Since three of the main begonia species have been transformed successfully with *Agrobacterium,* the next natural step would be continue along this road and introduce transgenic begonia with less need for chemical sprays. In Europe, there is great scepticism towards genetically modified food products. USA, Canada, China and other countries have been far more willing to see the benefits of genetic modifications. Maybe the consumers in Europe would be less sceptical to GM products if they could see the benefit for themselves of a product that they are not going to eat or easily spread into nature because they are not indigenous in the European environment. We believe that ornamentals could be a door opener, especially if the consumers could see the benefit themselves in getting a product that have had less chemical sprays or less susceptibility to diseases that normally would be detrimental to the crop.

However, genetic modifications merely open up a new toolbox for breeders, and the other breeding methods will still be important. Moreover, there are more biotechnological methods that could find potential uses in begonias, such as marker assistant (MAS) breeding and other genome techniques that could go into this important group of ornamentals. Begonia breeders have been in the forefront for more than a century and hopefully keep up their good traditions in the years to come.

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Chapter 10

IMPATIENS

Impatiens wallerana

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Abstract: *Impatiens wallerana* Impatiens continues to lead as one of the three most important bedding plants worldwide. It is available as both seed and vegetative products. The history of its development, marketing success, wide adaptability, a large palette of flower colors and forms, and unparalleled garden performance as a bedding plant for shade are described as an aid for continued crop development with breeding lessons for other flowering crops. Many other *Impatiens species* are available for commercialization as separate products (e.g. New Guinea impatiens, *I. hawkeri;* bush balsam, *I. balsamina*) or for use as parents for interspecific hybrids. The challenges of breeding a crop within the Balsaminaceae, with explosive seed dispersal mechanisms, are discussed. Important traits for continued crop improvement include seed germination, flower color, earliness, flower patterns, flower size, foliage color, pack performance, garden performance, double flowers, habit and branching. Insect and disease resistances, sun tolerance, and new flower colors are also important traits for new or improved series.

Key words: INSV, interspecific hybridization, self compatibility, sterility systems, transformation.

1. INTRODUCTION

Impatiens wallerana is one of the most important and most widely grown bedding plants in the world today. Previously known as *I. sultani* or *I. holstii*, it has several common names including “Busy Lizzie” and “Patience Plant”. It is one of two important groups of Impatiens in the Horticulture industry, with the New Guinea Impatiens (*Impatiens hawkeri*) group being the other. It should be noted as well that there are many other impatiens species grown to a lesser degree that can be

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found in the industry, and which will not be covered in this chapter. Among these, *Impatiens balsamina*, is frequently found in seed catalogues, and is typically described as suitable for "cottage gardens". Other species can be found from specialty nurseries and may be available from both seeds and cutting.

Despite being one of the most important bedding plants in the world, information on *I. wallerana* is relatively scarce. Various private companies have generated the vast majority of research and information, although most of this data is protected as "trade secrets". Even at the national level in the United States, the U.S. Department of Agriculture did not report statistics on I. wallerana until 1994 (see http://usda.mannlib.cornell.edu/reports/nassr/other/zfc-bb/). Sporadic data is available from the Census of Horticulture Specialties, starting in 1970 and continuing through 1998 on ten-year intervals. In 1970 the Census reports a total of 9.6 million plants were sold at a total value of US$1.9 million. By 1988 the total value of all impatiens grown in the United States was reported at UA$68 million, and by 1998 this number had reached almost US$200 million (http://usda.mannlib.cornell.edu/reports/nassr/other/zfc-bb/).

Impatiens wallerana is one of only a few bedding plants available in almost all colors. This, coupled with its preference for the shade and adaptability to containers, has contributed to a sustained high level of worldwide popularity for this plant. New breeding advances continue to add interest to the class. Its easy culture and the wide availability of seed or cuttings make this plant a favorite among both growers and consumers.

2. SPECIES ORIGIN & CENTERS OF DIVERSITY

The genus *Impatiens* contains over 600 species, half of which occur in the Indian subcontinent (Gill and Chinnappa, 1977). Jones and Smith (1966) proposed the Himalayan region as the center of origin. The major centers of diversity are in the highlands and mountains of tropical and sub-tropical Africa, Southeast Asia and the Indian sub-continent. There are only a few Holarctic species, and none indigenous to South America or Australia (Zinov'ea-Stahevitch, and Grant, 1984).

Impatiens wallerana, also known by the names *I. sultani* Hook. f., and *I. holstii* Engl. is native to areas of Eastern Equatorial Africa including Kenya, Tanzania, Mozambique Malawi and Rhodesia; as well as Zanzibar and Pemba Islands. Generally *I. wallerana* can be found in damp or moist shady locations in forests and forest margins or along river margins. Significant variation in leaf shape and flower color has been observed (Grey-Wilson, 1980a). As many as ten species are contained within the *I. wallerana* aggregate described by Grey-Wilson (1980a): *Impatiens cinnabarina* Grey Wilson, *I. hamata* Warb., *I. messumbaensis* G.M. Schulze, *I. pseudohamata* Grey-Wilson, *I. pseudoviola* Gilg., *I. serpens* Grey-

Wilson, *I. sodenii* Engl. and Warb. ex Engl. (=*I. oliveri*. and *I. magnifica*), *I. thamnoides* G.M. Schulze, *I. usambarensis* Grey-Wilson, and *I. wallerana* Hook. f.

3. HISTORY & DOMESTICATION

Though seven species of Impatiens were mentioned in 1753 in Linnaeus's 'Species Plantarum', it is likely that *Impatiens wallerana* didn't arrive in Europe until 1896. It was brought to England through the efforts of Dr. John Kirk, who was a physician and naturalist who spend many years travelling Africa as part of Dr. Livingstone's expeditions. *I. wallerana* was named after Horace Waller, a missionary and member of the Royal Geographic Society, who was responsible for publishing the journals of Dr. Livingstone (Anonymous, 1995).

Breeding in impatiens began in the late 1940's, with Bill Marchant and Bob Rieman among the first to work on this crop. Much of their germplasm was passed to Claude Hope who did his breeding work in Costa Rica at his farm "Linda Vista" (Figure 10-1) (Anonymous, 1987). Claude is widely regarded as the father of modern impatiens, conducting his first breeding work on *Impatiens wallerana* in 1961 (Hope, 1993). By this time Hope had already established himself as an accomplished breeder with significant work in petunias and snapdragon, and he was producing impatiens seed at this time for the Ball Seed Company.

Figure 10-1. Claude Hope, 'El Capitan' as he was known to his co-workers, working at his farm in Costa Rica.

Early breeding and domestication work progressed slowly, with open pollinated varieties first becoming available, and later, F_1 hybrids. Early breeding efforts were inhibited by the lack of a good red color, as well as the fact that white and purple had not yet been observed. In the 1950's, it was mostly open pollinated varieties, which were available, with only a few hybrids. The first hybrid on record is 'Pixie White', introduced in the 1958 Ball Seed Co. catalogue, followed in 1961 by the first members of the "Jewel" series (Anonymous, 1995). None of these were of particularly high quality by today's standards, but were significant achievements in their time. In 1960 Sluis en Groot offered for sale an F_1 hybrid impatiens of merit, "Imp". This was followed a few years later by the "Shadeglow" series offered by the Joseph Harris Company. The "Shadeglow" series had somewhat limited availability. Sluis en Groot followed up with the next significant improvements in the "Minarette" series. All of these series were rather tall, and though they did flower well, they were still on large-leafed plants, which had limited branching. At some time, while these series were being sold, Claude was able to breed a truly dwarf plant with a symmetric habit and good basal branching. In 1968 Claude was responsible for the introduction of the original "Elfin" series which was available in eight colors. This series was originally trialed at Michigan State and Purdue Universities, but was quickly made available for commercial sales. It was quickly followed up with the "Super Elfin" series which is still available today, though the genetics have continued to be improved. Only Sluis en Groot was also breeding impatiens at this time, and introduced series such as "Fantasia", "Futura" and "Shady Lady". Other companies which are today strong in Impatiens did not enter the F_1 Impatiens market until somewhat later (Hope, 1993).

In 1968 Goldsmith reviewed current breeding of F_1 hybrids and discussed breeding efforts in petunia, snapdragon, marigold, zinnia and geranium. He briefly mentioned that F_1 hybrid seed was available in impatiens. At that time, petunias were recognized as the most important bedding plant. In just twenty years impatiens went from a novelty crop to the number one bedding plant in the United States. Numerous seed-propagated series and cultivars are available on the market, from single to double flowered with numerous flower colors (Table 10-1). Popularity in Europe was increasing as well. At this time, five companies were acknowledged as breeding impatiens: Linda Vista/PanAmerican Seed, Goldsmith, Sluis en Groot, Harris Moran and Bodger Seeds (Anonymous, 1987). In 1992, the farm gate value of impatiens was estimated at US$200-250 million (wholesale) (Leue, 1995).

Table 10-1. Some major *Impatiens wallerana* series, their source, and number of flower colors on the world market as seed-propagated products.

Series Name	Breeder/Distributor Seed Company	Approximate number of flower colors
Single Flowered		
Accent	Goldsmith	27
Blitz 2000	Syngenta	9
Cajun	Syngenta	13

Series Name	Breeder/Distributor Seed Company	Approximate number of flower colors
Candy	Benary	16
Carnival	Daehnfeldt	17
Dazzler	PanAmerican/Ball	22
Deco	PanAmerican/Ball	8
Expo	PanAmerican/Ball	27
Impact	Sakata	13
Infinity	Sakata	13
Impulse	Syngenta	21
Super Elfin	PanAmerican	22
Tempo	Bodger	28
Double Flowered		
Carousel	PanAmerican	4
Confection	Harris Moran	4
Fanciful	PanAmerican	5
Tutu	Floranova	2
Victorian Rose	Goldsmith	1

4. BOTANY & CYTOLOGY

Impatiens are members of the family Balsaminaceae (order Geraniales), whose only other member is the monotypic genus *Hydrocera*. There are approximately 550-600 species within the genus *Impatiens* (Khoshoo, 1957, Gill and Chinnappa, 1977).

Though flower size and shape vary considerably, flowers are always zygomorphic. Stamens are fused and anthers project downward acting as a brush on the pollinator. The fused anther cone blocks access to the stigma and self-pollen generally does not come in contact. Anthers are shed as a single unit prior to the stigma becoming receptive. Ovary growth tends to push the anther cone off. However, self pollination (self compatibility) does occur in some species including *I. wallerana*, and certain species are cleistogamous (Clevenger, 1971); within a species these characters may be genotype specific. Pollinator access to the spur and nectary is gained from below the anthers. Impatiens are protandrous, with the anthers generally maturing one to three days prior to the stigma being receptive (Grey-Wilson, 1980a). Pollinators are known to include butterflies, birds, bees (Grey-Wilson, 1980a), along with moths and beetles (Clevenger, 1971).

Impatiens chromosomes have been one of the more widely studied aspects of the genus. This may be due to the fact that chromosomes can be counted from anthers on herbarium sheets since chromosomes go into a resting stage at Metaphase I of mitosis and stay in this stage until released from the plant (Gill and Chinnappa, 1977).

Within the genus there is a wide variety of chromosome numbers, including n=3-5, 6-7, 8, 9, 11, 13, 17 and their multiples (Gill and Chinnappa, 1977).

Zinov'ea-Stahevitch, and Grant (1984) studied 44 taxa within the genus and reported chromosome numbers of *n*=3,6,7,8,9,10,13,16,17,18,20 and 24. They acknowledge that numbers of up to n=33 have been found, and that these represent a number of dysploid, aneuploid and euploid series. Base chromosome numbers of *x*=7 (8 taxa), *x*=8 (14 taxa) and *x*=10 (7 taxa) were most common in their work, and this correlates with previously published data. Khoshoo (1957) proposed a base chromosome number for the genus at *n*=7. Jones and Smith (1966) proposed a base chromosome number of *n*=8 for Impatiens from the African continent. Jones and Smith acknowledged that *n*=7 and *n*=10 also appeared to be important base chromosome numbers.

As for *I. wallerana*, Zinov'ea-Stahevitch, and Grant (1984) determined that n=8, and 2*n*=2*x*=16. This report includes three distinctly different specimens within the species. Gill and Chinnappa (1977) had proposed a base chromosome number of *n*=10, though they acknowledge previous reports where *n*=8. Generally *n*=8 is widely thought to be the correct base chromosome number for *I. wallerana*. The ploidy levels of related African and Asian species are provided (Table 10-2) for use in interspecific hybridization with *I. wallerana.*

Table 10-2. Impatiens species (African and Asian) and their ploidy levels which may be useful to plant breeders in crossing with diploid *I. wallerana* (*n=8, 2n=2x*=16) (Data from Zinov'ea-Stahevitch and Grant, 1984).

Impatiens Species	Ploidy Level
cinnabarina Grey Wilson	*n=8, 2n=2x*=16
dalzellia Hk.f. et T	*n=8, 2n=2x*=16
pseudoviola Gilg.	*n=8, 2n=2x*=16
sodenii Engl. and Warb. ex Engl.	*n=8, 2n=2x*=16
usambarensis Grey-Wilson	*n=8, 2n=2x*=16
wallerana Hook. f.	*n=8, 2n=2x*=16
flanaganae Hemsl.	*n=16, 2n=2x*=32
gordonii Horne ex Bak.	*n=16, 2n=2x*=32
platypetala ssp. *nematoceras* (Miq,) Steen	*n=16, 2n=2x*=32
garneriana Wt.	*n=16, 2n=2x*=32
kleinii W. et A.	*n=16, 2n=2x*=32
levingei Gamble ex Hk.f.	*n=16, 2n=2x*=32
oppositifolia L	*n=16, 2n=2x*=32
viscida Wt.	*n=16, 2n=2x*=32
goughii Wt.	*n=16, 2n=2x*=32
linearifolia Warb.	*n=16, 2n=2x*=32
niamniamensis Gilg.	*n=16, 2n=2x*=32
schlechteri Warb.	*n=16, 2n=2x*=32
viscosa Bedd.	*n=16, 2n=2x*=32

5. HYBRIDIZATION MECHANISMS

5.1 Pollination Process

The *I. wallerana* flower consists of five fused anthers that surround an ovary that has a five lobed stigma at maturity. Mature anthers tend to fall off one to three days before the stigma is receptive, though some degree of self-pollination may occur. Generally emasculations are not necessary, but can be preformed if needed when the flower just begins to open. Care must be taken as it is very easy to damage the ovary. Pollinations can be attempted when the lobes become distinct on the stigma. There are many methods to apply pollen, including flower to flower. Seed matures in 14-21 days depending on temperature, and the swollen pods must be harvested before they "pop", forcibly dispersing their seed (Watts, 1980). Alternatives include "bagging" the fruit to collect the fully mature seed. Merlin and Grant (1985) describe a system of using a drawstring cotton crossing bag to prevent unwanted pollinations, and using a plastic sandwich bag with a twist tie to collect fully mature seed from "exploding" fruit. Several other methods have been tried by researchers, with the simple goal of preventing the widespread dispersal of seed. Generally there is minimal dormancy in impatiens seeds, and the seed may be sown soon after harvest.

5.2 Sterility Systems

Claude Hope discussed a genic male sterile system that was actually an obstacle to his early breeding work (Hope, 1993). While it was a frustration to his breeding efforts, Claude acknowledged the value of this system in hybrid seed production. Since impatiens are easily vegetatively propagated, sterile plants can be propagated and used as the female parent, thereby eliminating the risk of contamination through self-pollination (Hope, 1993). While less useful from a breeding perspective, female sterility is also known to exist in *I. wallerana*. Based on UV absorption curves, lack of chlorogenic acid in stigmatic exudate was implicated as a likely cause of female sterility by Namboodiri and Tara (1972).

Tara and Nanboodiri (1974) identified both male and female sterility within diploid mutant plants of *I. wallerana* found in India. Regarding male sterility, heritable irregularities were observed in most stages of meiotic and post-meiotic pollen formation, with most occurring during or after anaphase I. Among the abnormalities were chromosome bridges, laggards and unequal separation of chromosomes. Additional abnormalities were observed during cytokinesis. Up to 100% of the observed pollen grains were sterile. The explanation of female sterility is less complete, though missing stigmatic compound(s), possibly a phenol, absorbing UV light at 261nm is theorized to be the explanation.

Van Went (1981) studied aspects of cytoplasmic male sterility in *I. wallerana*. The limited information available suggests that microspore development in the sterile anther is blocked by a malfunctioning tapetum. In general, cytoplasmic male sterility is widely used by breeders of many crops to produce hybrid seed. Cytoplasmic male sterility in impatiens would provide additional assurances that harvested seeds were not contaminated with seeds resulting from self pollinations.

6. COMMERCIALLY IMPORTANT TRAITS & BREEDING OBJECTIVES

6.1 Germination

Seed germination has become one of the most important traits breeders must consider when working with impatiens. Most commercial hybrids are available with a 90% germination standard, and some are even available with a standard of 95% (PanAmerican Seed Co., 2003-2004). Maintaining this standard is required if a series is to be seriously considered by today's high tech growers. Impatiens is leading the trend of superb germination in all of the modern bedding crops.

Among the difficulties with this trait is the fact that most impatiens prefer, if not require light to germinate. In a study by Pereira De Souza and Pereira (1994) germination was absolutely dependent on light, irrespective of temperatures between 15 and 25C. The effect of red light was important, but it was reversed by far-red light indicating the involvement of the phytochrome system. The highest percentage germination was obtained under continuous red or white light. A single period of 8 hrs of red light irradiation was ineffective in promoting seed germination. Far-red light after the red light treatments reversed all positive effects on germination.

Other studies have shown that keeping imbibed seed in the dark for two or more days reduced final germination percentage. Light in the first 1-3 days promotes germination, but inhibits radicle extension in later stages of germination (Carpenter et al., 1994). Carpenter et al. went on to recommend that providing >75 μmol m^{-2} s^{-1} irradiance for one day is better than giving lower irradiance for several days. Darkness was recommended after the initial one to two days of light treatment.

The difficulty in providing these germination conditions is a breeding opportunity for impatiens breeders. It is conceivable that impatiens could be bred for improved germination under the dark conditions present in the germination chambers of some growers. Significant variation exists for germination ability, as can be evidenced by anyone who has crossed back to wild germplasm. However, it is likely that good germination is a quantitatively inherited trait. Improving dark

germination may be possible, but achieving a 90%+ germination will certainly be a challenge.

6.2 Flower Color

Impatiens wallerana can be found in virtually all colors except blue and yellow, though it was not always this way. In wild populations both white and purple were not generally observed, and reds were not of the quality we see today. Certain aspects of color inheritance appear to be relatively simple, with clear dominant and recessive relationships. However, with the incredible number of colors available, for instance six pinks in the Super Elfin series alone (PanAmerican Seed Company, 2003-2004), it is clear that numerous minor genes or modifiers play a substantial role. In fact, the question for the last fifteen years seems to be 'Do we really need all of these colors?'.

The biochemistry of color has at least been initially studied in other species of impatiens (Clevenger, 1964). In addition, Clevenger (1971) did initiate flower color studies on *I. wallerana*, but only minimal data has ever been published. Flavonoid pigments of *I. wallerana* are known to include leucocyanidin, leucodelphinidin, cyanidin, malvidin, peonidin, kaempferol and quercitin. It was also reported that there were different flavanol compositions among plants with the same flower color. Cyanidin appears to be a basic pigment, since it disappears as the others appear. Malvidin is the second most common and tends to be the most abundant pigment when it is present.

The genetics of flower color inheritance, at least on a practical level, is likely reasonably well understood by individual breeders within the many various private breeding companies. This information has been kept confidential as a trade secret and, though it would be interesting, no public disclosure of color inheritance is known to the author.

As is typical with most bedding plants, white is the most popular color. Strong colors such as red and rose are also popular, but preferred colors are often in the eye of the individual consumer.

6.3 Earliness

By today's standards, all commercial seed impatiens are expected to be "early" flowering. The differences between the earliest and later of the mainstream varieties may be on the order of about seven days under spring conditions. A seven to eight week crop time from sowing to flower is expected under ideal environments (PanAmerican Seed Company, 2003-2004). Having a substantive "plant mass" at first flower is also an important characteristic when plants are cropped in such a short time.

6.4 Flower Patterns

Generally flower patterns appear to add interest and an element of novelty to the impatiens market, but solid colors remain the most popular, except perhaps in the double impatiens market. In addition to the extensive array of flower colors displayed by *I. wallerana*, a number of flower patterns are also possible (Figure 10-2). Perhaps the most familiar of these is the "star" pattern, where solid white streaks longitudinally divide the pigmented sections of individual flower petals much like the "star" pattern in petunias (Figure 10-2). This pattern is popular and widely available, and seems to be especially popular in double flowered impatiens (Leue, 1996). Unfortunately this pattern is environmentally unstable, with both temperature and fertility playing a role. For individual growers, colors can range from near white all the way to the solid base color. This color instability across environments has led to some difficulties in obtaining Plant Breeder's Rights for some vegetatively propagated varieties exhibiting this pattern.

Other patterns have gained interest in more recent years including blush, picotee, mosaic and stardust (Figure 10-2). The blush pattern involves a large "eye" in the center of the flower where additional pigment is deposited especially on the lower two petals. Picotee (sometimes referred to as swirl) involves a darker pigment band around the outside of the petals. Picotee and blush patterns appear to be quantitatively inherited (Uchneat, unpublished data). Both the mosaic and stardust patterns add white areas that generally start near the center of the flower, and radiate outward to various degrees. The stardust pattern is more distinct in terms of where the pattern starts and stops, but both vary significantly. These two flower patterns appear to be more simply inherited, and in the case of the stardust pattern, a single recessive allele is responsible (Leue, 1999).

6.5 Flower Size

As with most ornamentals, large flowers are an important breeding objective. A flower size of approximately 4 cm would be typical for a current series, but the larger the flowers the better. Flower size can also be modified by the growing environment, with fertility and especially plant growth regulators affecting size.

Attempts have been made to further increase flower size through the manipulation of ploidy. Floranova released a tetraploid series "Bruno" which was sold as a large flowering series (http://www.floranova.co.uk/). As is common with diploids converted to tetraploids, some sacrifices were made. The Bruno series had reduced branching and never seemed to 'catch on' with growers. Howe (1998) indicated that in Florida trials, several diploid genotypes equaled the Bruno flower size. In fact several current diploid series are touted as having especially large flowers. Increases in chromosome number have caused increased flower size,

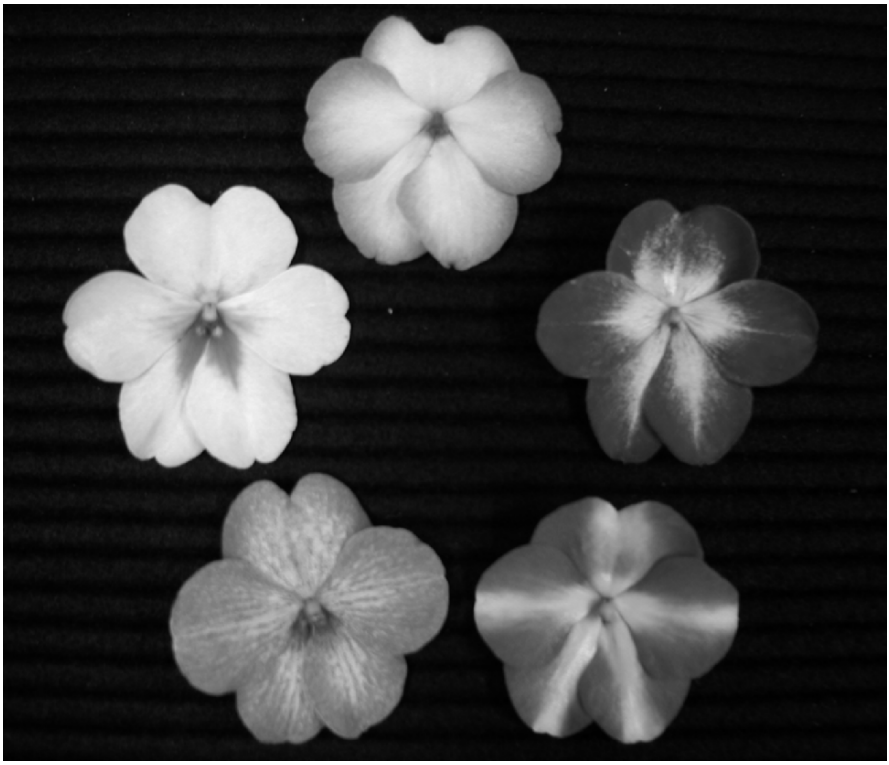

Figure 10-2. Flower pattern in *Impatiens wallerana*. From top (clockwise) picotee or swirl, stardust, star, mosaic and blush.

shoots, and leaves in other species of impatiens as well, including *I. grandis* and *I. parasitica* (Zinov'ea-Stahevitch, and Grant 1984).

It should be noted that not all emphasis has been on large flowers, as there has been some recent efforts to create novel small flowered varieties. The Firefly series from Goldsmith Plants (now merged with Fischer USA (http://www.fischerusa.com/fischer/default.aspx?tabid=24&artid=15), and the Pixie series from BallFloraplant Company (http://www.ballfloraplant.com/) are examples. These dwarf plants have significantly reduced flower size, down to two cm or less, and also have small leaves and a basal branching growth habit (Jonkers and Hanneke, 2000). An older small flowered series known as the "Hawaii" series can still be found at various specialty nurseries (Wolfe, 1987).

6.6 Foliage Color

Generally a dark green foliage color is desired in impatiens, as it imparts a rich, healthy look. Occasionally varieties can be plagued by yellowing foliage that may be only partially controlled by proper nutrition. Other interesting foliage color is possible. Within the Deco series, very dark to near black foliage is available. It is expressed the best in deep shade (PanAmerican Seed Company, 2003-2004). Though perhaps only a modest commercial success, it is a truly unique foliage feature available in the impatiens market. Variegated foliage is also available, though only through vegetatively propagated varieties such as the "Ice" series (Cole, 1991). Typically this variation shows up due to spontaneous mutations of green leafed varieties and it is not seed transmissible. In all cases it is thought to be a chimeric state involving lack of chlorophyll in specific cell layers. Typically these varieties are less vigorous due to their reduced chlorophyll levels.

6.7 Pack Performance

The ability of plants to "hold" in a pack, or to resist stretching is an important characteristic of any plant sold in a pack and subjected to standard retail conditions. Internode elongation contributes to stretch, and there is evidence that there is genetic variation for this trait (Wulff, 1998). In fact many companies now claim improved pack performance for impatiens. It is a popular anecdote that plants that perform well in the pack have less than optimal field or garden performance. This has led many to criticize impatiens breeders for putting too much emphasis on pack performance (Anonymous, 1987). Though it may be possible to breed for this characteristic, it should also be noted that ideal pack performance of impatiens can often be maintained through well-timed and carefully chosen plant growth regulator applications.

6.8 Garden Performance

Every year there are dozens of field trials around the world where various impatiens hybrids are trialed in comparison with one another. These often occur at Universities and Botanic Gardens, but various private companies run excellent trials as well. Many of these are open to the public. With so many impatiens hybrids available, it may be impossible to actually trial them all at the same time, making scientific comparisons difficult. Fortunately the results of these trials are placed on the internet with increasing frequency, improving the access to this information and allowing hybrid comparisons across years and locations.

While internet availability is increasing, there have been a few published evaluations of impatiens cultivars, the most recent two done in Florida (U.S.) (Howe, et al., 1991, Howe, 1998). The 1991 survey evaluated n=69 cultivars in the

spring and fall representing five different commercial breeding programs. The 1998 survey represented evaluations of 125 cultivars, both spring and fall trials, and represented at least seven commercial breeding programs. The latter evaluation concentrated on the newest introductions. Genotypes were evaluated for traits such as earliness, flower diameter, color, foliage, plant dimensions, uniformity, pest damage and overall appearance.

The 1991 survey indicated little differences among the 69 varieties with respect to overall ratings. In the 1998 trial, approximately 53 of the 125 entries were not significantly different, indicating the competitiveness of the current impatiens market. Accents were noted as being the most uniform series, and Tempos showed a lot of variation. More variation was apparent late in the season, and the authors attributed this to the fact that commercial companies are often thought to concentrate their breeding efforts on pack performance (Howe, 1998).

As a breeding objective, field performance is significantly complicated by genotype x environment issues. Genotypes that are more vigorous might be favored in the north, where cooler temperature and lower light levels are more of an issue. These varieties may be too vigorous in the south, and susceptible to stretching or a poor habit. The reverse may be true for compact varieties, which may be preferred in the south and too weak in the north. While this may be muted in a greenhouse environment, it is often obvious in the field. It is customary for breeding companies to release just one genotype for each color per series, and to expect that genotype to perform reasonably well in all environments. Widespread testing of hybrids is conducted to attempt to assure reasonable performance, but it is impossible to develop genotypes, which will excel in all environments. The genotype by environment issues are especially significant when considering an entire series and its field performance, when coupled with the fact that individual members of a particular series are often not genetically related.

An obvious solution to this situation could be to breed genetically related series, which will perform best in certain field environments, and poorly in others, and then to release an appropriate series for some of the more significantly different growing environments. This would likely be quite expensive, and is complicated by the fact that young plants are often produced in an area other than their final field location. Suffice it to say that breeding for garden performance is complicated and difficult, and that it will be a continuing challenge for today's breeders.

6.9 Double Flowers

Double flowered impatiens have become more popular recently, and are available from both seed (Table 10-1) and cuttings (Table 10-3). Early seed propagated series included series such as Rosette, Confections and Carousels, several of which are still available today (Table 10-1). Seed propagated double impatiens got a boost in 1997 when Victorian Rose from Goldsmith Seeds won an

AAS award (http://www.all-americaselections.org/). Since this time other seed double impatiens have entered the market such as the five colors of the Fanciful series, available from Ball Seed Co. (http://www.ballseed.com/) (Table 10-1).

Vegetatively propagated, double-flowered impatiens have been around for some time, but until recently they could only be found at specialty nurseries. In the mid-1990's the Fiesta series was introduced by Ball FloraPlant (http://www.ballfloraplant.com/), and this commenced a breeding trend towards double flowered asexually propagated impatiens (Table 10-3). There are now several companies offering this type of product.

As impatiens become more double they lose reproductive organs, and thus it has been impossible to breed an F_1 hybrid that is 100% fully double from seed. Both the Carousel series from PanAmerican Seed (http://www.panamseed.com/) and the Confection series from Floranova (http://www.floranova.co.uk/) segregate full double and semi-double flowered plants. Each color in these series segregates a different percentage of full double flowers, with the best percentage seeming to be slightly over 50%. It is clear that multiple genes are involved in "doubleness", but exactly how many is unknown.

Table 10-3. Impatiens series names, sources, and number of flower colors available as vegetatively-propagated (cutting) products.

Series Name	Breeder-Producer/Distributor Company	Approximate Number of Flower Colors
Cameo	Oglevee	6
Double Diamond	Goldsmith	7
Double-Up	Bodger Botanicals	7
Fiesta	Ball FloraPlant	17
Fiesta Ole	Ball FloraPlant	5
Golden	Oglevee	3
Rosebud	DS Cole	9
Tioga	Flower Fields	10
Ice	DS Cole	6
Novelty		
Firefly	Goldsmith	9
Pixie	Ball FloraPlant	6
Seashells	Bodger	4

6.10 Habit and Branching

The ideal habit in impatiens is subjective and environmentally dependent, though the trend in the past 20 years has been to compact plants with exceptional branching. In fact these were the early objectives of Claude Hope and the other original impatiens breeders. Without a doubt the various aspects of habit are under polygenic control, though single gene mutations for dwarfness have been observed in other impatiens species (Weigle and Butler, 1983). Among the secondary

benefits of additional branching is more flowering nodes and often better repeat flowering.

Among the vegetatively propagated double impatiens, a second class has developed based on habit. The popular 'Fiesta' series now has a more compact sub-group referred to as 'Fiesta Ole's' (http://www.ballfloraplant.com/). These are marketed as being more compact, easier to produce in small containers and requiring less plant growth regulators.

6.11 Series

One of the major challenges of breeders of all ornamental plants is that rarely is one color sufficient, and the true need is for a series of plants with similar habit and performance, but in a range of colors. In most commercial series today, a series is a collection of genotypes that share similar phenotypes. There is generally limited genetic similarity among individuals within a series.

In addition to the need for a series of colors, it is also common for one series to be used around the world. A difficult choice is then available to the breeder. If a backcrossing method is employed by the breeder, then a series with uniform genetics results, but it may not perform acceptably in all environments where the series is sold. However, if the breeder chooses individual genotypes that perform well in various environments, they are unlikely to perform as a uniform series in any one environment. To date, most breeders have not employed the backcross breeding method when trying to develop a series of colors.

7. INSECT AND DISEASE RESISTANCE

7.1 Western Flower Thrip/INSV

Western Flower thrips, *Frankliniella occidentalis* (Pergande), cause both physical damage and vector viruses, including Impatiens Necrotic Spot Virus (INSV), within impatiens. In the 1980's INSV became a major problem in North American greenhouses (Daughtrey et al., 1997). Characterized by chlorotic to necrotic spots, often with rings, tospoviruses were responsible for US$675,000 in loss in Pennsylvania greenhouses alone from 1989 to 1990. Forty one percent of this loss was due to infection on impatiens crops (Hausbeck et al., 1992). Warnock (2000) used thrips from several geographic locations around the United States to evaluate feeding damage on a group of commercial impatiens cultivars. Though resistance factors were unknown, variation among varieties was observed, with 'Cajun Carmine' being among the most resistant to thrips feeding damage, and 'Impulse Orange' serving as a susceptible control.

In further studies, nine cultivars were evaluated for resistance to thrips feeding damage. Some of the more resistant cultivars still supported high thrips populations suggesting multiple resistance mechanisms. Others had low thrips populations suggesting antibiosis. Some plants were heavily damaged to the point of no longer supporting a viable thrips population (Herrin and Warnock, 2000). Herrin and Warnock (2002) suggest that the variation observed in these nine cultivars suggests breeding for improved thrips resistance is a realistic possibility. Follow-up studies by Loughner (2002) showed results inconsistent with previous reports, indicating the difficulties likely to be encountered in any directed breeding program for Western Flower Thrip resistance.

In addition to the study of the virus vector, resistance to INSV and the closely related tomato spotted wilt virus (TSWV) have also been studied, though not in *I. wallerana*. Interesting research was conducted by Sherman et al., (1996) where resistance to TSWV was engineered into chrysanthemum. It remains to be seen if this type of system could be adapted to benefit impatiens.

7.2 Bacteria

Though initially isolated from New Guinea Impatiens, *Pseudomonas syringae* has been shown to cause a foliar blight of *I. wallerana* (Cooksey and Koike, 1990). Since this condition is rarely reported from growers, it is likely that this pathogen is not causing much economic loss, and possibly requires specific, infrequent environmental conditions for disease development. In all likelihood certain areas of the world may struggle with bacterial problems on impatiens, but they are not a limiting factor in production in the North American and Northern European markets.

7.3 Fungi

Occasional problems are reported with various damping-off pathogens such as *Pythium* and *Rhizoctonia*. It is likely that in most situations these pathogens can be controlled through modified culture. Breeding for resistance to these organisms is likely not justified, and would be daunting at best. In addition to the inherently difficult job of breeding for resistance to these opportunistic pathogens, any resistance gains would need to be transferred to an entire series of perhaps twenty or more colors. However, should durable single resistance genes ever be found, they would be welcomed within the industry.

8. GENES IDENTIFIED

Very little information is available regarding specific genes that have been identified in *I. wallerana*. In fact, only a few genes have been identified within the

whole genus of *Impatiens* (Clevenger, 1971; Leue, 1999; Weigle and Butler, 1983). Many commercial traits, such as habit, seed germination, foliage color, thrips resistance, etc., are probably polygenic in nature. In all likelihood color inheritance is widely understood by various private companies; however there is little information that is public knowledge. The inheritance of flower color has been studied in *I. balsamina* (Clevenger, 1971).

The "stardust" gene, which confers a star pattern in the middle of the flower is controlled by a single recessive allele. Other bicolor flower patterns including the "star" and "picotee" patterns are thought to be polygenic in nature (Leue, 1999).

Weigle and Butler (1983) identified a dwarf mutant in *I. platypetala* following an ethyl-methansulfonate treatment. This mutation was controlled by a single gene. From early research a dwarf genotype was important to early impatiens breeding breakthroughs by Claude Hope, though it is unclear if this phenotype was controlled by a single gene.

9. INTERSPECIFIC CROSSING

Within the genus *Impatiens*, the ability for interspecific crossing varies widely among the various species. Members of the New Guinea group appear to easily make interspecific crosses among related taxa, but within the *I. wallerana* group, somewhat limited interspecific crossing has been achieved. In fact, Grey-Wilson (1980b) indicates that *I. wallerana* and *I usambrensis* appear to be evolutionarily isolated from other species based on pollen morphology, even though they share floral morphology with species such as *I. hamata*. Interspecific crossing in the wild appears to be limited as well, at least partly due to geographic isolation. However, *I. wallerana* and *I. usambarensis* are known to have naturally hybridized in the wild (Grey-Wilson, 1980a; Grey-Wilson, 1980b).

With the wonderful horticulture discovery of the New Guinea group of impatiens in the early 1970's, there was significant interest in attempting controlled interspecific pollinations. Beck et al. (1974) attempted crossing *I. holstii* (*I. wallerana*) with several New Guinea and Java impatiens species, including those with a chromosome number of $2n=2x=16$. No success was reported for any of these crosses, and significant chromosome morphology differences were reported.

In 1985, Merlin and Grant completed extensive hybridization studies within the genus. Both *I. gordonii* Horne ex Bak.(= *I. thomasetti* Hook. f. ex Helmsa.) and *I. usambarensis* easily hybridized with *I. wallerana* in both directions. *I.gordonii* has larger flowers than *I. wallerana* and hybrids produced are fertile. Attempted interspecific hybridizations with *I. sodenii*, *I. pseudoviola*, *I harlandii*, *I flacida*, *I. platypetala* ssp *aurantica*, *I. cinnabarina* were unsuccessful. Reciprocals were also tried except that *I. pseudoviola* was never used as a female parent and *I. wallerana*

was not used as a female parent for *I. cinnabarina*. Ovule culture was not tried in this study, but suggested as a way to complete more of these crosses.

An interesting method was used by Stephens (1997) to predict *in vivo* success of interspecific crosses using *in vitro* pollen evaluations. The predictive nature of this system worked acceptably when *in vitro* pollen germination was above 10%. This work was completed using the New Guinea group of impatiens (*I. hawkeri*).

Attempts have been made at embryo and ovule culture to facilitate crosses among impatiens species, which would not otherwise develop viable progeny. Arisumi (1980) described attempts to make interspecific crosses between *I. wallerana* and a number of species. Embryo development is rather limited, and ovule culture is recommended for attempted recovery of a number of interspecific crosses. *I. wallerana* crossed with *I. auricoma*, *I. flaccida*, and *I. hookeriana* all developed to the globular stage (embryo), and crosses with *I. uguenensis* developed to the heart stage. Interestingly, Arisumi (1980) also refers to crosses between *I. wallerana* and *I. thomassetii* as being successful without embryo rescue, but the true identity of *I. thomassetii* has eluded the author, though it appears it may be from the Seychelles region.

Further research was conducted by Arisumi on ovule culture (Arisumi, 1985). Interspecific plants were rescued in the following crosses involving *I. wallerana*:

I. wallerana x *I. auricoma*

I. auricoma x *I. wallerana*

I. wallerana x *I. epiphytica*

I. wallerana x *I. niamniamensis*

I. wallerana x *I. hawkeri* N463, N47, N514 and N568 (all New Guinea cultivars)

Cultured ovules that had made it to 6 to 11 days had much higher rates of survival than those explanted before the sixth day post-pollination. All of the resulting hybrids were sterile and shared morphological characteristics of both parents. In his closing paragraph Arisumi speculates more hybrids could be embryo rescued with improved methodology.

Gager (1987) further studied interspecific hybridization in Impatiens. Studying pollen tube growth and development, high rates of ovule penetration were observed when *I. wallerana* was used as a female parent, and *I. auricoma* was the pollen source. Crosses between *I. wallerana*, *I. auricoma, I. repens* and *I. capensis* all showed signs of interspecific incompatibility including wandering pollen tube growth, corkscrewing of the pollen tube, etc. Though many of these pollen grains ultimately penetrated the ovule, little seed was produced.

Of all of these interspecific hybridization opportunities, only hybrids between *I. wallerana* and *I. auricoma* have been commercialized (Parker, 1999; Anonymous, 1998) (see also U.S. Plant Patents 10,967; 10,973; 10,974; 10985 and 10,997 at http://www.uspto.gov/main/search.html). These hybrids are currently being sold under the "Bodger Botanicals" line and the series name of "Seashells"

(http://www.bodger.com/). Unfortunately it is likely that the poor genetics from the *I. auricoma* parent has limited the performance of these varieties. Further germplasm collection and additional breeding effort on the *I. auricoma* parent would likely benefit these hybrids. These interspecific hybrids are breakthrough varieties in the sense that they are the first commercially available yellow impatiens involving crosses with *I. wallerana*.

10. BIOTECHNOLOGY

Where "biotechnology" begins and ends in terms of techniques is a bit ambiguous. Obviously ovule rescue and tissue culture techniques have been useful in the development of *I. wallerana* germplasm, though many would not consider this to be a biotech approach.

The ability to develop Agrobacterium mediated transgenic impatiens has been recently demonstrated in a U.S. Utility Patent by Chou (2000). Though the utility patent discusses options for disease and insect tolerance, tolerance for environmental stresses, habit, fragrance and color, there are currently no transgenic impatiens on the market. Opportunities my exist for TSWV resistance as discussed in an earlier section. The final affect of this technology remains to be seen.

It is unknown if anyone is using marker-based selection, or other similar technology to advance their breeding programs, though one could envision many opportunities here.

11. FUTURE OPPORTUNITIES

The size and diversity of initial *I. wallerana* collections is not known. Though it is known that wild *I. wallerana* from Costa Rica in the 1950's was the start of much current commercial breeding, including genes for dwarf and "blush" types (Leue, 1995), it is uncertain how much additional collecting has happened since these early days, and whether or not any of this has made it into current commercial varieties. Also unknown is the value of potential untapped diversity which still remains in wild gene pools, especially those in Africa.

11.1 The Missing Flower Colors

Though it seems like impatiens come in every available color, there are some colors currently not available in *I. wallerana*, most notably yellow and blue. While there have been significant efforts at creating yellow impatiens through interspecific crossing, no true yellow *I. wallerana* currently exists. Recently introduced cream colored impatiens, (i.e. 'Showstopper Buttercream'), while not yellow, do offer a

hint of possibility. How much more "yellow" could be derived from these genetics is not known. Since primary hybrids between *I. wallerana* and *I. auricoma* are yellow, there is perhaps some hope that breeders will ultimately accomplish their goal of creating true yellow impatiens (Leue, 1995). In addition to *I. auricoma*, there are many other impatiens species which have a yellow flower color, including many species from the Himalayan region (Akiyama et al., 1991).

Blue impatiens are another story. To the best of the author's knowledge there have been no serious attempts at creating a blue impatiens, and the methodology to do so has not been described. However, there are a few interesting leads. In a 1995 Report of the Herbaceous Ornamental Crop Germplasm Committee (http://www.ars-grin.gov/npgs/cgc_reports/herbscgc1995.htm), Leue describes a Himalayan species, *I. decipiens*, which reportedly has a metallic blue flower color. Other Himalayan species have been described with purple flowers, and these may approach the color commonly called "blue" within various cultivars of *Petunia* x*hybrida*. Little is known about many of these species, and they have been only rarely collected and studied. Of particular interest are species such as *I. puberula* DC. and *I. cybifera* Hook. f. (Akiyama et al., 1991).

11.2 Sun Tolerance

Impatiens are firmly established as the most significant shade tolerant bedding plant, but the idea of making them sun tolerant has crossed the minds of many breeders. Though *Impatiens* continue to "burn" in the sun, increased sun tolerance may be a future opportunity for impatiens breeders. A recent study by Wulff (1998) demonstrated a possible genetic component to light tolerance. Half-sib families subjected to varying ratios of red to far-red light varied for traits such as plant weight, leaf area, and internode elongation. It may be possible to exploit such variation to improve the plants ability to withstand the various stresses of full sun conditions. Setting up a breeding program with this objective should be relatively simple and straight forward, provided there is enough variation in existing gene pools.

Interspecific crossing may also provide opportunities for increasing sun tolerance. *I. usambarensis* has been mentioned as possibly having more sun tolerance, and it is known that this species can intercross with *I. wallerana* (Leue, 1995). Other interspecific hybrids, such as those with *I. auricoma* show no increase sun tolerance, and perhaps even greater sensitivity to high levels of light.

Related to sun tolerance is the ability of the plants to withstand high temperatures. This includes in both production and landscape situations. In a study by Lee et al., (1989), conducted in Florida, variation was noted for tolerance to high temperatures, with 'Accent Pink', 'Dazzler Pink', and 'Dazzler White' being rated the most tolerant of nineteen cultivars tested. Further studies such as these are needed before a sun/heat tolerance breeding program could be undertaken.

12. CURRENT TRENDS AND STATUS

As an established core crop of the United States, European and World bedding plant market, seed propagated impatiens suffer from being considered a commodity crop. With so many series and colors available, and more coming each year, many growers consider them boring and low margin items. This is unfortunate in a crop, which has come so far, and has so much more to offer. As suppliers continue to supply very high quality seed, above 95% germination in some instances, at very reasonable prices, this trend is likely to continue. Creative marketing and product packaging will be necessary to counter this trend.

The one area of impatiens that has seen significant growth and interest is the area of vegetatively propagated varieties. Most of these varieties are double flowered, and though double flowers are not a recent introduction, the significant improvements introduced in the Fiesta series in the mid-1990's sparked renewed interest in this group. Other vegetative lines have created significant interest, such as the "Seashells", though sales have not followed due to mediocre product performance.

The author, for one, has faith that there are many more interesting and exciting breeding opportunities in this product class. Over the next decade and millennium one can only guess at the number of new and exciting products that might be made available through the creative work of the world's plant breeders.

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Chapter 11

PETUNIA

Petunia x hybrida

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Abstract: Petunias are popular seed- and vegetatively-propagated bedding plants with numerous phenotypes (Grandifloras, Multifloras, Millifloras, Waves, Hedgifloras, etc.), series, and cultivars available on the world market. The history of petunia development as a crop is highlighted with implications for a wide variety of other floricultural crops. Hybrid seed production transformed the crop; continued domestication by flower breeders produced a wide range of flower colors and flower patterns. In the 1970s the petunia market flattened out. Not until plant collectors found new phenotypes in wild species (with the wave habit, for instance) did interspecific hybridization revitalize the crop. Petunias are one of the few floriculture crops which have well-established linkage maps, trisomics, transformation/regeneration protocols, precise elucidation of taxonomic and cytogenetic relationships between taxa, and advanced molecular genetics. In particular, elucidation of the genes involved in the flavonoid biosynthesis pathway has enabled precision in the creation of new flower colors or modification of pigment production and expression.

Key words: *Calibrachoa,* cytogenetics, flavonoids, flower color, genetic engineering, molecular biology, taxonomy.

1. INTRODUCTION

In 1944, a severe summer drought killed record numbers of bedding plants throughout the United States. Petunias became national news because of their drought tolerance (Haughton, 1978). This tolerance established their widespread popularity, which lead to a major effort to develop new cultivars with novel flower colors and improved garden performance. These improved cultivars resulted in

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petunia becoming the number one bedding plant in the 1960's in terms of both sales and number of plants produced.

Until recently, only two types of petunia existed (grandiflora and multiflora) (Dole and Wilkins, 2004). The grandiflora types produced a small number of more open flowers on compact plants with large leaves; while the multiflora types produced a large number of smaller flowers on large branched plants with small leaves. Originally, the multiflora types were called hybrida types.

The grandiflora type first appeared in 1881 as a mutation in the multiflora 'Defiance Strain' (Ewart, 1984; Weddle, 1976). It was later discovered that the grandiflora genotype was due to a single dominant gene (*GG*) which in the homozygous condition was nearly lethal (Reimann-Philipp, 1962; Seidel, 1962). Another large flowered mutation (superbissima) was discovered in 1888. The superbissima genotype was due to tetraploidy (Vilmorin and Simonet, 1927). A superbissima type called 'California Giant' was developed by Mrs. Theodosia Shepherd in California and became the leading petunia grown during the 1930's. Both grandiflora and superbissima types were grown as potted plants, not as bedding plants. They did not have the growth habit or the vigor necessary for a bedding plant.

Breeding methods for petunia radically changed during the 1930's. Prior to 1930, most cultivars were developed through mass selection and superior clones were vegetatively propagated (Ewart, 1984; Weddle, 1976). After 1930, breeding focused on individual plant selection, inbreeding and seed propagation of superior strains. The result was the introduction by Ernst Benary of Germany of the first inbred cultivars ('Setting Sun', 'Lace Veil', 'White Cloud', etc.). Due to the difficulties in breeding both the grandiflora and superbissima types, inbred cultivars first appeared in the multiflora types.

Another important development in petunia breeding was the development of F_1 hybrids (Ewart, 1984; Weddle, 1976). During the 1940's, Charles Weddle of PanAmerican Seed Company (W. Chicago, Illinois, U.S.A.) developed the first method of commercially produced grandiflora types from seed by crossing an inbred multiflora type with a line-bred grandiflora type. The first F_1 hybrid grandiflora ('Ballerina') was introduced by Weddle in 1952 (Weddle, 1976). Cultivars with purple, violet, blue, pink, and white flowers were easy to create; however, cultivars with red flowers took longer to develop. The first inbred red-flowered multiflora cultivar ('Fire Chief") was introduced by S. Sinclair of Bodger Seeds (Lompoc, California, U.S.A.) in 1950. Claude Hope, a co-founder of PanAmerican Seed Company, used 'Fire Chief' to create the first red-flowered F_1 grandiflora cultivar ('Comanche'). 'Comanche' won an All-America Selections (AAS) award in 1953 (http://www.annualflowers.com/manual/aas/aaselect.html).

By 1970, it was difficult to distinguish multiflora from grandiflora types as all cultivars produced large flowers on compact plants (Maatsch and Nolting, 1968). Many of the characteristics (i.e., disease, stress and pest tolerance) which led to the

popularity of petunias were lost in breeding. These factors, along with changes in consumer preferences, led to the major decline in their popularity. During the 1990s, petunia breeding programs shifted back to the wild species and older cultivars to redevelop the multiflora types with a new phenotype—prostrate or groundcover plants. In order to distinguish these redeveloped multiflora types from the other types, many different names have been given to them - Wave™ (PanAmerican), Cascadias® (Danziger), Surfinia® (Suntory), Trailblazer® (Novartis), Ramblin® (Goldsmith), Petitunia® (Danziger), Tiny Tunias™ (Bodger), Tidal Wave™ (Pan American), Kahuna® (Novartis), Trumpets™ (Bodger), and Supertunia® (Suntory). The plants in all of these series have the stress tolerance, plant vigor and floriferousness of the original multiflora types. They differ slightly in growth habit, flower size, and method of propagation. Depending upon the series, plants are either propagated as vegetative clones, inbred lines or F_1 hybrids. As a result of these redeveloped multiflora types, the popularity of petunia is increasing once again. Currently, petunias rank as the number two bedding plants in sales within the U.S. (http://usda.mannlib.cornell.edu/reports/nassr/other/zfc-bb/).

2. COMMERCIAL PRODUCTION

Commercial propagation is primarily by seed, although in recent years there has been an upsurge in vegetatively propagated cultivars. Depending upon the species or cultivar, light may be required for germination (Toole, 1973). The light requirement can be overcome by treatment with 100 ppm gibberellic acid (Cathey, 1984). Because many of the wild species are more difficult to germinate than *P. x hybrida,* gibberellic acid treatment is routinely used in their germination.

The germination process for bedding plants has been divided into four stages (Nau, 1991). During stage 1, the radicle emerges from the seed. The cotyledons emerge during stage 2 and the first leaves during stage 3. During stage 4, the seedlings grow large enough to transplant. For petunia, the optimal conditions for each stage of growth has been determined (Table 11-1). Seedlings are usually grown in a peat-lite soil mix with a pH between 5.8 and 6.5 and an electrical conductivity between 0.75 and 1.25 (Nau, 1991; Ewart, 1984). Mature plants are fertilized weekly with a 20-10-20 or a 20-0-20 balanced fertilizer at 200-300 ppm nitrogen.

Petunia plants respond to daylength in two ways. Long days accelerate flowering and induce a long terminal growth habit with few lateral branches, while short days inhibit flowering and produce a rosette growth habit (Piringer and Cathey, 1960). Daylength also controls how plants respond to CO_2 enrichment (Reekie et al., 1997). Under short day conditions, elevated CO_2 levels increase the height and decrease branching, while under long day condition, elevated CO_2 has no effect. Elevated CO_2 levels also increase flower production under both photoperiods. In order to maximize flowering and minimize undesirable growth habits, it is

important to restrict long day lighting to that time in development when plants are sensitive to flower induction. Both temperature and light intensity can influence when plants reach this stage of development (Adams et al., 1999). A plant that is quick to flower with a short main stem and lateral branches can be produced using a positive DIF (high day / low night temperatures) under a red light enriched long-day photoperiod (Kuboto, et al., 2000).

Table 11-1. Cultural conditions for seed germination and subsequent growth of *Petunia x hybrida* (Nau, 1991).

Stage	Duration (days)	Day temp. (°C)	Night temp. (°C)	Light intensity ($\mu mols^{-1}m^{-2}$)	Photoperiod (hrs)	Fertilizer (ppm N)
1	3-5	24-28	24-28	50-100	>13	0
2	10-14	24-28	22-24	50-100	>13	50
3	10-14	24-28	16-20	50-100	>13	150
4	7-10	24-28	16-18	100-200	>13	150
Mature plants	---	24-28	16-18	200-400	>13	300

Recently, new cultivars have been developed that are vegetatively propagated. These cultivars are propagated via rooted cuttings grown under long day conditions. Virus diseases have become a serious problem in the vegetatively propagated cultivars (Lesemann and Dalchow, 1995). At least 11 known viruses have been detected in commercially propagated plants (Lesemann, 1996).

3. TAXONOMY

3.1 Petunia

The early literature on *Petunia* is quite confusing since the taxonomists of the era did not have ready access to foreign herbaria specimens or the scientific literature (Ando, et al., 1992). The first *Petunia* species was collected by P. Commerson in Uruguay, but described by J. Lamarck (1793) as *Nicotiana axillaris*. Unaware of the description of *N. axillaris*, A. de Jussieu in 1803 established the genus *Petunia* and described the taxon collected by Commerson in Argentina as *Petunia nyctaginiflora.* The name petunia was derived from the aboriginal term for tobacco, petun. It was soon discovered that *N. axillaris* and *P. nyctaginiflora* were very closely related and *P. nyctaginiflora* was transferred to *Nicotiana* as *N. nyctaginiflora.* By 1825, it was well recognized that these two plants were the same taxon and were not a *Nicotiana species.* The taxon was commonly known as *P. nyctaginiflora*. At this time, the modern nomenclature rules used today were not in place. It was not until 1888, when Britton, Sterns and Poggenburg (1888) properly changed the name of this taxon to *P. axillaris* (Lamarck) Britton, Sterns et Poggenburg.

L. Parodi discovered another taxon closely related to P. axillaris in Argentina. This taxon was distinguished from *P. axillaris* by its stamens of equal length and long narrow corolla tube and acute corolla apices; while *P. axillaris* has didynamous stamens and a short wide corolla tube. In 1930, W. C. Steere (Steere, 1930) described this taxon as *P. parodii.* In 1979, Cabrera reduced the status of this taxon to a subspecies, *P. axillaris* subsp. *parodii* (Steere) Cabrera. T. Ando (1996) described a second subspecies from Bolivia, P. axillaris subsp. subandina Ando. Its didynamous stamens and a long narrow corolla tube distinguished this subspecies.

The second *Petunia* taxon to be described was collected in Argentina by J. Tweedie. This taxon was described by W. Hooker (1831) as *Salpiglossis integrifolia*. It was clear that this taxon was not a *Salpiglossis*; therefore in 1833, it was renamed *Nierembergia phoenicea* by D. Don (Don, 1833) and *P. violacea* by J. Lindley (Lindley, 1833). Once again because of nomenclature rules at the time, this species was commonly known as *P. violacea.* Schiz and Thellung (1915) using modern rules changed the name to *P. integrifolia* (Hooker) Schinz et Thellung.

R.E. Fries published the first monograph on *Petunia* in 1911 (Fries, 1911). In this monograph, he recognized two subgenera (*Pseudonicotiana* and *Eupetunia*). In *Pseudonicotiana,* the corolla is hypocrateriform and the filaments are attached to the middle of the tube. In *Eupetunia*, the corolla is infundibuliform and the filaments are attached below the middle of the tube. Fries placed *P. axillaris* in *Pseudonicotiana* and *P. integrifolia* in *Eupetunia*.

Fries also separated *P. integrifolia* into three distinct species (*P. violacea*, *P. inflata* Fries and *P. occidentalis* Fries). *P. integrifolia* was not the accepted name in 1911. *Petunia violacea* had the largest flowers and pendent pedicels, while *P. occidentalis* had the smallest flowers and erect pedicels. The flower size and position of the pedicel of *P. inflata* were intermediate between *P. violacea* and *P. occidentalis*. In addition, Fries recognized a diminutive subspecies of *P. violacea* (*P. violacea* subsp. *depauperata* Fries) with very small flowers and leaves.

L.B. Smith and R. J. Downs (1966) recognized *P. integrifolia* (*P. violacea*) and *P. inflata* as the same taxon (*P. integrifolia* var. *integrifolia*) and *P. violacea* subsp. *depauperata* and *P. occidentalis* as the same taxon (*P. integrifolia* var. *depauperata* (Fries) Smith et Downs). In addition, at an earlier date they had recognized four *P. integrifolia* taxa as distinct species (*P. reitzii* Smith et Downs, *P. saxicola* Smith et Downs, *P. littoralis* Smith et Downs, and *P. scheideana* Smith et Downs) (Smith and Downs, 1964).

H.J. Wijsman (1982) reported that both flower size and pedicel position in *P. integrifolia* could be characterized by geographical distribution. The more western ecotypes had smaller flowers and more erect pedicels. Therefore, he concluded that various taxons were subspecies of a single broadly defined species. He recognized three different subspecies (*P. integrifolia* subsp. *integrifolia* (Fries) Wijsman, *P. integrifolia* subsp. *inflata* (Fries) Wijsman, and *P. integrifolia* subsp. *occidentalis* (Fries) Wijsman) and a single variety (*P. integrifolia* subsp. *integrifolia* var.

depauperata (Fries) Wijsman). Wijsman did not report on the relationship of *P. reitzii*, *P. saxicola*, *P. littoralis*, and *P. scheideana* to *P. integrifolia.*

Ando, et al. (1995) completed an extensive morphological comparison using living material of the all the described *Eupetunia* species. They concluded that all of the species, except *P. littoralis*, were clearly distinct. *P. littoralis* could not be distinguished from *P. integrifolia* subsp. *integrifolia* var. *depauperata*. In addition, all of the *P. integrifolia* subspecies in their native habitats were separately distributed and readily distinguished from one another.

T. Ando and G. Hashimoto recognized during the 1990's (Ando and Hashimoto 1993, 1994, 1995, 1996) four *P. integrifolia* taxa as distinct species (*P. bonjardinensis* Ando et Hashimoto, *P. altiplana* Ando et Hashimoto, *P. guarapuavensis* Ando et Hashimoto, *P. interior* Ando et Hashimoto, *P. bajeensis* Ando et Hashimoto and *P. riograndensis* Ando et Hashimoto). In addition, they recognized a *P. scheideana* taxon as a distinct species (*P. mantiqueirensis* Ando et Hashimoto) (Ando and Hashimoto, 1994).

An additional *Pseudonicotiana* species, *P. exserta* Stehmann, was described by Stehmann (1987). The following is a dichotomous taxonomic key for the identification of the *Petunia* species:

1. Pedicel deflexed in fruiting stage
    2. Stigma above anthers of longest stamen
        3. Corolla tube long and narrow; main stem erect, subglabrous; leaves oblong-lanceolate, hirsute, base petiolate, apex acute
        P. mantiqueirensis
        3. Corolla tube short and campanulate; main stem erect, hirsute; leaves oblong-elliptical, subglabrous, base attenuate, apex obtuse
        P. bonjardinensis
    2. Stigma at same level as anthers of longest stamen
        3. Main stem erect, glabrous; leaves oblong-lanceolate, subglabrous, base petiolate, apex acute; corolla infundibuliform, tube paler than limb ***P. scheideana***
        3. Main stem erect, hirsute; leaves oblong-lanceolate, subglabrous, base petiolate, apex acute; corolla infundibuliform, tube paler than limb ***P. guarapuavensis***
    2. Stigma between anthers of longest stamen and shortest stamen
        3. Main stem prostrate
            4. Leaves prostrate, broad spatulate, subglabrous, apex rotundate, base attenuate; stem hirsute, branched at lower unflowered internodes; corolla narrow campanulate, tube paler than limb
            P. altiplana
            4. Leaves erect, linear to oblong, apex obtuse, base attenuate; stem unbranched at lower unflowered internodes

5. Stem and leaves glabrous; corolla tube broad infundibuliform, tube darker than limb ***P. littoralis***
5. Stem and leaves hirsute
6. Corolla limb ≈30 mm wide, tube broad infundibuliform, tube darker than limb ***P. i. ssp. depauperata***
6. Corolla limb ≈50 mm wide, tube narrow infundibuliform with cylindrical base, tube same intensity as limb ***P. riograndensis***

3. Main stem erect
4. Corolla tube short, broad infundibuliform
5. Anther lobes with channel; stem hirsute; leaves oblong, subglabrous, apex acute, base attenuate-cuneate; tube paler than limb ***P. interior***
5. Anther lobes without channel; stem hirsute; leaves oblong, hirsute, apex acute, base attenuate
6. Corolla tube not intruded, tube darker than limb, limb mouth round ***P. i. ssp. integrifolia***
6. Corolla tube intruded, tube lighter than limb, limb mouth reniform ***P. bajeensis***
4. Corolla tube long, narrow infundibuliform with cylindrical base, same color intensity as limb
5. Leaves linear-lanceolate, hirsute, apex attenuate, base petiolate; stem hirsute; corolla limb purple ***P. reitzii***
5. Leaves elliptical, subglabrous, apex subacute, base petiolate; stem subglabrous; corolla limb red ***P. saxicola***

1. Pedicel not deflexed in fruiting state
2. Corolla hypocrateriform, tube cylindrical
3. Stamen and stigma above corolla tube; corolla limb red ***P. exserta***
3. Stamen and stigma within corolla tube
4. Corolla limb white
5. Corolla tube ≈40 mm long, limb ≈25 mm wide ***P. a. ssp. axillaris***
5. Corolla tube ≈60 mm long, limb ≈45 mm wide ***P. a. ssp. parodii***
4. Corolla limb purple ***P. secreta***
2. Corolla infundibuliform; limb colored
3. Corolla tube base cylindrical, limb ≈20 mm wide ***P. occidentalis***
3. Corolla tube base infundibuliform, limb ≈35 mm wide ***P. i. ssp. inflata***

The garden petunia is considered a complex hybrid between *P. axillaris* and *P. integrifolia.* The first report the *P. axillaris* x *P. integrifolia* interspecific hybrid was by Atkins in 1834 and described by R. Sweet (Sweet, 1835) as *Nierembergia* Atkinsiana. Many forms of the hybrid using different parents and backcrosses quickly appeared in gardens (Loudon, 1840). At this time, all the purple-flowered garden hybrids were called "*P. violacea.*" By 1900, the true species had disappeared from cultivation (Ferguson & Ottley, 1932). Even the herbarium specimen of *P. integrifolia* at the Royal Botanic Garden at Kew was determined to a *P. axillaris* x *P. integrifolia* hybrid (Anonymous, 1918)! In 1863, R. Vilmorin coined the term *P. x hybrida* to represent the garden petunia (Vilmorin, 1863).

Several molecular marker studies of *Petunia* have been made using species-specific repeated DNA (Shepherd et al., 1990), rDNA (Benabdelmouna and Abirached-Darmency, 1997; Kabbaj et al., 1995; Zeboudj et al., 1994), and randomly amplified DNA (Cerny et al., 1996; Benabdelmouna et al., 1999). However, in all of these studies, the species that was actually used is uncertain. For example, phylogenetic analysis using rDNA suggested that *P. integrifolia* was very distantly related to *P. violacea* (Kabbaj et al., 1995), but these are the same species! Furthermore, the complex taxonomy of *Petunia* has caused many cultivated species to be misidentified. For example, a botanical garden in Europe is currently distributing seed of an *Eupetunia* x *Pseudonicotiana* hybrid as *P. violacea.*

Molecular heterogeneity of introns may be used to characterize the *Petunia* species (Griesbach et al., 2000). A comparison was made of the Rsa 1restriction digest profile of the chalcone synthase intron of several accessions each of *P. integrifolia subsp. integrifolia var. depauperata* and *P. altiplana*. Further studies still in progress have included accessions of *P. littoralis*. All the accessions were collected from different known locations. Each species had a uniform and unique restriction digest profile.

3.2 Calibrachoa

In 1985, Wijsman and De Jong separated *Petunia* into two genera based upon chromosome number, flower morphology and breeding behavior. The type species (*P. parviflora* Juss.) and all those species with a chromosome number $2n = 18$ remained in *Petunia*; while those species with a chromosome number of $2n = 14$ were transferred to *Stimoryne* Rafin. *Stimoryne* was selected because it was the oldest known name other than *Petunia* for a $2n = 14$ species. *Petunia integrifolia* was described by Rafinesque-Schmaltz (1836) as *Stimoryne purpurea*.

This resulted in the highly undesirable name change for the garden petunia. Therefore, Wijands, et al. (1986) proposed that the second described species (*P. nyctaginiflora* Juss.) be conserved as the lecotype. The I.N.G. Committee agreed (Brummitt, 1989). Wijsman (1990) then transferred the $2n = 18$ species to *Calibrachoa* Llave et Lex. *Calibrachoa* was selected because it was the oldest

known name other than *Petunia* for a $2n = 18$ species. *Calibrachoa parviflora* was described by La Llave and Lexarza (1825) as *Calibrachoa procumbens*.

Besides the obvious difference in chromosome number, *Calibrachoa* can also be distinguished from *Petunia* by their woody stems, conduplicate aestivation with two petals covering the other three, and seed coats with straight anticlinal walls. *Petunia* have non-woody stems, imbricate aestivation, and seed coats with wavy anticlinal walls.

4. CYTOGENETICS

4.1 Karyotype

The first karyotype for *Petunia* was established by Marthaler (1936) who described seven pairs of chromosomes (Table 11-2). Using relative length and arms ratios, it is possible to distinguish all the chromosomes except IV and V (Benzer et al., 1971; Maizonnier and Cornu, 1971). These two chromosomes can be distinguished by either their quinacrine fluorescence pattern (Smith et al., 1973) or DNA content (White and Rees, 1987). Chromosome II has a secondary constriction on its short arm due to nucleolar organizer.

Pollen diameter (Ferguson and Coolidge, 1932), chloroplast number in guard cells (Mitchell et al., 1980), stomata length (Santos and Handro, 1983) and microfluorimetry (Galbraith et al., 1981) have been used to simplify the determination of ploidy level. Under certain instances, these techniques may not be reliable. For example, chloroplast counts only measure the polyploidy level of the epidermis and will not be able to determine the ploidy of the gametes (Kamo and Griesbach, 1989).

Table 11-2. Chromosome measurements for *Petunia x hybrida* (Marthaler, 1936; Smith et al., 1973; White and Ress, 1987).

Chromosome number	Centromere index (long arm length:total chromosome length)	Relative length (% of total chromosome length)	DNA amount (pgs)
I	0.52	8.7	0.29
II	0.63	8.0	0.35
III	0.66	7.0	0.38
IV	0.52	6.8	0.41
V	0.58	6.9	0.41
VI	0.59	6.9	0.43
VII	0.53	6.2	0.47

4.2 Ploidy

The first giant petunia was recognized in 1888; however it was not until much later that the tetraploid nature of this mutation was discovered (Dermen, 1931; Kostoff, 1930; Matsuda, 1934; Steere, 1932; Vilmorin and Simonet, 1927). Once it was known that colchicine could artificially induce polyploidy (Blakeslee and Avery, 1937), a number of researchers immediately reported on its use in petunia (Györffy, 1938; Nebel and Ruttle, 1938; Nishiyama, 1938; Simonet, 1938).

Most naturally occurring polyploids are the result of unreduced or *2n* gametes (Matsuda, 1927). In petunia, a few chromosomes frequently remain at the plate during metaphase of the first meiotic division. If there are more than one or two lagging chromosomes, the nuclear membrane forms around them and then expands to include the chromosomes at the poles. The resulting gamete is diploid. The frequency of unreduced gametes depends upon the genotype and the environment. It is not uncommon to find 3% of the pollen to be diploid (Matsuda, 1927).

Unreduced diploid (*2n*) gametes are viable and often lead to triploid progeny. Unlike in many plants, triploid petunias can be quite fertile (Seidel, 1962; Smith et al., 1975). One of the reasons for this high degree of fertility, is the lack of multivalent formation (Singh, 1989). Even in autotetraploids, only bivalents are found. Of 365 progeny from triploid x diploid crosses, approximately 12% was diploid, 33% was trisomic diploid (2*n*+1), 25% was double trisomic diploid (2*n*+1+1), 12% was triple trisomic diploid (2*n*+1+1+1), 9% was triple monosomic triploid (3*n*-1-1-1), 5% was double monosomic triploid (3*n*-1-1),and 4% was monosomic triploid (3*n*-1). Over 80% of the progeny from triploid x tetraploid crosses was either tetraploid (4*n*) or monosomic tetraploid (4*n*-1). Of the 13 progeny from triploid x triploid crosses, each had a different chromosome number , which ranged from triple monosomic triploid (3*n*-1-1-1) to tetraploid (4*n*).

When triploid females are crossed with diploids, a very small percent of the progeny (0.04%) are haploid (Straub, 1973). Haploids can also be obtained from pollen or anther culture. Depending upon the genotype, up to 10% of cultured anthers can produce plantlets (Hanson, 1984). Most of the regenerated plantlets are not haploid, but triploid. In four different studies (Engvild, 1973; Raquin,1973; Sangwan and Norreel 1975; Wagner and Hees , 1974), the frequency of haploid plant ranged from 0 to 30%. Due to these difficulties, haploids are best obtained through triploid x diploid crosses.

Haploid petunias are not sterile and can produce up to 30% viable pollen (Maizonnier, 1974). Nearly all of the progeny is diploid, the result of unreduced gametes. Diploid plants can also arise through tissue culture. In culture, haploid plants are unstable and frequently give rise to diploid shoots. These ‘doubled -haploid’ diploids are extremely useful for genetic studies for they are homozygous for all genes. A ‘doubled -haploid’ petunia, ‘Mitchell’, was widely distributed during the 1980's a model plant for genetic studies (Ausubel et al., 1980). ‘Mitchell’

arose from the anther culture of a plant from the cross *P. axillaris* x (*P. axillaris* x *P. x hybrida* 'Rose du Ciel'). The use of 'Mitchell' as a model plant was superceded by *Arabidopsis.*

Aneuploidy is extremely well tolerated in petunia. Trisomic plants appear healthy, grow well, and are fertile (Table 11-3; Maizonnier, 1984; Reddi and Padmaja, 1982). The extra chromosome in a trisomic is only transmitted through the female gamete (Cornu and Maizonnier, 1983). The rate of transmission varies from 3 to 37% depending upon the chromosome and genetic background. Each trisomic has a unique set of characteristics and an identification key has been developed (Table 11-3). Trisomics have been extensively used for genetic mapping (Table 11-4). *Petunia* is one of the few floricultural crops which has a linkage map established.

Table 11-3. Key to the seven primary trisomic plants (I-VII) in *Petunia x hybrida* (adapted from Maizonnier, 1984). A '+' denotes that the characteristic is significantly higher in the trisomics than the diploids; a '-' indicates that the character is significantly lower in the trisomics; a '=' means there is no significant difference.

Characteristic	I	II	III	IV	V	VI	VII
Number 5 leaf (from cotyledon) length/width	+	-	-	=	-	-	-
Plant height at first flower	-	-	=	=	+	+	=
Pedicel length	-	-	+	=	=	=	=
Sepal length	+	-	+	-	-	=	=
Corolla length	+	-	+	-	-	-	-
Corolla diameter	-	-	+	-	-	-	-
Corolla length/diameter	+	+	=	-	+	=	=
Petal length	=	-	+	-	-	-	-
Tube height	+	-	+	-	-	-	-
Tube diameter	-	-	+	+	=	-	+
Gynoecium length	-	-	+	-	-	-	-
Short stamen length	-	-	+	-	-	-	-
Time to flower	=	+	+	-	=	+	+

Table 11-4. Gene linkage groups on chromosomes I – VII in *Petunia x hybrida* (Cornu and Maizonnier, 1983; Gerats, 1991).

I	II	III	IV	V	VI	VII
An9	*Fl*	*Ht1*	*An3*	*An8*	*An1*	*An4*
Ht2	*Fa*	*Mt1*	*An6*	*Hf2*	*An2*	*An11*
Hfl	*Co*	*Mf1*	*An11*	*Mt2*	*Dg1*	*Jaf13*
Ph1	*Lg2*	*Ph4*	*Ph2*	*Mf2*	*Dw2*	*Ph3*
Ph5	*Lu1*	*PrxA*	*Bl*	*Gf*	*Rf2*	*Vs5*
Ch1	*Ws*	*Alf*	*Gp*	*Fn*	*Le2*	*Do1*
Ea	*Do2*	*Ch2*	*Apt*	*Ve1*	*Rt*	*Ls*
PrxB	*Dw7*	*Ch3*	*Rf1*	*Sdh*	*Tu*	*Ms3*
Lg1	*Px*	*Fu1*	*St1*	*Po*	*Ms4*	*Dw4*
Vs1	*Si*	*Dg5*	*Dw1*	*Un*	*Le2*	*GpiB*
Vs4	*Cr*	*PrxD*	*Dg2*	*Us*	*PrxF*	*Lg5*
Le1	*Ftr*	*Vs2*	*PrxC*	*Sp1*	*PrxG*	*LabB*
Le3	*Rm1*	*Yg3*	*Vs3*	*Yg1*	*PrxH*	

I	II	III	IV	V	VI	VII
Nl1	*ChsG*	*S*		*Fu2*	*Rf2*	
Dg6	*ChsL*			*G*	*DfrC*	
Wi	*DfrB*			*ChsA*		
				ChsB		
				ChsD		
				ChsF		

4.3 Transposable Elements

Petunias with variegated flowers (i.e., flowers with flecks of a different color) have been frequently described (Cornu, 1977; Bianchi et al., 1978; Dale, 1941; Malinowski,1928; Straub, 1973). These variegated flowers are the result of a transposable element unstably inserting into a gene. When a transposable elements "jumps" out of a gene, the wild type function is usually restored. Background tissue expresses the mutant phenotype (transposon inserted into the gene), while the sector tissue expresses the restored wild type phenotype.

In *Zea mays*, three major families of transposable elements (Activator-Dissociation (*Ac-Ds*), Mutator (*Mu*), and Suppressor-Mutator (*Spm*)) have been defined (Fincham and Sastry, 1974). Two transposable elements (*dTph1* and *Ps1*) have been described in petunia. The *dTph1* element is 284 bp, a member of the *Ac-DS* family, and trans-activated by the *Act1* gene (Gerats, et al., 1990; Huits, et al., 1995). There are between 2 and 25 copies of *dTph1* within the genome. The *Ps1* element is 9900 bp and a member of the *Spm* family (Snowden and Naploi, 1998). There are between 2 and 4 copies of *Ps1* within the genome.

The members of each transposable element family can be placed into one of two groups. Autonomous elements can transpose without the help of additional genes; while nonautonomous elements require an autonomous member of the family for transposition. In the *Ac-Ds* family, *Ac* transposes autonomously and *Ds* transposition requires trans-activation by *Ac* (Federoff, 1989). The DNA sequence of *Ds* is identical to *Ac* except for a 200 bp central deletion. *Ac* is 4565 bp with an 11bp imperfect terminal inverted repeat and encodes a transposase, which is sufficient for transposition. Upon *Ac* insertion, a 8 bp duplication is created in the target gene. In the presence of an active *Ac*, *Ds* becomes demethylated allowing it to be transcribed. The *Ds* transcription production can either cause transposition or chromosome breakage. The broken chromosome usually mends itself by forming a U-shaped dicentric chromosome with two centromeres. This unusual chromosome leads to the breakage-fusion-bridge cycle. During this cycle, the U-chromosome breaks during mitosis at a random point between the two centromeres. The resulting daughter cells will either contain gene duplication(s) or deletion(s).

Unlike the *Ac* element, the *Spm* element encodes at least two different functions that are derived by alternative splicing. Both products are required for transposition (Masson et al., 1989). *Spm* can exist in one of three forms (stable inactive called

cryptic, unstable active called programmable, and stable active) (Banks, et al., 1988). There are two phases in control of *Spm* activation. During the setting phase, the element's activity is determined (active vs. inactive). During the program phase, the element's degree of stability is determined. Active elements are transcribed, while inactive elements are untranscribed the result of upstream methylation. When an active *Spm* is introduced into a genome containing a cryptic element, the cryptic element is partially demethylated but remains transcriptionally inactive. When an active *Spm* is introduced into a genome containing a programmable element, the programmable element is extensively demethylated allowing it to be transcribed. Upon *Spm* insertion, a 3 bp duplication is created in the target gene.

Transposable elements have been used to 'tag' genes involved in flower color (Gerats et al., 1989). Tagged genes are very useful in genetic and biochemical studies. Within the same tissue, one has both the mutant and wild type phenotype in isogenic cells. This makes it possible to identify new genes, determine gene function and to physically isolate a gene. *Petunia x hybrida* 'V26' was transformed with a plasmid containing *Ac* (Chuck et al., 1993). In one of the resulting transgenic plants, *Ac* was inserted into the *An1* (previously studied as *Ph6*) regulatory gene. Studies showed that *An1* regulated both vacuolar pH and anthocyanin genes (Griesbach, 1998).

Through classical breeding, *dTph1* was used to tag 40 alleles of seven genes (Houwelingen et al., 1998). The seven genes affected were *An3* (encodes flavaone-3β-hydroxylase), *An11* (encodes an anthocyanin regulatory gene), *Alf* (causes aberrant leaf and flower morphology), *Nam* (causes no apical meristem), *Ph4* (vacuolar pH gene), *Ph3* (vacuolar pH gene), and *Ph7* (vacuolar pH gene). One of these genes (*Ph7*) was not previous described.

4.4 Self Incompatibility

In several plants, reproductive mechanisms have evolved to prevent self-pollination. One such mechanism is self incompatibility (SI). SI can be due to either the gametophyte or sporophyte genotype. In sporophytic SI, the pollen's response is determined by its parental genotype, while in gametophytic SI the pollen's response is determined by its own genotype. Only the *Eupetunia* express gametophytic SI (Dowd et al., 2000; Robbins et al., 2000). The *Pseudonicotiana* species are self-compatible producing seed upon self-pollination. SI is controlled by a multiallelic *S*-locus. Fertilization is prevented when the *S*-allele of the pollen matches at least one of the two *S*-alleles expressed in the pistil. Incompatible pollen usually germinates, but does not grow past the upper third of the style.

In *Petunia*, 19 different *S* alleles have been described (Ai et al., 1991; Broothaerts et al., 1989; Linskens and Straub, 1978; Mather, 1943; Robbins et al., 2000; Wang et al., 2001). Phylogenetic analysis suggested that the different alleles arose through intragenic recombination (Wang et al., 2001). The *S*-alleles within the

pistol encode *S*-RNases which have two highly variable regions (HV_a and HV_b) and five conserved regions (C1 through C5). The exact nature of the pollen *S*-alleles have not yet been elucidated; however, they are physically linked to the pistil *S*-alleles (McCubbin, et al., 2000). A potential pollen *S*-allele encoded protein has been identified (Sims and Ordanic, 2001). This protein (PhSBP1) is only expressed in pollen and binds to *S*-RNases with a high degree of specificity. PhSBP1 contains a RING-HC domain, which functions as an E3 ubiquitin ligase. This suggests that PhSBP1might function by degrading *S*-RNases.

4.5 Interspecific Hybridization

Within the two subgenera (*Pseudonicotiana* and *Eupetunia*), geographic isolation is the primary reproductive isolating mechanism. Within a subgenus, the species are allopatric producing fertile progeny between any interspecific combination (Griesbach, unpublished data). The species in the different subgenera are syntopic. The range of the *Pseudonicotiana* and *Eupetunia* species overlaps, but hybrids are not commonly found. Lack of hybridization is due to the difference in pollinators (Ando et al., 2001). The *Pseudonicotiana* species are pollinated by nocturnally active hawkmoths (*Manduca contracta* and *M. diffusa*), while the *Eupetunia* species are pollinated by a diurnally active bee (*Hexantheda* sp.).

All *Pseudonicotiana* x *Eupetunia* hybrids are fertile, but vary in pollen viability (Table 11-5) (Watanabe et al., 1996). Even within a subgenus, pollen viability of interspecific hybrids can vary from 100% to less than 50% that of a sib-mated species control (Tsukamoto et al., 1998). Interestingly, the fertility of hybrids between species in the subgenera are not significantly less fertile than hybrids among species within a subgenus.

Petunia axillaris x *P. integrifolia* is the most studied *Pseudonicotiana* x *Eupetunia* hybrid. The cultivated petunia (*P. x hybrida*) was derived from this hybrid. Significantly higher success rates occur when *P. axillaris* is used as the female parent. Morphologically the hybrid is intermediate between the parents except in flower color. In most instances, the hybrid produces purple flowers the same intensity as the *P. integrifolia* parent. In advanced generations, aberrant segregation ratios occur. It was concluded that these aberrant ratios were caused by differential pollen growth (Mather and Edwardes, 1943). Aberrant segregation can also be caused by recombination genes (Robet, et al., 1991). A major gene (*Rm1*) and several modifier genes have been found which enhance recombination frequencies of specific chromosome fragments.

Table 11-5. Cross compatibility of interspecific hybrids using *Petunia axillaris* as the female parent (Watanabe, et al., 1996, 2001).

Male parent species	No. pollinated flowers / No. of seed capsules	Pollen viability (%)
P. axillaris	1.00	98
P. exserta	0.67	98
P. integrifolia	0.33	79
P. reitzii	0.90	75
P. interior	0.57	70
P. guarapuavensis	0.34	69
P. altiplana	0.14	68
P. mantiqueirensis	0.91	67
P. scheideana	0.44	61
P. saxicola	1.00	60
P. bonjardinensis	0.82	59

4.6 Somatic Hybridization

Petunia is consider one of the model plants for tissue culture (Hanson, 1980). Techniques for plant regeneration from almost any tissue have been reported (Izhar and Zelcher, 1984). Unlike in most plants, petunia protoplasts are relatively easily regenerated into whole plants (Cocking 1975). The crucial step in somatic hybridization is in selection. Once protoplasts are fused, hybrid cells need to be selected from the parental unfused cells. Two approaches have been used to select hybrids; both approaches use two steps. The first step involves plating the mixture of parental and fused cells on a medium, which prevents one of the parental cells from dividing. In the second step, a component is added to the medium to prevent the other parental cells from dividing. Complementation allows only the hybrid cells to divide. In the other approach, step one remains the same. In step two, a chlorophyll deficient mutant is used as the other parent. Through complementation only the hybrids cells produce green callus.

Several wide hybrids between sexually incompatible species have been created, but only three have resulted in whole plants (Sink, 1984a, b). The widest hybrids created were *P. x hybrida* + *Salpiglossis sinuata* and *P. axillaris* subsp. *parodii* + *Salpiglossis sinuata* (Lee et al., 1994). Putative hybrids were identified by chromosome and isozyme analysis. Both hybrids had abnormal leaf development and very poor, if any, root growth. Neither hybrid produced flowers.

The other wide hybrid between sexually incompatible species was *P. axillaris* subsp. *parodii* (2*n*=14) + *Calibrachoa parviflora* (2n=18) (Powers, et al., 1980). Fifty plants of this hybrid were regenerated from a single callus. All of the plants were aneuploids with 31 chromosomes. A 32 chromosome count was expected (14 +18). The missing chromosome was the result of a reciprocal exchange between a *P. axillaris* subsp. *parodii* and *C. parviflora* chromosome with the larger interchanged chromosome being subsequently lost (White and Rees, 1987). During

meiosis, the hybrid behaved as an allotetraploid producing only bivalents between homologous chromosomes. The chiasma frequency of each genome was different in the hybrid background compared with its parental species background. There was an increase in the chiasma frequency of *P. axillaris* subsp. *parodii* bivalents in the hybrid relative to the species parent. The opposite situation occurred for *C. parviflora* bivalents.

The two most widely studied somatic hybrids (*P. axillaris* subsp. *parodii* + *P. integrifolia* subsp. *inflata* and *P. axillaris* subsp. *parodii* + *P. x hybrida*) are between sexually compatible species (Powers, et al., 1976, 1979). Both hybrids can be sexually produced and are fully fertile. The shape of the flowers and leaves of the somatic hybrids are slightly different from that of tetraploid forms of the sexually produced hybrids.

A large number of different *P. axillaris* subsp. *parodii* + *P. x hybrida* somatic hybrids have been analyzed. They fall into one of two types (Izhar and Zelcher, 1980). In the most common type, the hybrids contained both nuclear genomes. In the second type, only a single genome was present. In the hybrids with a single parental nuclear genome, the cytoplasmic genome contained plastid (cpDNA) and mitochondrial (mtDNA) genomes from both parents. The term 'cybrid' was coined for these hybrids. Cybrids have been used to determine the inheritance of mtDNA. In *P. axillaris* subsp. *parodii* + *P. x hybrida* cybrids, novel mtDNA fragments have been found that were due to intergenomic recombination (Rothenberg and Hanson, 1988).

The *P. axillaris* subsp. *parodii* + *P. integrifolia* subsp. *inflata* somatic hybrids were not as stable as their sexually produced counterparts (Schnabelrauch et al., 1985). Many of the plants produced branches with slightly abnormal leaves and flowers. A few plants were unstable aneuploids producing varying chromosome numbers over time. These plants expressed various degrees of abnormal floral and leaf morphology, as well as floral anthocyanin and leaf chlorophyll variegation. It was proposed that the instability was due to cytoplasmic/nuclear incompatibility analogous to hybrid dysgenesis. In plants, with abnormal leaf and flower development, an occasional branch is produced with normal flowers and leaves. cpDNA and mtDNA rearrangements and segregation was found to occur over time (Clark et al., 1986). All of the hybrids contained only the *P. axillaris* subsp. *parodii* cpDNA genome. On the other hand, most of the hybrids contained only the *P. integrifolia* subsp. *inflata* mtDNA genome. Additional evidence for hybrid dysgenesis is found in the fact that the sexual hybrid can only be created when *P. axillaris* subsp. *parodii* is used as the female parent. The sexual hybrid is completely stable.

The instability of the *P. axillaris* subsp. *parodii* + *P. integrifolia* subsp. *inflata* hybrid was increased by treating regenerating leaves with griseofulvin (Griesbach, et al, 1983). Four percent of the regenerated plants had a reduced chromosome number. Experiments in fungi, demonstrated that griseofulvin reduces the

chromosomes in two stages. During the first stage, the chromosome number is increased. During the second stage, several abnormal cell divisions occur in which chromosomes are randomly lost (North, 1977). In several of the reduced chromosome number plants, new phenotypes were expressed that were similar to what was seen in an F_2 segregating population.

5. FLOWER COLOR

5.1 Chemistry

In *Petunia*, the pigments responsible for flower color are the flavonoids. These pigments can be artificially subdivided into two groups - the anthocyanins and the co-pigments. The anthocyanins contain sugar and acyl moieties attached to the basic flavonoid ring structure (Asen, 1976). The anthocyanins minus their sugars and acyl moieties are called anthocyanidins. There are five different anthocyanidins (pelargonidin, cyanidin, peonidin, delphinidin, petunidin and malvidin) which are all found in *Petunia.* In addition, three different acyl moieties (coumaric, ferulic, and caffeic acids) and two different sugars (glucose and rhamnose) are also found (Ando et al., 1999; Gonzalez, et al., 2001; Griesbach et al., 1991; Muszynski, 1964, 1968). The exact structure for a number anthocyanins has been determined (Fukui, et al., 1998; Slimestad, et al., 1999; Tatsuzawa, et al., 1999, 2000; Gonzalez, et al., 2001). For example, one of the anthocyanins found in *P. x hybrida* 'Festival' was malvidin 3-O-(6-O-(4-O-(4-O-(6-O-feruloyl-β-D-glucopyranosyl)-*E*-*p*-coumaroyl)-α-rhamnosyl)-β-D-glucopyranoside)-5-β-D-glucopyranoside (Gonzalez, et al., 2001).

The co-pigments found in *Petunia* are flavonols. Like the anthocyanins, both sugar and acyl moieties are attached to the basic flavonoid ring structure. Two flavonols (quercetin and kaempferol) with caffeic acid and glucose attached have been found in *Petunia* (Birkofer and Kaiser, 1962; Griesbach and Asen, 1990). The most complex co-pigment found was quercetin 3-O-caffeoylsophoryl-7-glucoside. Its exact structure has not yet been determined.

At physiological pHs, the anthocyanins are not very stable and are nearly colorless (Asen, 1976). The addition of co-pigments to the anthocyanins increases both the stability and intensity of the anthocyanin's color. For example, a solution containing 10 mM of the anthocyanin cyanidin 3,5-diglucoside and 30 mM of the co-pigment quercetin has an absorption eight times greater than a solution containing only the anthocyanin (Asen et al., 1972). This effect is called co-pigmentation.

Within the cell, the anthocyanins and co-pigments occur bound together as a chemical complex. In *Commelina communis*, the anthocyanin/co-pigment complex contains six anthocyanins, six co-pigments, and two magnesium molecules (Kondo, et al., 1992). To date, this is only anthocyanin/co-pigment complex in which the

exact structure has been elucidated. In the anthocyanin/co-pigment complex, hydrophobic interactions between the aromatic rings of the anthocyanin and co-pigment molecules result in visible color (Brouillard, 1988).

It is generally assumed that red flowers contain predominantly cyanidin and blue flowers mostly delphinidin. Although this is usually true, there are many exceptions. For example, the red flower color of *P. exserta* is the result of delphinidin; while in red flowered *P. x hybrida* cultivars, the pigment is cyanidin (Ando et. al., 2000). pH is one of the major reasons why flowers containing the same anthocyanin can be different colors (Stewart, et al., 1975). As the pH becomes more alkaline, the color of a specific anthocyanin/co-pigment complex becomes bluer. All the anthocyanins except pelargonidin have the capability of producing blue flowers (Asen, 1976).

In *P. x hybrida*, floral vacuolar pH ranged between 5.2 and 6.5 units (Griesbach, 1996). Five genes (*Ph1, Ph2, Ph3, Ph4,* and *Ph5)* have been identified that control the pH (deVlaming et al., 1983; Chuck et al., 1993). The *Ph1* and *Ph2* genes only affect flower color. The other genes are pleiotropic affecting other processes as well (i.e. fertility). In the presence of dominant alleles of a *Ph* gene, the pH is lowered. The genes are co-dominantly inherited with each allele reducing the pH by about 0.4 unit (Griesbach, 1998). The *Ph* genes encode tonoplast Na+/H+ exchanger proteins (*NHX1*) (Reuveni et. al., 2001; Yamaguchi et al., 2001).

5.1.1 Flavonoid Biosynthesis

Flavonoids are the pigments responsible for flower color in *Petunia*. The flavonoid biosynthetic pathway (fig. 2) is very well understood (Holton and Cornish, 1995; Mol, et al., 1998; Winkel-Shirley, 2001). All of the enzymes and their corresponding genes have been studied in detail. The first studies identified genes that were involved in the inheritance of flower color. As biochemical data became available, these genes were assigned specific functions (Wiering and deVlaming, 1984).

In *Petunia*, three genes (*Chs, Chi* and *An3*) are involved in creating the basic flavonoid ring. *Chs* encodes the chalcone synthase multigene family with eight complete (*ChsA, B, D, F, G, H, J* and *L*) and four incomplete (*ChsC, E, I* and *K*) copies per haploid genome (Koes et al., 1987 and 1989). *ChsA* gene is the only gene transcribed to a significant extent in flower tissue. In leaf tissue, *ChsA* is silenced due to the methylation of one of its EcoRII sites (O'Dell et al, 1999). In floral tissue this site is not methylated. Each complete *Chs* gene consists of two exons separated by an intron of variable size and sequence (Koes et al., 1989). The incomplete *Chs* genes do not contain an intron.

Chi encodes the chalcone flavanone isomerase multigene family with two copies (*ChiA* and *ChiB)* per haploid genome (van Tunen, et al., 1988). *ChiA* is expressed in all floral tissue and contains no introns; while *ChiB* is only expressed in anthers and contains three introns. The *Po* mutation is the result of a mutation in the regulatory

region of *ChiA* abolishing promoter activity in anthers but not in corollas (van Tunen, et. al., 1991). The last gene involved in creating the basic flavonoid ring structure is *An3*. *An3* encodes a 2-oxoglutarate-dependent dioxygense (Britsch, 1990). In the presence of oxygen and ferrous ions, this enzyme catalyzes the decarboxylation of 2-oxoglutarate releasing carbon dioxide and succinic acid. *An3* has been cloned and the active site of the enzyme determined (Lukacin, et al., 2000).

In corolla cells, three different genes (*Ht1, Hf1* and *Hf2*) are responsible for hydroxylating the flavonoid ring to create a dihydroflavonol (Stotz, et al., 1985). The *Ht* genes encoded encode a cytochrome P450-dependent monooxygenase, which hydroxylates the carbon at the 3' position (Brugliera, et al.,1999). The *Hf* genes also encode a cytochrome P450-dependent monooxygenase which hydroxylates the carbon at the 5' position instead of the 3' position (de Vetten, et al., 1999; Shimada, et al., 2001). The 5' hydroxylase requires the presence of an additional protein (cytochrome b_5) encoded by *DifF*. Cytochrome b_5 acts as the electron donor between NADPH and cytochrome P450-dependent monooxygenase.

The conversion of dihydroflavonols into anthocyanins requires the concerted action of three enzymes (Nakajima, et al., 2001; Saito, et. al., 1999; Turnbull, et. al., 2000). This is a very complex step in the pathway, which involves two different reactions—the reduction of the double bonded oxygen on the carbon at the 4 position and the glucosylation of the hydroxyl group at the 3 position. The first enzyme (dihydroflavonol reductase) is encoded by *An6* and catalyzes the conversion of dihydroflavonols to leucoanthocyanins (Huitts, et. al., 1994). *An6* contains five introns. Besides *An6*, there are two other dihydroflavonol reductase genes (Beld, et al., 1989). The second enzyme in this complex reaction (anthocyanidin synthase, a 2-oxoglutarate-dependant oxygenase) is encoded by *An17* and converts leucoanthocyanins into 3-flaven-2,3-diols (Weiss, et al., 1993). The last enzyme in the reaction (UDP-glucose : anthocyanin 3-O-glucosyltransferase) creates the anthocyanin-3-glucoside (Kho, et. al., 1978). The 3-O-glucosyltransferase gene in *Petunia* has been cloned and exists in two copies (Yamazaki, et al., 2002).

Dihydroflavovols can also be converted into flavonol glycosides. *Fl* encodes flavonol synthase which is a 2-oxoglutarate-dependant oxygenase (Holton, et al., 1993). The *Petunia* flavonol synthase has a greater K_m for dihydrokaempferol and dihdroquercetin than for dihydromyricetin (Gerats, et. al., 1982; Forkman and Ruhnau, 1987). In addition, this enzyme has a greater K_m for dihydrokaempferol and dihdroquercetin than dihydroflavonol reductase. Therefore in Fl^+ genotypes, quercetin glycosides accumulate at the expense of cyanidin-based anthocyanins. To a lesser extent, myricetin glycosides accumulate at the expense of delphinidin-based anthocyanins in Fl^+ genotypes.

The 3-glucosyl anthocyanin is the substrate for the *Rt* encoded enzyme which adds a rhamnose to the glucose at the 3 position to create a rutinoside (Brugliera, et. al., 1994; Kroon, et al., 1994). The 3-rutinoside is now the substrate for *Gf* which encodes an enzyme which attaches acylates to the 3-rutinoside with either caffeic

acid or coumaric acid (Jonsson, et al., 1984a). Once the acyl group is attached, UDP-glucose : anthocyanin 5-O-glucosyltransferase adds glucose at the 5 position (Jonsson, et al., 1984a). The 5-O-glucosyltransferase gene in *Petunia* has been cloned and the enzyme exhibits strict substrate specificity for the anthocyanin 3-acylrutinoside (Yamazaki, et al., 2002). The last steps in the pathway involve the methylation of the acylated rutinoside.

There are four different anthocyanin-O-methyltransferase genes in *Petunia* (*Mt1, Mt2, Mf1,* and *Mf2*) (Jonsson, et al., 1983). Each gene controls a distinct and independent enzyme which is capable of methylating both the 3' and 5' positions on the anthocyanin molecule (Figure 11-1). Each enzyme, however, had a distinct substrate specificity. The *Mf1* (8 μM) and *Mt2* (6 μM) encoded enzymes had an approximately three-fold lower K_m values for cyanidin and petunidin as the substrate than the *Mf2* (21 μM) and *Mt1* (25 μM) encoded enzymes. Each enzyme also had a different efficiency in methylating delphinidin - *Mf1* (175 pkat·mg protein^{-1}), *Mf2* (100 pkat·mg protein^{-1}), *Mt1* (60 pkat·mg protein^{-1}), and *Mt2* (30 pkat·mg protein^{-1}). The *Mt* encoded enzymes preferred cyanidin as a substrate, instead of either petunidin or delphinidin . When delphinidin was the substrate, the *Mt* encoded enzymes produced mainly petunidin; while the *Mf* encoded enzymes produced mainly malvidin. There was, however, a differential effect on substrate inhibition. High concentrations of delphinidin reduced the amount of malvidin produced, but not the amount of petunidin produced. In addition, a dosage effect was suggest for *Mf* / *Mt* gene expression. The greater the number of Mf^+ / Mt^+ genes, the higher the relative concentration of malvidin. High concentrations of petunidin coupled with low concentrations of delphinidin promoted malvidin synthesis (Jonsson, et al., 1984b).

In *Petunia* flowers, the genes encoding the enzymes that are expressed early in the anthocyanin biosynthetic pathway (chalcone synthase, chalcone-flavone isomerase, flavanone 3-hydroxylase, etc.) are controlled by a different set of regulatory genes than those genes encoding the enzymes expressed late in the pathway (dihydroflavonol reductase, anthocyanin rhamnosyltransferase, anthocyanin methyltransferase, etc.) (Quattrocchio, et al., 1993). At least four regulatory genes (*An1, An2, An4*, and *An11*) are required for the transcription of the genes expressed late in the pathway. *An1* encodes a basic helix-loop-helix (bHLH) transcription factor that is active in all parts of the flower (Spelt et al., 2000). *An2* and *An4* encode MYB-domain transcription factors (Quattrocchio et al., 1999). *An2* is only active within the limb, while *An4* is only active within the anthers. *An11* encodes a regulatory protein with five WD-repeat units that is active in all parts of the flower (de Vetten et al., 1997).

These regulatory genes operate in a complex regulatory hierarchy which is still not completely understood. *An11* encodes a cytoplasmic protein that regulates the expression of *An2,* as well as other non-anthocyanin related genes (de Vetten, et al., 1997). It appears that *An11* links cellular and/or environmental signals with

transcription of *An2*. However, *An2* does not directly regulate the transcription of any anthocyanin structural gene. *An2* controls the expression of *An1* which directly activates the transcription of the structural genes within the limb and tube (Spelt, et al., 2000). Besides regulating anthocyanin biosynthesis, *An1*, *An2*, and *An11* also control vacuolar pH (Mol, et al., 1998). *An1* (previously studied as *Ph6*) regulates the expression of *Ph1* and *Ph2* (Griesbach, 1998).

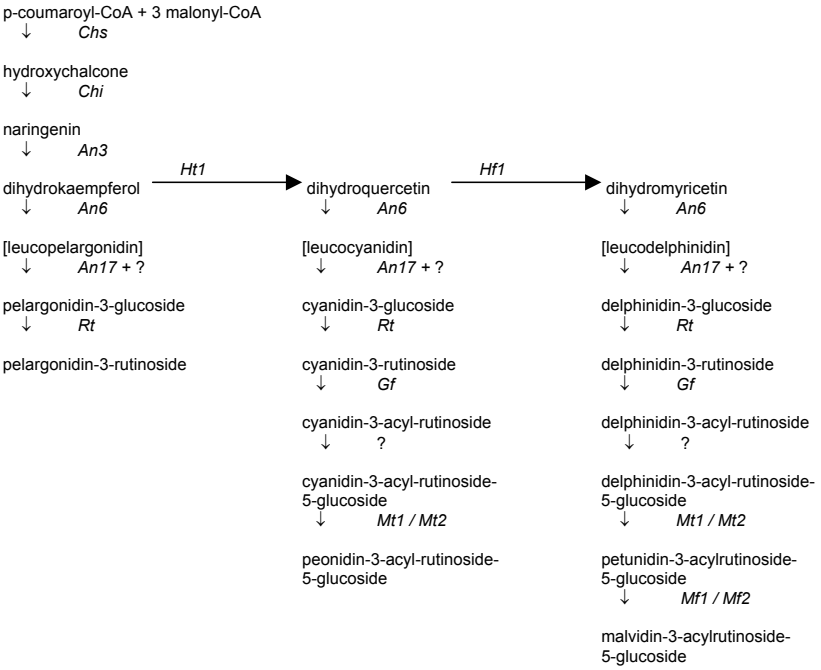

Figure 11-1. The flavonoid biosynthetic pathway, with the identified corresponding genes, operating in *Petunia x hybrida*.

5.1.2 Flavonoid Genetic Engineering

One of the first practical examples of plant genetic engineering involved the development of a novel flower color in petunia through the engineering of dihydroflavonol reductase (DFR). The *Petunia* DFR has an extremely low substrate specificity for dihydrokaempferol; therefore, pelargonidin is rarely found (Huitts, et. al., 1994). Mutants with a defective *Ht1* gene would be expected to produce unpigmented flowers (Figure 11-1). Two leaky *ht*$^{-}$ *hf1*$^{-}$ *mf1*$^{-}$ mutants (RL01 and

W80) and one leaky *ht- hf1- hf2-* mutant (Skr4 x Sw63) were used as parent plants in transformation. Mutants RL01and W80 both produced one tenth the normal amount of anthocyanin that was composed of 28% delphinidin, 63% cyanidin and 9% pelargonidin (Griesbach, 1993; Johnson, et al., 1999). Mutant Skr4 x Sw63 produced one tenth the normal amount of anthocyanin that was composed of 24% pelargonidin, 7% peonidin, 4% petunidin and 65% malvidin (Tanaka, et al., 1995).

A *Cymbidium* DFR gene was introduced into mutant W80 (Johnson et al., 1999). The *Cymbidium* enzyme only recognizes dihydroquercetin as a substrate. Therefore, only cyanidin and peonidin are produced (Tatsuzawa et al., 1996). When the *Cymbidium* gene was expressed in W80, there was no change in flower color.

A *Zea mays* DFR gene (*A1*) was introduced into mutant RL01 (Meyer, et al., 1987). The *Zea* enzyme recognizes both dihydroquercetin and dihydrokaempferol as a substrate, but has a much stronger affinity for dihydroquercetin. Pelargonidin is only produced in the absence of dihydroquercetin. When *A1* was expressed in RL01, there was a ten-fold increase in the total amount of anthocyanin, as well as an increase in the relative amount of pelargonidin from 9% to 55% (Griesbach, 1993).

A *Gerbera* DFR gene (*Gdfr*) was introduced into RL01 (Elomaa et al., 1995). The *Gerbera* enzyme recognizes both dihydroquercetin and dihydrokaempferol as a substrate, but has a much stronger affinity for dihydrokaempferol. Pelargonidin is produced irrespective of what precursor is present (Asen, 1984). When the *Gdfr* was expressed in RL01, the total amount anthocyanin and the relative amount of pelargonidin did not differ significantly from RL01 plants expressing *A1*.

A *Rosa* DFR gene was introduced into Skr4 x Sw63 (Tanaka et al., 1995). The *Rosa* enzyme recognizes both dihydroquercetin and dihydrokaempferol, but has a stronger affinity for dihydroquercetin. A small amount of pelargonidin is always produced (Asen 1982). When the *Rosa* gene was expressed Skr4 x Sw63, there was a ten-fold increase in the total amount of anthocyanin, as well as, an increase in the relative amount of pelargonidin from 24% to 97%.

In order to identify the DNA sequence leading to substrate specificity, chimeric DFR genes were constructed and introduced into *P. x hybrida* 'W80' (Johnson, et al., 1999). It was determined that the substrate binding region was between amino acids 132 and 158 with amino acid 134 critical in substrate specificity. A switch from asparagine to leucine at position 134 caused a change in substrate preference from dihydroquercetin to dihydrokaempferol.

When grown in the greenhouse, RL01 plants expressing *A1* produced solid orange flowers; while when grown outdoors, they produced weakly pigmented flowers with reduced levels of *A1* expression (Meyer, et al., 1992). Reduced expression (gene silencing) was due to DNA methylation within either the *35S* promoter in the *A1* construct or in *A1* itself (Meyer and Heidmann, 1994).

Unlike plants expressing *A1*, plants expressing *Gdfr* very rarely produced weakly pigmented flowers with reduced levels of transgene expression. In those rare plants with reduced expression, only the 35S promoter was methylated. *Gdfr* itself was

never methylated. It was suggested that the increase in the stability of *Gdfr* expression over *A1* expression was due to the lower GC content (39%) of *Gdfr* than *A1* (60%). Dicotyledonous plants have a lower GC content than monocotyledonous plants. Transgene silencing can be induced by differences in the GC content between the foreign and host DNA (Fagard and Vaucheret, 2000).

There are two mechanisms for gene silencing (Fagard and Vaucheret, 2000). In transcriptionally gene silencing (TGS), there is a decrease in mRNA synthesis because of promoter methylation. As described above, the *A1* gene was silenced through TGS. In post-transcriptionally gene silencing (PTGS), there is decrease in the mRNA because of sequence specific degradation. The exact mechanism(s) for gene silence are still unknown; however, both TGS and PTGS involve the production of small double stranded RNA (dsRNA) molecules (Sijen et al., 2001).

The introduction of a transgene can also lead to PTGS of its endogenous homologous gene. The silenced phenotype is dependent upon the orientation of the transgene. *Petunia x hybrida* plants expressing a chalcone synthase (*Chs*) transgene in the same orientation as the endogenous gene (co-suppression) produced flowers with reduced pigmentation at the junctions between adjacent petals (Napoli, et al., 1990). While *P. x hybrida* plants expressing a chalcone synthase (*Chs*) transgene in opposite orientation as the endogenous gene (anti-sense suppression), produced a different phenotype with white petal margins and reduced pigmentation throughout the flower (van der Krol, et al., 1988). Through breeding it was possible to combine both phenotypes in a single plant (Que, et al., 1998). It was suggested that sense and anti-sense suppression occur in different cellular compartments or at different times in development.

Genetic engineering has been used to study the expression of flavonoid regulatory genes. In genetic complementation studies, the *Zea mays* regulatory genes *Lc* (encodes a bHLH protein) and *C1* (encodes a MYB protein) together were able to up-regulate the *An6* promoter and increase anthocyanin production in a white-flowered *an2*$^{-}$ mutant (Quattrocchio, et al., 1993). *Lc* alone had no effect. It was suggested that structural gene activation requires the interaction of a bHLH protein with a MYB protein (Bradley, et al., 1998). In the complementation study, the *Lc* encoded bHLH protein by itself was not able to up-regulate the *An6* promoter because of the absence of a MYB protein due to the *an2*$^{-}$ mutation.

Regulatory genes are highly conserved (Solano, et al., 1997). All animal c-MYB-domain proteins bind to a single specific DNA sequence (AAAC(G/C)GTTA) called MBSI, while the *Petunia* encoded MYB-domain protein binds to both the MBSI sequence and another sequence (AGTTAGTTA) called MBSII. A single amino acid change from glutamine to leucine at position 71 in the animal protein switched its single DNA binding specificity to dual binding.

6. FUTURE BREEDING & GENETICS

Significant genetic and breeding progress has been accomplished in the half century since F_1 hybrid seed-propagated petunias were introduced. Petunia is one of the few floricultural crops which now has a linkage map established. Significant research has been conducted to elucidate the genes controlling pigmentation in the flavonoid biosynthetic pathway, tissue culturing (regeneration/transformation protocols), and genetic engineering. As such, the research conducted with petunia can be used as a model for the continued genetic development of other new and domesticated floriculture crops. Future breeding and genetics will progress in a wide variety of arenas, allowing for continued development of new flower colors, patterns, plant habits, and other significant phenotypic changes.

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Chapter 12

ZINNIA

Zinnia elegans, Z. angustifolia

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Abstract: Zinnias are widely cultivated worldwide both as cut flowers and bedding plants. The seed-propagated crop has a diverse range of plant habits, flower colors, and flower types. Zinnias are noted for their garden performance and flower power. Recent innovations through breeding have created disease-tolerant cultivars.

Key words: Breeding, cytology, polyploidy, self incompatibility, F_1 hybrids.

1. INTRODUCTION

Zinnias are multipurpose annuals and are cultivated worldwide for use as bedding plants and cut flowers. Cultivars exhibit considerable phenotypic diversity for plant habit, flower morphology, and ray floret color. This multitude of plant types lends to their utility and versatility as garden subjects. Zinnias are one of the most popular annuals grown in North America and have maintained a high ranking in total unit sales of seed packets sold by seed companies in the United States. Breeders made many noteworthy improvements in zinnias during the 20th century, as evidenced by conferral of 37 All-America Selection awards on zinnias since the inception of the awards program in 1933 (All-America Selections, 2003). This chapter will focus on current techniques and future challenges in the improvement of zinnias.

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2. TAXONOMY

The genus *Zinnia* (family Asteraceae, tribe Heliantheae) consists of 19 species of annual herbs or perennial shrubs or subshrubs (McVaugh, 1984; Torres, 1963a). The genus is named in honor of Johann Gottfried Zinn (1727-1759), professor of anatomy and botany at the University of Göttingen (Germany), who described the species now known as *Z. peruviana* (L.). *Zinnia* species are restricted to North America with one notable exception, *Z. peruviana*, which extends from the southern United States to Argentina. The center of diversity for the genus is Mexico.

Zinnia species are classified into two subgenera: *Diplothrix* and *Zinnia* (McVaugh, 1984; Torres, 1963a). Subgenus *Diplothrix* consists of six species of perennial cespitose shrubs or subshrubs distributed in the southwestern United States and northern Mexico. The base chromosome number for subgenus *Diplothrix* is n = 10; diploid and polyploid taxa have been documented (Torres, 1963a). The only species in subgenus *Diplothrix* cultivated extensively as an ornamental is the Rocky mountain zinnia, *Z. grandiflora* Nutt. This species is a perennial subshrub cold hardy to U.S. Zone 4 and drought tolerant (Borland, 1989). Plants are 10-22 cm in height and have linear leaves about 2.5 cm long. Inflorescences (capitula) are 2.5-3.8 cm in diameter and have bright yellow ray florets. *Zinnia grandiflora* is a polyploid ($2n = 4x = 21$) whose territory extends from Kansas and southeastern Colorado south to Sonora, Coahuila, Durango, and Zacatecas (Torres, 1963a). In its native range, *Z. grandiflora* flowers from late spring or early summer to frost.

Taxa in subgenus *Zinnia* are distributed in the warm temperate and tropical regions of North America and have a base chromosome number of n = 12 (Torres, 1963a,b). The 13 species in subgenus *Zinnia* are classified into three sections: *Mendezia*, *Tragoceros*, and *Zinnia* (McVaugh, 1984; Torres, 1963a,b). Section *Zinnia* contains three species: *Z. haageana* Regel, *Z. peruviana*, and *Z. violacea* Cav. (formerly *Z. elegans* Jacq.). The chromosome number is $2n = 2x = 24$ for all three species (Torres, 1963a).

Zinnia haageana (Mexican zinnia) is an erect or decumbent herbaceous annual up to 60 cm in height, with lanceolate leaves, and 2-4.5 cm wide capitula with a single whorl of solid or bicoloured ligules (McVaugh, 1984; Torres, 1963a). Cultivated forms often have several whorls of ray florets. The species is found from Jalisco to Guerrero at elevations from 900 to 2000 meters, and flowers from July to November. It is endemic to grasslands, grassy or rocky hills, wet meadows, wet fields, roadsides, and disturbed habitats.

Zinnia peruviana (type species for the genus) is an erect herbaceous annual, 70-100 cm in height, with lancelinear to broadly ovate or elliptic leaves, and 4-5 cm wide capitula with ray florets in a single whorl (McVaugh, 1984; Torres, 1963a). Ligules are either yellow or scarlet red. It is distributed from southeastern Arizona and western Mexico to Peru and Argentina at elevations from sea level to 3000 meters. Plants flower from April to October in North America (Torres, 1963a). The

species is found in numerous habitats throughout its range. Plants can yield prolific numbers of seed and it has escaped from cultivation in some temperate and subtropical regions with summer rainfall (Beeks, 1954). *Zinnia peruviana* is not cultivated widely, perhaps because it exhibits little phenotypic variation relative to *Z. violacea*.

Zinnia violacea (formerly *Z. elegans*) is the most widely cultivated zinnia species and most important in terms of economic value. It is an herbaceous annual that is grown for its large, showy capitula and diversity of ray petal (ligule) colors and shapes (Beeks, 1954). Plants are erect, 9-200 cm in height, sparsely branched, with large ovate to lanceolate leaves, and 4-7 cm wide capitula (McVaugh, 1984; Torres, 1963a). Capitula of cultivated plants range from 2.5 to 15 cm in diameter and have one to several whorls of ray florets. The species is distributed from Sinaloa and Durango to Guerrero at elevations from 600 to 1800 meters, and flowers from March to November. It is endemic to openings in woodlands, grassy and weedy places, old fields, roadsides, ditches, and in open oak forest or tropical deciduous forest. Landrace types are widely grown in Mexican household gardens.

Section *Mendezia* in subgenus *Zinnia* contains eight species that are endemic to central and southern Mexico, but are found primarily in the western coastal states (McVaugh, 1984; Torres, 1963a). Six of the species are herbaceous annuals whereas the remaining two species are perennials. Chromosome numbers of $n = 10$, 11, and 12 have been reported for taxa in Section *Mendezia* (Keil et al., 1988; Olorode, 1970; Terry-Lewandowski et al., 1984; Torres, 1963a). *Zinnia angustifolia* H.B.K. (formerly *Z. linearis* Benth.) is the sole species in section *Mendezia* that is cultivated extensively. *Zinnia angustifolia* (narrow leaf zinnia) is an herbaceous annual exhibiting an erect or decumbent habit. Plants are 20-40 cm in height, profusely branched, with linear to oblong-elliptic leaves and masses of small capitula (1.5-3.5 cm in diameter) with orange or white ray florets in a single whorl (McVaugh, 1984; Torres, 1963a). Its range extends from Sonora and Chihuahua to Michoacán at elevations from 60 to 2100 meters, and flowers from July to January. It is endemic to rocky or grassy hills, lava-flows, clearings and woodland openings, disturbed habitats, oak or oak-pine forests, tropical deciduous forests, and savannahs. Chromosome counts of $2n = 2x = 22$ (Keil et al., 1988; Olorode, 1970; Powell and Turner, 1963; Terry-Lewandowski et al., 1984; Turner et al., 1961) and $2n = 2x = 24$ (Torres, 1963a; Turner et al., 1962) have been reported for *Z. angustifolia*.

Section *Tragoceros* contains several species of herbaceous annuals with a base chromosome number of $n = 11$ (Torres, 1963b). *Tragoceros* was maintained as a separate genus until 1970, when Olorode and Torres (1970) showed that *Tragoceros* species would cross with taxa in *Zinnia* Section *Mendezia*. Species in Section *Tragoceros* have small flowers (ligules <1 cm in length) and none are cultivated as ornamentals.

3. ORIGIN AND BRIEF HISTORY

In pre-Columbian times, the indigenous peoples of Mexico cultivated at least four plant genera for their showy flowers: dahlia (*Dahlia*), marigold (*Tagetes*), tiger flower (*Tigridia*), and tuberose (*Polianthes*) (Parsons, 1992). However, it is unlikely that zinnias received much attention as ornamentals until after their introduction to Europe (Parsons, 1992). *Zinnia peruviana* was introduced from Peru to France in the early 1700s and seeds were distributed to several European botanists in 1753 (Beeks, 1954). Seeds of a single *Z. violacea* accession were sent from Mexico to the Royal Botanical Gardens in Madrid about 1790 and were disseminated to other European gardens beginning in 1796 (McVaugh, 1984). *Zinnia haageana* was introduced from Mexico to France in 1825 (Beeks, 1954). *Zinnia angustifolia* was introduced from Mexico to England in 1838 (Beeks, 1954).

3.1 *Zinnia Violacea*

The original accession of *Z. violacea* exhibited purple ray florets in a single whorl. No changes in plant form or ligule color were noted until 1829 when a variety with scarlet ligules (‘Coccinea’) was introduced (Beeks, 1954). In subsequent generations, ‘Coccinea’ segregated for ligule color, thus providing a broader range of flower colors. The first double-flowered *Z. violacea* cultivar (‘Flore Pleno’) was developed in India and introduced to Europe in 1858. Capitula of ‘Flore Pleno’ were 5-7.5 cm in diameter and ligules were “purple, deep rose, light rose, rose striped, red, orange red, orange, buff, and various shades of these colors" (Anonymous, 1860). ‘Nana Flore Pleno’, released in 1866, was similar to ‘Flore Pleno’ but plants were more dwarf. In 1874, the German horticulturists Haage and Schmidt released their version of ‘Flore Pleno’, a dahlia-flowered type with double flowers. Herr C. Lorenz of Erfurt, Germany developed a strain with large double flowers named ‘Robusta Grandiflora Plenissima’; this strain was released in 1886 and eventually became known as the ‘Giant Mammoth’ strain. Plants of this cultivar were 70-100 cm tall with capitula up to 15 cm in diameter. ‘Giant Mammoth’ was a breakthrough in zinnia breeding and it was the progenitor of the large-flowered *Z. violacea* cultivars available today. ‘Pompon’ and ‘Lilliput’ types were developed in the 1870s. The first very dwarf cultivar (‘Tom Thumb’) was released before 1900. Mutations in ligule morphology appeared in the early 1900s. Quill-rayed (‘Fantasy’) types were developed around 1900 and ‘Cactus’-flowered zinnias with fluted ligules were introduced around 1914 (Beeks, 1954). The ‘Dahlia-Flowered’ strain was developed by Bodger Seeds and released in 1919 (Bodger Seeds Ltd., 1935). Plants are ≈100 cm tall and produce fully double capitula 11-13 cm in diameter. This strain is actually a series with 18 cultivars, each one with a unique ligule color. ‘Giants of California’, released in 1926, was also developed by Bodger Seeds. Plants were taller than the ‘Dahlia-Flowered’ types and produced longer,

stronger stems that florists prefer for cut flowers. The 'Giants of California' included 14 cultivars in a broad range of flower colors. 'Thumbelina', released by Bodger Seeds in 1963, was the first cultivar to breed true for dwarf habit and won an All-America Selections Gold Medal.

An apetalous male sterile ("femina") mutant was discovered in 1948 by John Mondry of W. Atlee Burpee Co. and its subsequent utilization in breeding led to the introduction of true F_1 hybrids. In 1960, W. Atlee Burpee Co. released 'Trail Blazer', the first F_1 hybrid zinnia cultivar utilizing male sterility for seed production. Numerous F_1 hybrid cultivars have been introduced since 1960. Among the most notable is 'Peter Pan', a dwarf F_1 hybrid series of seven separate colors, introduced between 1971 and 1980.

3.2 *Zinnia Haageana*

Following the introduction of a single-flowered *Z. haageana* accession to France in 1825, seeds were disseminated throughout Europe and grown in gardens. Horticultural interest in *Z. haageana* increased when Haage and Schmidt released a double-flowered cultivar ('Flora Pleno') in 1871 (Beeks, 1954). Three improved cultivars of *Z. haageana* have been introduced: 'Persian Carpet', 'Old Mexico', and 'Chippendale'. These first two cultivars won All-America Selections awards in 1952 and 1962, respectively. *Zinnia haageana* has not received as much attention by breeders as *Z. violacea*.

3.3 *Zinnia Angustifolia*

Although *Z. angustifolia* arrived in Europe in the 1830s, it apparently remained a little-known annual until the early 1900s. This species was unknown in the United States until an orange-flowered cultivar was introduced from Australia in the early 1930s by John Bodger (Bodger Seeds Ltd., 1935). Orange remained the only ligule color available for *Z. angustifolia* cultivars sold domestically until the early 1990s when cultivars with white ('Star White', 'Crystal White') and yellow ('Star Yellow') ligules were released. Interestingly, a *Z. angustifolia* cultivar with white ligules has been sold commercially in India since the 1960s (Bose and Panigrahi, 1969).

4. BREEDING OBJECTIVES

The most important selection criteria used for breeding zinnias are: rapid and uniform seed germination, strong stems, adequate branching, upright habit, freedom from disease problems, reduced leaf size (for *Z. violacea*), uniformity of flowering, mature plant vigor (rapid plant establishment after transplanting to outdoors),

number and size of capitula, a high percentage of plants with fully double flowers (for *Z. violacea*), long flowering period, and novel ligule colors and/or growth habits.

5. BREEDING TECHNIQUES

5.1 Reproductive Biology

Zinnia capitula contain one or more outer whorls of fertile, pistillate ray florets and inner whorls of hermaphroditic (fertile) disc florets. Species in Section *Tragoceros*, however, have disc florets that are staminate and sterile (McVaugh, 1984). Ray florets of *Z. violacea* have a showy ligule that contains flavonoids and/or carotenoids in epidermal cells (Boyle and Stimart, 1989a). Disc florets of *Z. violacea* are tubular, small (4-5 mm in diameter at the apex) and less showy than ray florets. Most *Z. violacea* cultivars have yellow disc florets, but one cultivar ('Scabiosa Flowered') produces elongated disc florets that are the same color as ray florets. Ovaries are inferior and each contains a single ovule. Within a capitulum, florets mature sequentially from the outermost to the innermost whorl. The anthers in disc florets form a tube. Anthers dehisce pollen to the inside of the tube and the elongating style pushes the pollen to the apex of the disc floret. Disc florets are dichogamous with several hours elapsing between pollen presentation and stigma receptivity. The stigmatic surface, located on the inner surface of both stylar lobes, is not exposed for pollination until after the stylar lobes have reflexed.

Most *Zinnia* species are obligate outbreeders. Self-incompatibility (SI) is prevalent in the genus and has been documented in at least 10 species: *Z. acerosa* (DC.) A. Gray, *Z. angustifolia*, *Z. anomala* A. Gray, *Z. citrea* Torres, *Z. grandiflora*, *Z. greggii* Robins. & Greenm., *Z. juniperifolia* (DC.) A. Gray, *Z. leucoglossa* Blake, *Z. littoralis* Robins. & Greenm., and *Z. violacea* (Boyle and Stimart, 1986; Olorode, 1970; Samaha and Boyle, 1989; Torres, 1964). *Zinnia peruviana* is apparently self-compatible (Torres, 1964) as are some lines of *Z. violacea*. Samaha and Boyle (1989) investigated the genetics of self-incompatibility in *Z. angustifolia* and proposed a single-locus, multiallelic system under sporophytic control. The data suggested the presence of a linear dominance series of *S*-alleles in the pollen and either a linear dominance series or a combination of dominance and independent action of *S*-alleles in the style. Examination of pollinated styles using ultra-violet epifluorescence microscopy revealed that pollen loads were low and callose was deposited in stigmatic papillae following incompatible crosses, whereas pollen loads were higher and little or no callose accumulated in stigmatic papillae after compatible crosses (Samaha et al., 1989).

5.2 Pollination and Fruit Development

In *Z. angustifolia* and *Z. violacea*, pollen germination and pollen tube penetration of the stigma occurs within 10 to 20 minutes after cross-compatible pollination, and pollen tubes traverse the full length of the style in the majority of florets by 30 minutes after pollination (Boyle and Stimart, 1986). Unpollinated pistils of *Z. violacea* can remain turgid for ≥3 weeks after the stylar lobes reflex. Pollen is capable of germinating on stigmas up to 20 days after the stylar lobes reflex but the likelihood of seed set diminishes as pistils age (Miyajima, 1995). Miyajima (1995) found that percent seed set was ≈100% when *Z. violacea* florets were pollinated on the day stylar lobes reflexed (day 0) but decreased to ≈50% by day 8, and to ≈20% by day 13. Like in other Asteraceae taxa, pollen of zinnia is trinucleate and is probably short-lived (<1 day at ≥20 °C) (Hoekstra, 1973, 1983).

Zinnias are relatively easy to cross-pollinate. Disc florets need to be removed with a pair of broad-tipped forceps to prevent selfing unless the female parent is either fully double or a femina type (see section 6.1). Pollen is then applied to pistillate ray florets on the emasculated parent. Forceps are used to remove disc florets from the paternal parent and pollinate the stigmas of the pistillate ray florets.

Each *Zinnia* floret produces a single seed (achene). The time required for seed maturation is dependent on environmental conditions (primarily mean daily temperature) plus the nutritional and moisture status of the maternal parent during fertilization and seed development. Generally, capitula require about 7 to 8 weeks from pollination to mature seed.

Seed of open-pollinated cultivars and most F_1 hybrids are produced in field plots. Since zinnias are predominantly outcrossed, Hawthorn and Pollard (1954) recommended an isolation distance of 400 m between zinnia stock-seed plantings and at least 200 m between commercial zinnia varieties for seed production. Seed yields of open-pollinated *Z. violacea* cultivars range from 170 to 355 $kg \cdot ha^{-1}$ (Hawthorn and Pollard, 1954).

5.3 Seed Cleaning and Storage

Capitula may be harvested manually, as is done for some F_1 hybrids, or plants can be severed at the base and allowed to dry on tarps in the field. In either case, seed is threshed when the seeds have dried sufficiently. Commercially, zinnia seeds are processed using a screening-fanning mill, indent separator, and/or gravity separator to remove trash. Since achenes from ray florets are larger and shaped differently than achenes from disc florets (Miyajima, 1998), seeds of these two types are often separated, cleaned, and bulked for the final product. Small batches of seed, like those generated during breeding, can be cleaned manually.

Zinnia seeds can be stored for prolonged intervals without an appreciable decline in germination if the storage temperature and relative humidity are satisfactory. The

germination percentage declined only 13% when seeds of *Z. violacea* were stored for 16 years at 4-5 ^{o}C and 35% to 40% relative humidity (Bass, 1980). Stanwood and Bass (1981) reported that air-dried *Z. violacea* seeds were not damaged when cooled to -196 ^{o}C in liquid nitrogen. These results indicate that *Z. violacea* seeds are tolerant of desiccation like other orthodox seeds.

6. GENERATION OF GENETIC VARIANTS

6.1 Intraspecific Crosses

Most zinnia cultivars are derived from outcrossing and/or inbreeding within the same species. Commercial cultivars are either open-pollinated types or F_1 hybrids. Currently, about half of the *Z. violacea* cultivars but none of the *Z. angustifolia* and *Z. haageana* cultivars sold commercially are F_1 hybrids. To date, male-sterile genotypes have not been discovered in *Z. angustifolia* and *Z. haageana*, so it is not feasible to produce commercial F_1 hybrids of these two species.

In *Z. violacea*, capitula of male-sterile plants (femina) are composed exclusively of pistillate ray florets that are apetalous (without ligules). Apetalous male-sterile plants are commonly referred to as feminas. Male sterility is a multigenic trait in *Z. violacea* and probably controlled by three unlinked recessive genes (Cowen and Ewart, 1990). Male-fertile lines can be heterozygous or homozygous at each locus so that several different segregation ratios (normal phenotype:femina) are possible in full-sib families. Femina plants are homozygous recessive at all three loci.

Since the release of the first F_1 zinnia hybrid in 1960 ('Trail Blazer'), the dominant trend in *Z. violacea* breeding has been towards development of F_1 hybrids. F_1 hybrid seed of *Z. violacea* is produced using maternal lines segregating for male-fertile and femina plants (usually a 1:1 ratio) and paternal lines with 100% male-fertile plants. Since femina plants are devoid of ligules and pollen, visits by biotic pollen vectors, primarily insects, are less frequent and consequently seed yields are lower than on plants with a normal phenotype.

Inbreeding is performed primarily to make pure-breeding *Z. violacea* lines for F_1 hybrid seed production. Either sibcrosses or a combination of sibcrosses and selfing are used to produce inbred lines. Five to six generations of inbreeding are required to obtain lines that are sufficiently homozygous for F_1 hybrid seed production. Inbreeding depression is in direct proportion to inbreeding so it becomes difficult to go beyond the S_4 generation by selfing alone.

6.2 Interspecific Crosses

Interspecific crosses have played an important role in the development of zinnia cultivars. Four interspecific hybrids have been reported: *Z. haageana* x *Z. violacea*, *Z. peruviana* x *Z. violacea*, *Z. angustifolia* x *Z. violacea*, and *Z. angustifolia* x *Z. haageana*. About 1875, Haage and Schmidt released several double-flowered cultivars that were putative hybrids of *Z. haageana* (as ♀) and *Z. violacea*. These hybrids were given the collective name *Z. darwinii*. One cultivar ('Vitata') resembled *Z. violacea* in plant size but had large capitula with striped ligules, a trait unknown previously in *Z. violacea* but present in *Z. haageana*, thus supporting their hybrid claim. Beeks (1954) traced the history of zinnia cultivars released before 1950 and concluded that several other cultivars introduced in the late 1800s and early 1900s may have been interspecific hybrids of *Z. haageana* and *Z. violacea*. 'Navajo' (also known as 'Gaillardia Flowered'), 'Sombrero', and 'Whirligig' are modern-day cultivars that are likely to be hybrids of *Z. haageana* and *Z. violacea* (Weddle, 1945).

Shahin et al. (1971) crossed diploid ($2n = 2x = 24$) accessions of *Z. peruviana* (as ♀) and *Z. violacea* and obtained F_1 hybrids. The F_1 hybrids were more vigorous than either parent and most hybrids resembled *Z. violacea* more than *Z. peruviana*. Intercrosses between F_1 hybrids and backcrosses to both parents failed to produce viable seeds. Microscopic examination of pollen mother cells revealed irregular meiosis. There have been no further reports of interspecific hybrids between these two species.

Ramalingam et al. (1971) obtained hybrids of *Z. angustifolia* ($2n = 2x = 22$) and *Z. violacea* ($2n = 2x = 24$) using *Z. angustifolia* as the female parent. The chromosome number in all F_1 hybrids was $2n = 2x = 23$. The authors reported "a very low seed set" but did not present any data on the characteristics of the resulting progeny. Research on the *Z. angustifolia* x *Z. violacea* hybrids was resumed at the University of Maryland (College Park, Maryland) in the 1980s. Crosses between *Z. angustifolia* and *Z. violacea* yielded infertile allodiploids ($2n = 2x = 23$), but partially fertile, true-breeding allotetraploids ($2n = 4x = 46$) were produced by treating allodiploids with colchicine (Boyle and Stimart, 1982; Terry-Lewandowski et al., 1984).

Boyle and Stimart (1987) found the *Z. angustifolia* genotype used as a maternal parent had a significant effect on the percentage of emerged allodiploid seedlings and the percentage of morphologically normal allodiploids. The *Z. violacea* cultivars used as pollen parents had no apparent effect on these traits. Variation in the percentage of emerged allodiploid seedlings was due to differences in the extent of hybrid embryo breakdown and percentage of nongerminating seeds. Crossing studies suggested the percentage of emerged allodiploid seedlings and percentage of morphologically normal allodiploids were controlled by multiple nuclear genes from *Z. angustifolia*. These results imply that thorough sampling of the *Z. angustifolia*

gene pool would likely result in identification of genotypes with greater crossability to *Z. violacea*. Additional studies (Boyle and Stimart, 1989b) showed that *Z. angustifolia* maternal genotype also affected leaf length, leaf width, capitulum diameter, number of ray florets, and days to flowering of F_1 allodiploids. Thus, selection of elite *Z. angustifolia* genotypes for use as maternal parents may be an effective means of producing interspecific hybrids with shorter stature, earlier flowering, and/or larger capitula.

Allotetraploids of *Z. angustifolia* and *Z. violacea* have been given the collective name *Z. marylandica* Spooner, Stimart & Boyle in honor of the institution where they were first characterized (Spooner et al., 1991). The first commercial *Z. marylandica* cultivar ('Rose Pinwheel') was developed by W. Atlee Burpee Company and introduced in 1987; five additional cultivars in the 'Pinwheel' series have since been introduced. Recently, Sakata Seed Company introduced three *Z. marylandica* cultivars: 'Profusion Orange', 'Profusion Cherry', and 'Profusion White'. All-America Selections Gold Medals were awarded to 'Profusion Orange' and 'Profusion Cherry' in 1999; 'Profusion White' earned an All-America Selections Gold Medal in 2001 (All-America Selections, 2003). *Zinnia marylandica* hybrids have had a greater commercial impact than any other *Zinnia* interspecific hybrid.

True-breeding lines of *Z. marylandica* ($2n = 4x = 46$) were backcrossed for one generation with diploid and autotetraploid *Z. angustifolia* ($2n = 4x = 44$) and *Z. violacea* ($2n = 4x = 48$) (Boyle, 1996). In most cases, backcrosses were more successful with *Z. angustifolia* and *Z. violacea* as autotetraploids than as diploids. BC_1 progeny were obtained by crossing *Z. marylandica* (as ♀) with autotetraploid *Z. angustifolia* or autotetraploid *Z. violacea*. However, no embryos were observed in capitula collected from field-grown BC_1 plants. BC_1 hybrids of *Z. marylandica* and autotetraploid *Z. violacea* produced larger capitula and a greater number of ray florets than allotetraploid *Z. marylandica*, and displayed novel pigment combinations in the ligules (Boyle, 1996). Backcross hybrids of *Z. marylandica* and *Z. violacea* ($2n = 4x = 48$) may have commercial potential as seed-propagated bedding plants. Linderman and Ewart (1990) obtained two interspecific hybrids from crosses between *Z. angustifolia* 'Classic' (as ♀) and *Z. haageana* 'Persian Carpet'. Only one of the hybrids produced flowers. The flowering plant ($2n = 2x = 23$) produced little stainable pollen (0.68%) and formed two to five bivalents per cell (mean: 3.67), indicating partial homology between the two genomes. Backcrosses between the F_1 hybrid and its parents failed to produce any progeny.

6.3 Polyploidy

Two types of polyploid zinnias have been developed: 1) autotetraploid ($4x$) cultivars of *Z. violacea* and *Z. haageana* (Cook, 1938); and 2) allotetraploids (or amphidiploids) of *Z. marylandica* ($2n = 4x = 46$) (Boyle and Stimart, 1982; Terry-

Lewandowski et al., 1984). In *Z. violacea*, autotetraploid plants were produced in the 1930s (Cook, 1938) and the first autotetraploid cultivar ('State Fair') was released by Ferry Morse Seed Company in the 1950s. Several other autotetraploid cultivars have been introduced since that time. 'Old Mexico', an autotetraploid *Z. haageana* cultivar, was released in 1962. All autotetraploid zinnia cultivars released to date are open-pollinated. Relative to diploids, tetraploid zinnias have larger flowers and thicker, stronger stems but also have poorer seed germination, less branching, delayed flowering, and fewer capitula. Difficulties also occur with producing autotetraploid lines with a uniform phenotype due to additional alleles affecting ligule color and plant habit.

Polyploidy was used to restore fertility in allodiploid ($2n = 2x = 23$) *Z. marylandica*. Allodiploids are highly sterile due to irregular meiosis, but bivalent associations predominate in the induced allotetraploids ($2n = 4x = 46$) and functional gametes are produced (Terry-Lewandowski et al., 1984). Polyploidy is induced by treating zinnia seedlings with colchicine through several methods. Gupta and Koak (1976) immersed *Z. violacea* seeds in colchicine solutions [0.05%, 0.1%, or 0.2% (w/v)] for 6 hours and then applied cotton plugs saturated with colchicine to apices of the seedlings for 8 hours. Colchicine concentrations used for saturating the cotton plugs were the same as those used for seed immersions. Autotetraploids were obtained using 0.2% colchicine but no polyploid seedlings were obtained with more dilute colchicine solutions. Bose and Panigrahi (1969) produced polyploids of *Z. angustifolia* by applying cotton plugs saturated with 0.2% colchicine to seedling apices. Colchicine treatments commenced when the seedlings had two leaves and were applied 4 hours per day for 3 consecutive days. Allotetraploids of *Z. marylandica* were produced by treating axillary buds with 0.1% colchicine daily for 5 consecutive days following shoot tip removal (Terry-Lewandowski et al., 1984).

6.4 Heritable Variation Induced by *in vitro* Culture

Traditional methods of breeding for crop improvement are ineffective for *Z. marylandica* due to bivalent associations of chromosomes that predominate in the induced allotetraploids (Terry-Lewandowski et al., 1984). *Zinnia marylandica* is classified as a segmental allopolyploid due to chromosome associations during meiosis (Stebbins, 1950). Fully homologous chromosomes preferentially pair and homoeologous pairing is eliminated, allowing limited to no segregation in subsequent generations (Terry-Lewandowski et al., 1984). Self-pollination of *Z. marylandica* ($2n = 4x = 46$) produced seed and subsequently plants with essentially clonal uniformity. Backcrossing to diploid *Z. violacea* or *Z. angustifolia* or crossing with tetraploid *Z. violacea* 'State Fair' resulted in sterile progeny (Boyle, 1996), thus obviating the use of traditional sexual breeding methods for generating variants.

Somaclonal variation is variability resulting from tissue culture (Larkin and Scowcroft, 1981). This phenomenon has been reported in over thirty species of

agronomic and ornamental plants regenerated from *in vitro* culture (Scowcroft, 1985). Stieve (1991) obtained adventitious shoots from cultured cotyledons of *Z. marylandica* seedlings. Optimum conditions for adventitious shoot formation included excision of 16-day old cotyledons and placement (adaxial surface down) on Murashige-Skoog medium salts and organics (Murashige and Skoog, 1962) supplemented with 0.2 μM thidiazuron. Embryos cultured on 22.2 μM thidiazuron produced more callus and took longer to form adventitious shoots.

Phenotypic variation in *Z. marylandica* progeny was evaluated using plants propagated by seed or adventitious techniques (described above). Adventitious plants regenerated directly from tissue culture exhibited substantial variation in ligule color and morphology, fertility, and plant height, while seed-propagated (control) plants displayed a uniform phenotype. About ten percent of the plants derived from either 0.2 or 22.2 μM thidiazuron showed morphological variation when compared to plants propagated from seed (Stieve et al, 1992). Variant characteristics included tallness; dwarfness; increased seed set; enlarged capitula; fasciated capitula; and ligule curvature, distortion, striping, spotting, and color variations. All variants except those with striped or dark red-orange ligules were transmitted sexually, indicating that genetic rather than epigenetic changes had occurred. New traits such as pink ligules, crested disc florets, green spots on ligules, upwardly curved ligules, and tubular ligules, not observed in initially isolated plants derived from tissue culture, arose in progeny derived by selfing regenerated plants. These observations suggest that some of the genetic changes that occurred during or after culture were recessive and not expressed immediately following regeneration, or that regenerated plant genomes were unstable and genetic rearrangement continued after regeneration. Application of this culture technique on other *Zinnia* taxa, particularly those with limited phenotypic variation, may yield useful mutants for plant breeding programs.

7. BREEDING FOR SPECIFIC TRAITS

7.1 Flower Color

Zinnias are well known for their great diversity of flower colors (Fig. 12-1). The pigments responsible for flower color in *Z. angustifolia* and *Z. violacea* are located primarily in the upper epidermis of ligules and to a lesser extent in the lower epidermis (Boyle and Stimart, 1989a). For *Z. violacea*, variation in ligule color is attributable to presence or absence of carotenoids in chromoplasts and flavonoids in vacuoles, and vacuolar pH. For *Z. violacea*, quantitative differences in the amounts of two anthocyanidins, pelargonidin and cyanidin, and differences in epidermal cell pH account for pink, lavender, rose, maroon, and violet shades when carotenoids are

Figure 12-1. Flower color variation in *Zinnia violacea* 'Orange King' (upper left), 'Enchantress' (lower left), 'Crimson Monarch' (center), 'Canary Bird' (upper right), and 'Purity (lower right).

absent, and for orange, scarlet, and red shades when carotenoids are present. Presence of carotenoids but absence of anthocyanidins results in yellow ligules while absence of carotenoids and anthocyanidins results in white ligules. Variation in ligule color in *Z. angustifolia* is due to quantitative differences of flavonoids in vacuoles with no apparent effect due to epidermal cell pH. Carotenoids do not contribute to ligule color in *Z. angustifolia* and most *Z. marylandica* allotetraploids (Boyle and Stimart, 1989a; Boyle, 1996).

Ligule color in *Z. violacea* is controlled by two major genes (Boyle and Stimart, 1988). Presence of the anthocyanidins pelargonidin and cyanidin is controlled by a single dominant gene (*An1*). Carotenoid expression is conditioned by a recessive gene (*ca*) governing its presence and other genes controlling the distribution of carotenoids in ligules. Thus, white ligules are devoid of anthocyanidins (*an1 an1*) and carotenoids (*Ca* _). Pollard (1939) identified seven additional genes affecting ligule color in *Z. violacea*; additional research is needed to elucidate the effects of these genes on pigment biosynthesis. In *Z. angustifolia*, presence of orange and/or yellow pigments in ligules is controlled by a single dominant gene (*An2*). Analysis of floral pigmentation in *Z. marylandica* suggests that *An1* and *An2* are nonallelic and control different steps in anthocyanidin biosynthesis (Boyle and Stimart, 1988).

Presence of carotenoids and anthocyanidins is required for scarlet-orange ligule color in *Z. violacea* (Boyle and Stimart, 1989). Although carotenoids are not produced in most allotetraploid *Z. marylandica*, scarletorange flower color can be obtained in *Z. marylandica* when yellow-orange anthocyanidins from *Z. angustifolia* occur with magenta-colored cyanidin from *Z. violacea.* Equivalent color phenotypes may thus be produced in other zinnias by different. combinations of genetic backgrounds.

7.2 Flower Doubleness

Degree of doubleness is determined by the number and arrangement of ray florets per capitulum (Fig. 12-2). Single capitula contain only one whorl of ray florets whereas semi-double types have two or more whorls of ray florets. Fully double capitula produce numerous whorls of ray florets that cover the entire receptacle but yield few or no disc florets. Thus, there is an inverse relationship between the numbers of disc florets and ray florets (Lien, 1968; Miyajima and Nakayama, 1994).

Flower doubleness is an important trait in *Z. violacea* and is controlled by multiple genes (Gotoh, 1954; Lien, 1968). One problem associated with producing F_1 hybrids yielding a high percentage of plants with fully double capitula is

Figure 12-2. A fully double capitulum with multiple whorls of ray florets (left) and a single capitulum with one whorl of ray florets (right).

discerning the degree of doubleness in the apetalous maternal line. Degree of doubleness of apetalous types cannot be determined by observation, so test crosses must be performed to determine their genotype. Also, fully double flowers yield little pollen because few disc florets are produced. Therefore, paternal lines must yield a high proportion of semi-double types to ensure adequate pollen for seed production.

Achenes originating from disc and ray florets are distinguishable by their shape and size. Miyajima (1998) observed that ray florets in the first whorl of the capitulum produced wider achenes than ray florets that develop later.

Consequently, he was able to differentiate thin and wide ray achenes and sort achenes into three classes: those originating from disc florets and those from the thin and wide ray florets. Achenes from commercial cultivars were sorted into the three types, plants were grown, and their capitula were classified as either single, semi-double, or fully double. Miyajima (1998) found that the percentage of plants with single, semi-double, or fully double capitula was correlated with seed morphology. The percentage of plants with fully double capitula and the number of ray florets per capitulum were greatest for plants from thin ray floret seeds and least for plants from disc floret seeds. These results indicate that it should be feasible to increase the percentage of double-flowered progeny in a seed lot by removing some or all of the disc floret seeds.

7.3 Disease Resistance

Zinnia violacea is subject to attack by three major pathogens: *Alternaria zinniae* Pape (alternaria blight), *Erysiphe cichoracearum* DC. ex Merat (powdery mildew), and *Xanthomonas campestris* pv. *zinniae* Hopkins & Dowson (bacterial leaf and flower spot) (Andersen, 1971; Jones and Strider, 1979; Lipschutz, 1965; Torres, 1963a). These pathogens incite moderate to severe epiphytotics during greenhouse production or in outdoor plantings resulting in plant losses and/or decreased ornamental value. *Alternaria zinniae* and *X. campestris* pv. *zinniae* are seed-borne pathogens and are thus a major concern for both seed producers and commercial growers. *Zinnia angustifolia* is highly resistant to each of these pathogens (Andersen, 1971; Jones and Strider, 1979; Lipschutz, 1965; Torres, 1963a).

One of the most important attributes of *Z. marylandica* cultivars is their disease resistance. Terry-Lewandowski and Stimart (1983) found that *Z. marylandica* exhibits high levels of resistance to *A. zinniae* and *E. cichoracearum* and moderate to high levels of resistance to *X. campestris* pv. *zinniae*. The inheritance of resistance to *E. cichoracearum* in interspecific hybrids and induced amphiploids of *Z.violacea* and *Z. angustifolia* was examined in leaf and ligule tissue (Terry-Lewandowski and Stimart, 1985). Resistance to *E. cichoracearum* was complexly inherited in both leaves and ligules of sterile hybrids and induced amphiploids. Two

major dominant genes conferred resistance in ligule tissue of derived amphiploids. Data obtained from F_1 hybrid progeny of intercrossed amphiploids indicate the trait is not cytoplasmically inherited. It appears that genes conferring ligule resistance act independently from those controlling leaf resistance with resistance genes being inherited from *Z. angustifolia*. These results demonstrate why plant breeders have been unable to develop mildew-resistant *Z. violacea* cultivars despite six decades of research.

Boyle and Wick (1996) screened seed-generated BC_1 progeny of *Z. marylandica* crossed to either *Z. angustifolia* (4*x*) or *Z. violacea* (4*x*) for resistance to *A. zinniae*, *E. cichoracearum*, and *X. campestris* pv. *zinniae*. All BC_1 families exhibited high levels of resistance to *A. zinniae* and *E. cichoracearum*. BC_1 families of *Z. marylandica* x *Z. angustifolia* (4*x*) were highly resistant to *X. campestris* pv. *Zinniae* while BC_1 families of *Z. marylandica* x *Z. violacea* (4*x*) were susceptible to this pathogen. These results suggest that *Z. marylandica* allotetraploids require one *Z. angustifolia* genome to confer resistance to alternaria blight and powdery mildew, but at least two *Z. angustifolia* genomes are required to provide resistance to bacterial leaf and flower spot.

7.4 Plant Height

Cultivars of *Z. violacea* display great diversity in mature plant height, ranging from 20 cm ('Thumbelina') to >100 cm ('Giants of California', 'Giant Cactus-Flowered'). Pollard (1939) reported a single recessive gene controlling dwarfness in *Z. violacea*, but Lien (1968) reported that multiple genes affect plant height in this species. In her work, Lien (1968) classified *Z. violacea* plants into six categories: normal, dw 1, dw 2, dw 3, dw 4, and dw 5. Normal plants were the tallest (≥ 40 cm in height), followed by dw 1 (30-40 cm), dw 2 (20-29 cm), dw 3 (16-19 cm), dw 4 (12-15 cm), and dw 5 (6-11 cm). Ten plants from each height category were selfed and S_1 progeny were evaluated for plant height. Each S_1 population was evaluated in two different environments: ground beds inside a cloth house or as potted plants within a greenhouse. Plant height was affected by genotype and environment; plants grown in the cloth house were shorter than their counterparts grown in the greenhouse. The height difference was attributed to longer internodes on greenhouse-grown plants. All S_1 progeny from normal parents exhibited a normal phenotype while S_1 progeny from dw 1, dw 2, dw 3, dw 4, and dw 5 parents segregated for plant height. Progeny from dw 5 individuals displayed less variation in plant height than progeny from dw 1, dw 2, dw 3, or dw 4 individuals. Lien (1968) stated that pure lines of dw 1 and dw 2 were difficult to produce, but further inbreeding would likely yield pure-breeding lines from dw 3, dw 4, and dw 5 individuals.

Open-pollinated cultivars, as well as inbred lines used for producing F_1 hybrid seed, must be reasonably uniform for plant height. Crossing two inbred lines with dissimilar plant heights will yield uniform F_1 hybrids but also add proprietary protection for seed producers due to segregation for this trait in the F_2 and subsequent generations.

7.5 Leaf Morphology

Zinnias rank high among annuals sold in seed packets through seed displays but comprise only about 1% of the bedding plants sold in cell packs or pots. Bedding plant growers attribute this low percentage to high seed cost and difficulties producing high quality plants [L.C. Ewart (personal communication), 1995]. *Zinnia violacea* has relatively large leaves, and plants growing in small pots or cell packs often require frequent irrigation when the transpiration rate is high. Also, the large leaves inhibit air circulation within plant canopies, thus increasing the probability of disease problems in high-density plantings. Cowen and Ewart (1988) selected *Z. violacea* genotypes with narrow leaves and developed inbred lines that were uniform for this character. Crosses between narrow-leaf and wide-leaf (normal phenotype) lines revealed that leaf width was controlled primarily by additive gene effects. These results indicate direct phenotypic selection of narrow-leaf plants would be effective for developing pure-breeding lines. However, the difference between the parental averages $[(P_1 + P_2)/2]$ and their F_1 progeny was highly significant, indicating that dominant gene action also contributed to expression of leaf width. Development of narrow-leaf F_1 hybrids of *Z. violacea* may reduce disease problems and watering requirements, thus resulting in higher-quality plants.

8. FUTURE BREEDING EFFORTS

Although zinnia breeding programs have made substantial progress in the past half century, modern cultivars still exhibit numerous shortcomings. A future challenge will be to develop disease-resistant cultivars of *Z. violacea*. Some landrace types and primitive cultivars of *Z. violacea* from Mexico appear to have better disease resistance than commercial cultivars [L.C. Ewart (personal communication), 1995)], and this germplasm should be evaluated for its breeding potential. Development of commercially-acceptable *Z. violacea* cultivars with improved disease resistance would be beneficial to growers and consumers. Another desirable goal would be to develop *Z. violacea* cultivars with improved drought tolerance. Alternatively, breeding *Z. violacea* cultivars with narrow leaves (Cowen and Ewart, 1988) may reduce the watering requirements and thus improve plant performance in retail display areas and gardens.

Utilization of *Z. angustifolia* as a landscape plant has increased steadily during the past decade; two worthy goals for this species would be to develop double-flowered cultivars and expand the flower color range.

Only a relatively small portion of the germplasm resources present in the genus *Zinnia* has been exploited by plant breeders. Some *Zinnia* species that are not cultivated presently may have commercial value. Representative germplasm of these species should be collected and evaluated in numerous environments. Also,

the meteoric rise in popularity of *Z. marylandica* hybrids demonstrates the potential of zinnia interspecific hybrids. However, few of the myriad possible crosses between *Zinnia* species have been attempted. Crosses between taxa in Section *Mendezia* and Section *Zinnia* appear to have the most commercial potential. Previous work (Boyle and Stimart, 1987) has shown the value of exploiting genetic variation within species for increasing the likelihood of obtaining interspecific hybrids. Thus, it would probably be beneficial to acquire germplasm from several populations in different parts of ranges of each species for use in interspecific hybridization programs.

More fundamental studies on the biochemistry and physiology of important traits and identification of genes controlling these traits would aid breeders in their quest for improved varieties. For example, additional information on the physiology and genetics of ligule color in intra-and interspecific hybrids may aid in the breeding of novel flower colors. Another area of research that warrants further study is the use of somaclonal variation for generating mutants in zinnia.

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FLOWERING POTTED PLANTS

Chapter 13

CACTI

Schlumbergera truncata, S. x buckleyi, Hatiora gaertneri

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Abstract: Ornamental flowering potted cacti are used for several floral holidays throughout the year, including Easter, Christmas, and Thanksgiving (U.S.). Their continued popularity has warranted extensive breeding and development of novel phenotypes, particularly upright growth habit, early flower initiation, upfacing flowers, flower coloration, floral longevity, and disease resistant cultivars. Most *Hatiora* and *Schlumbergera species* possess a gametophytic self incompatibility system. The use of intra- and inter-specific hybridization, breeding for disease resistance, polyploidy, and mutation breeding are presented with a focus on creating novel transformations for this crop in the future.

Key words: Easter Cactus, Christmas Cactus, Thanksgiving Cactus, Breeding, Genetics.

1. INTRODUCTION

The Cactaceae family comprises about 1800 species of succulent perennials that are distributed throughout most of the New World from Canada to southern Argentina and Chile, with eight species restricted to the Galápagos Islands and one species [*Rhipsalis baccifera* (J.S. Miller) Stearn] found in tropical regions of the New World and Old World (Anderson, 2001). More than 300 species of cacti are cultivated as ornamentals (Anderson, 2001). Most of these species are grown on a small scale and are sold by a few specialist growers. Two Cactaceae genera, however, are cultivated widely as flowering potted plants: *Hatiora* Britton & Rose (formerly *Rhipsalidopsis* Britt. & Rose) and *Schlumbergera* Lemaire (Boyle, 1997a). Cultivars of *Hatiora* and *Schlumbergera* have been grown as conservatory specimens and houseplants for more than a century (McMillan and Horobin, 1995; Schumann, 1902).

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Hatiora and *Schlumbergera* are minor crops in North America, Japan, and Australia but are economically important glasshouse crops in northern Europe. *Schlumbergera* plants can be forced into flower year-round and, in the northern hemisphere, flowering *Hatiora* plants can be obtained from January to June. Accurate scheduling of these crops was made possible by research on the effects of temperature and photoperiod on flower initiation and development (Lange and Heins, 1992; Peters and Rünger, 1971; Rünger and Poole, 1985). During the past 30 years, breeders have developed *Hatiora* and *Schlumbergera* cultivars with better growth habits, increased branching, larger and more upright flowers, novel flower colors, and greater postproduction longevity (Cobia, 1992; Meier, 1995). This chapter will focus on the taxonomy and reproductive biology of these two genera and the breeding methodology, selection criteria, and future breeding challenges.

2. TAXONOMY

2.1 *Schlumbergera*

The genus *Schlumbergera* contains six species of epiphytic or epilithic shrubs native to eastern Brazil (Barthlott and Taylor, 1995; Hunt, 1969). *Schlumbergera* species can be classified in two groups based on their morphology. *Schlumbergera kautskyi* (Horobin & McMillan) N.P. Taylor, *S. orssichiana* Barthlott & McMillan, *S. russelliana* (Hooker) Britton & Rose, and *S. truncata* (Haworth) Moran have flattened, crenate or truncate phylloclades (stem segments) with areoles confined to the margins and apices. In contrast, *S. microsphaerica* (K. Schumann) Hövel and *S. opuntioides* (Löfgren & Dusén) D. Hunt have cylindric, terete or obovoid phylloclades with areoles distributed over the entire surface (Barthlott and Taylor, 1995). All *Schlumbergera* species examined thus far are diploid ($2n = 2x = 22$) (Barthlott, 1976; Barthlott and Rauh, 1977; Remski, 1954). Sugiura's (1936) aberrant count of $2n = 24$ for *Epiphyllum truncatum* (= *S. truncata*) is likely to be erroneous.

Schlumbergera truncata (= zygocactus, crab cactus or Thanksgiving cactus) is the most common species in cultivation (Figure 13-1). The remaining five *Schlumbergera* species are rare in cultivation. Three interspecific hybrids have been bred: *S.* x *exotica* Barthlott & Rauh (= *S. opuntioides* x *S. truncata*); *S.* x *reginae* McMillan & Orssich (= *S. orssichiana* x *S. truncata*); and *S.* x *buckleyi* (Buckley) Tjaden (= *S. russelliana* x *S. truncata*). *Schlumbergera* x *buckleyi* has been utilized extensively for breeding of commercial *Schlumbergera* cultivars, whereas *S.* x *exotica* and *S.* x *reginae* have only recently been incorporated into breeding programs.

Plants of *S. truncata* have phylloclades with dentate margins and zygomorphic flowers with yellow pollen, white filaments, and terete ovaries (Figure 13-1). *Schlumbergera russelliana* has small phylloclades with crenate margins and actinomorphic flowers with pink pollen, light purple filaments, and ribbed ovaries. Generally, *S.* x *buckleyi* hybrids exhibit traits that are intermediate between the two parents (Figure 13-2) (Hunt, 1981; Tjaden, 1964). *Schlumbergera* cultivars that are

Figure 13-1. Flowers and phylloclades of two primitive cultivars of *Schlumbergera truncata*, 'Abendroth 2' (left) and 'Delicatus' (right).

Figure 13-2. A *Schlumbergera* x *buckleyi* F_1 hybrid seedling with deep purple ovaries.

grown commercially are either *S. truncata* or complex *S.* x *buckleyi* hybrids. Holiday cactus is a collective name for zygocactus and Christmas cactus (Boyle, 1997a), and this term will be used henceforth when referring to *Schlumbergera* cultivars grown as potted plants.

2.2 *Hatiora*

The genus *Hatiora* consists of five species of epiphytic or epilithic shrubs that are native to southeastern Brazil (Barthlott, 1987; Barthlott and Taylor, 1995). Two subgenera are recognized: *Hatiora* and *Rhipsalidopsis* (Barthlott, 1987). Subgenus *Rhipsalidopsis* contains three species - *H. gaertneri* (Regel) Barthlott, *H. rosea* (Lagerheim) Barthlott, and *H. epiphylloides* (Campos-Porto & Werdermann) F. Buxbaum - with flattened or angular phylloclades and angled pericarpels. Subgenus *Hatiora* contains two species - *H. salcornioides* (Haworth) Britton & Rose and *H. herminiae* (Campos-Porto & Castellanos) Backeberg ex Barthlott - with terete or cylindric phylloclades and terete pericarpels. *Hatiora gaertneri*, *H. rosea*, and *H. salicornioides* are diploid species with a chromosome number of $2n = 2x = 22$ (Barthlott, 1976; Gadella et al., 1979). There have been no reports on chromosome numbers for *H. epiphylloides* and *H. herminiae*.

Hatiora epiphylloides and *H. herminiae* are vulnerable or endangered in the wild (Taylor, 1997) and are represented in few living collections. *Hatiora gaertneri*, *H. rosea*, and *H. salicornioides* are cultivated as ornamentals. *Hatiora salicornioides* is grown as a foliage plant, the chief aesthetic features being its unique jointed stems and small (≈1 cm long) yellow-orange flowers. *Hatiora gaertneri* and *H. rosea* share many morphological features such as actinomorphic (symmetrical) flowers, ribbed ovaries, and phylloclades with slightly scalloped margins, but they differ in other respects. *Hatiora gaertneri* has scarlet-red flowers 4.5-7.5 cm in diameter and phylloclades 4-7 cm long whereas *H. rosea* has rose-pink flowers 3-4 cm in diameter and phylloclades 2-4 cm in length. *Hatiora gaertneri*, *H. rosea*, and their interspecific hybrid (= *H.* x *graeseri* Barthlott ex D. Hunt) are commonly known as Easter cactus. The plants are aptly named because they flower in early spring in the northern hemisphere, i.e., near Easter. Most of the 100+ Easter cactus cultivars in existence are complex *H.* x *graeseri* hybrids (Boyle, 1995; Meier, 1995). Flowers and phylloclades of *H.* x *graeseri* range from *H. rosea*-like to *H. gaertneri*-like in morphology and size, but the flower color range extends beyond that of either species (Figs. 13-3 and 13-4). In the remainder of this chapter, the term Easter cactus will be used when referring to *Hatiora* cultivars grown as a flowering potted plants.

Holiday cactus and Easter cactus differ in floral morphology and time of flowering but their growth habits are similar. Plants in both genera produce a series of leafless, flattened phylloclades, and flowers develop primarily on the tips of apical segments and occasionally on subapical segments.

Figure 13-3. Flowers of *Hatiora x graeseri* 'Amherst'.

Figure 13-4. Diversity for flower color among *Hatiora* x *graeseri* cultivars.

3. ORIGIN AND BRIEF HISOTRY

Schlumbergera truncata and *S. russelliana* were introduced to cultivation around 1817 and 1839, respectively (McMillan and Horobin, 1995; Tjaden, 1964). The first *S.* x *buckleyi* hybrids were produced prior to 1852 by Wilbraham Buckley in London, England (Hunt, 1981; Tjaden, 1964). *Schlumbergera opuntioides*, native to the Itatiaia Mountains in southeast Brazil, was discovered in the late 1800s (Barthlott and Rauh, 1975). *Schlumbergera* x *exotica* probably originated in the 1940s (Horobin and McMillan, 1985). *Schlumbergera orssichiana* was introduced around 1974 (Barthlott and McMillan, 1978) and the first *S.* x *reginae* F_1 hybrids were produced in 1979 (Horobin and McMillan, 1985). Neither *S.* x *exotica* nor *S.* x *reginae* are cultivated as extensively as *S.* x *buckleyi* or *S. truncata*.

The first specimens of *H. gaertneri* and *H. rosea* were sent from Brazil to Europe about 1882 and 1911, respectively (Lagerheim, 1912; Regel, 1884; Schumann, 1901). In the late 1920s, Alfred Gräser of Nürnberg, Germany crossed *H. gaertneri* (as female) with *H. rosea* and produced the first *H.* x *graeseri* F_1 hybrids (Gräser, 1956; Tjaden, 1986).

Cultivars of holiday cactus or Easter cactus bred prior to 1950 do not possess traits deemed important for pot plant production, such as upright growth habit, early flower initiation, and upfacing flowers. Hence, the cultivars of holiday cactus and Easter cactus that are grown commercially were developed after 1950 (Tables 13-1 and 13-2).

'Weihnachtsfreude' (also known as 'Christmas Cheer') is one of the most popular holiday cactus cultivars ever developed. This cultivar was bred by Rudolf Zenneck of Germany and was introduced by Firma Königer in 1953. During the 1950s, Alfred Gräser of Nürnberg, Germany, released several cultivars of holiday cactus ('Laterne', 'Lilofee', and 'Noris') and Easter cactus ('Electra', 'Frühlingszauber', and 'Ostergruss') (Meier, 1995; McMillan and Horobin, 1995). In the 1950s and 1960s, Harry Johnson, cactus breeder extraordinaire and owner of Johnson Cactus Gardens in Paramount, California, introduced several cultivars of holiday cacti ('Amelia Manda', 'Paramount Pink', and 'Parna') and Easter cacti ('Andre', 'Crimson Giant', 'Rainbow', and 'Sutter's Gold') (Meier, 1995; McMillan and Horobin, 1995). A few of the aforementioned cultivars are still in cultivation but most have been replaced by superior cultivars.

The firm B.L. Cobia Inc. of Winter Garden, Florida, was probably the first commercial nursery to establish a large-scale breeding program to develop cultivars of holiday cactus for commercial greenhouse production. Cobia's *Schlumbergera* breeding program commenced in the 1960s and yielded many cultivars (Table 13-1). Perhaps the most renowned of Cobia's holiday cactus cultivars is 'Gold Charm', the first yellow variety. According to Cobia (1992), 'Gold Charm' required 15 years from the initial concept to variety release, and in excess of 50,000 seedlings were evaluated in the effort to produce a commercially acceptable plant with yellow flowers. Other well-known Cobia cultivars include 'Christmas Charm', 'Christmas Fantasy',

'Christmas Flame', 'Lavender Doll', and 'White Christmas'. Cobia began breeding Easter cactus in the 1980s and two cultivars have been released (Table 13-2).

Most of the holiday cactus and Easter cactus cultivars grown commercially were bred by J. de Vries Potplantencultures (Aalsmeer, The Netherlands), Gartneriet PKM (Odense, Denmark), or Gartneriet Thoruplund (Odense, Denmark) (Tables 13-1 & 13-2 These three companies produce finished plants of both crops, and they have developed upright cultivars that are adapted to production at close spacing. Recently, J. de Vries Potplantencultures, Gartneriet PKM, Gartneriet Rohde, and Danish breeder Jorn Hansson formed a partnership (CB Cactus) specializing in breeding *Hatiora* and *Schlumbergera*. CB Cactus has released several cultivars, including the Dancer series (Table 13-1) and 'Sirius' (= 'Wit'), the first white-flowered Easter cactus (Table 13-2).

Amateur hybridizers have developed numerous cultivars of holiday cactus and Easter cactus, but few of these are grown on a large scale. See Meier (1995) and McMillan and Horobin (1995) for comprehensive lists of *Hatiora* and *Schlumbergera* cultivars.

Table 13-1. Some modern cultivars of holiday cactus (*Schlumbergera*). Data from Cobia (1992) and personal correspondence with the breeders listed.

Cultivar	Breeder	Year introduced
Beach Dancer	CB Cactus, DK	2000
Christmas Charm	B.L. Cobia, USA	1977
Christmas Fantasy	B.L. Cobia, USA	1986
Christmas Flame	B.L. Cobia, USA	1988
Christmas Magic II	B.L. Cobia, USA	1986
Cyber Dancer	CB Cactus, DK	2000
Dark Eva	CB Cactus, DK	1996
Dark Marie	Gartneriet PKM, DK	----
Dasher	Bay City Flower Co., USA	1990
Eva	Gartneriet PKM, DK	----
Exotic Dancer	CB Cactus, DK	2000
Gina	Gartneriet PKM, DK	----
Gold Charm	B.L. Cobia, USA	1982
Golden Dancer	CB Cactus, DK	2000
Holiday Splendor	B.L. Cobia, USA	1991
Jolly Dancer	CB Cactus, DK	2000
Kris Kringle	B.L. Cobia, USA	1974
Kris Kringle II	B.L. Cobia, USA	1988
Lavender Doll	B.L. Cobia, USA	1974
Lavender Doll II	B.L. Cobia, USA	1986
Madisto	Gartneriet PKM, DK	----
Madonga	Gartneriet PKM, DK	----
Malibu	Gartneriet PKM, DK	----
Malindi	Gartneriet PKM, DK	----
Naomi	J. de Vries, NETH	1991

Cultivar	Breeder	Year introduced
Peach Parfait	B.L. Cobia, USA	1974
Red Radiance	B.L. Cobia, USA	1977
Rudolph	Bay City Flower Co., USA	1990
Sleigh Bells	B.L. Cobia, USA	1991
Stephanie	J. de Vries, NETH	1991
Thor-Alex	Gartneriet Thoruplund, DK	1992
Thor-Alice	Gartneriet Thoruplund, DK	1990
Thor-Bella	Gartneriet Thoruplund, DK	2001
Thor-Britta	Gartneriet Thoruplund, DK	1993
Thor-Nille	Gartneriet Thoruplund, DK	1994
Thor-Olga	Gartneriet Thoruplund, DK	1998
Thor-Tina	Gartneriet Thoruplund, DK	1990
Thor-Vida	Gartneriet Thoruplund, DK	1994
White Christmas	B.L. Cobia, USA	1973
Zaraika	J. de Vries, NETH	1991

Table 13-2. Some modern cultivars of Easter cactus (*Hatiora*). Data from personal correspondence with the breeders listed.

Cultivar	Breeder	Year introduced
Amherst	Univ. Massachusetts, USA	2002
Andromeda	Gartneriet PKM, DK	----
Ashley	B.L. Cobia, USA	1995
Auriga	Gartneriet PKM, DK	----
Capella	CB Cactus, DK	1995
Cassiopeia	Gartneriet PKM, DK	----
Castor	CB Cactus, DK	1995
Cetus	Gartneriet PKM, DK	----
Elisa	J. de Vries, NETH	2001
Elsie	Univ. Massachusetts, USA	2004
Evita	J. de Vries, NETH	1983
Heather	B.L. Cobia, USA	1995
Ian	J. de Vries, NETH	1995
Leah	J. de Vries, NETH	2001
Louise	Univ. Massachusetts, USA	2004
Mira	-	----
Nina	J. de Vries, NETH	2001
Orion	Gartneriet PKM, DK	----
Phoenix	CB Cactus, DK	2000
Purple Pride	J. de Vries, NETH	1985
Red Pride	J. de Vries, NETH	1985
Rio	Univ. Massachusetts, USA	2001
Romy	J. de Vries, NETH	2001

Cultivar	Breeder	Year introduced
Sagitta	Gartneriet PKM, DK	----
Savannah-94	J. de Vries, NETH	2001
Sirius	CB Cactus, DK	2002
Thor-Alina	Gartneriet Thoruplund, DK	2001
Thor-Anet	Gartneriet Thoruplund, DK	2001
Thor-Anne	Gartneriet Thoruplund, DK	1990
Thor-Ina	Gartneriet Thoruplund, DK	1993
Thor-Rona	Gartneriet Thoruplund, DK	1991
Thor-Siff	Gartneriet Thoruplund, DK	2000
Tricky Pink	J. de Vries, NETH	2001
Zetus	Gartneriet PKM, DK	----
Ziff	Gartneriet PKM, DK	----
Zoë	J. de Vries, NETH	2001
16	J. de Vries, NETH	2001
79	J. de Vries, NETH	2001

4. BREEDING OBJECTIVES

The selection criteria used for breeding holiday cactus and Easter cactus are listed in Table 13-3. The most important criteria are: rapid rooting, growth and flowering; upright habit; adequate branching; resistance to the most common pathogens; development of two or more flower buds per apical phylloclade; large, brightly-colored flowers; acceptable flower longevity; and the ability to withstand the rigors of shipping and retail display. It is noteworthy that nearly all of these criteria apply equally to breeding of *Hatiora* and *Schlumbergera* cultivars.

5. BREEDING TECHNIQUES

5.1 Reproductive Biology

All species of *Hatiora* and *Schlumbergera* have hermaphroditic flowers that are borne primarily on apical phylloclades (Barthlott and Taylor, 1995). Individual flowers have numerous stamens and pollen grains are tricellular at the time of shedding. Style length varies from 11 to 17 mm in Easter cactus and from 45 to 70 mm in holiday cactus (Boyle, unpublished data). Style and stamens are exserted in both genera. Stigma lobes are usually connivent in holiday cactus but are free and spreading in Easter cactus. The stigma surface is papillate and devoid of exudate. Ovaries are inferior and each contains 25-300+ ovules.

Most species in *Hatiora* and *Schlumbergera* are self-incompatible (SI) and yield few if any seed when selfed or crossed with a different clone with the same incompatibility phenotype. SI has been documented in all *Schlumbergera* species except for *S. kautskyi* and *S. microsphaerica* (Boyle, 1996, 1997b; McMillan and Horobin, 1995). Among the five *Hatiora* species, only *H. herminiae* is known to be self-compatible (SC) (Boyle et al., 1994; Boyle, 2003). Genetic studies of two Cactaceae genera (*Schlumbergera*, *Echinopsis*) indicate that the SI system is gametophytic and controlled by a single locus (Boyle, 1997b; Boyle and Idnurm, 2001). Incompatibility groups have been elucidated for 19 cultivars of holiday cactus and 10 cultivars of Easter cactus (Boyle, 2003). Following incompatible pollinations, pollen germinates normally but pollen tubes cease growth in the style. A few clones of holiday cactus and Easter cactus are SC (Parks, 2002).

Table 13-3. Selection criteria used in breeding commercial cultivars of *Hatiora* and *Schlumbergera*.

Selection criterion	Rationale
Freedom from injury when phylloclades are removed from stock plants for propagation	Maximal yields of propagules and decreased losses during production
Rapid rooting and plant growth	Reduced cropping time
Erect growth habit	Close spacing of pots
Low apical dominance (self-branching)	Reduced need for pinching and increased yield of phylloclades from stock plants
Low competitive ability	Reduced variation within and between pots
Resistance to diseases	Decreased losses during production
Rapid flower initiation and development	Reduced cropping time
Multiple flower buds per phylloclade	Increased aesthetic quality
Large flowers with wide petals	Increased aesthetic quality
Wide range of flower colors	Increased consumer interest
Satisfactory postproduction longevity	Increased consumer satisfaction
Short vernalization requirement for flower initiation[a]	Reduced cropping time and longer marketing period

[a]Applies to Easter cactus only

5.2 Crossing Procedures

The widespread occurrence of SI in holiday cactus and Easter cactus (Boyle, 1996, 2003; Boyle et al., 1994) indicates that emasculation is not necessary for routine crosses of most clones. However, selfing tests should be performed on individual clones to determine if emasculation is necessary.

Flowers of holiday cactus and Easter cactus are relatively large and therefore easy to manipulate for crosses. Stigmas become receptive to pollination a few days before anthesis (Boyle et al., 1994). Seed yields are maximal 0-2 days after anthesis and decline thereafter. If flowers require emasculation then stamens must be removed prior to pollen shedding. Emasculation should occur 1-3 days before anthesis and is achieved by making a longitudinal slit through the perianth with fine point forceps followed by removal of the corolla and stamens. Any floral debris adhering to the stigma lobes can be removed with a stream of tap water applied with a wash bottle.

Generally, flowers are pollinated by collecting whole fresh flowers and gently brushing the anthers across the stigma lobes. If the amount of pollen is limited, one or a few stamens can be removed from flowers with fine point forceps. Anthers do not readily abscise from the filaments, and thus it is easier to pinch off filaments ≈1 cm below the anthers instead of removing anthers only. To minimize the possibility of accidental contamination, forceps used for pollination should be dipped briefly in 70% ethanol and dried thoroughly before starting a new cross. Pollinated flowers can be labeled using paper jewelry tags, but it is cheaper, faster, and more convenient to write the name of the male parent and pollination date on the phylloclade subtending the pollinated flower using a fine tip permanent marker (Figure 13-5). Pollinated flowers (fruit) can be accidentally detached if paper tags become snagged on benchtops; this will not occur if the cross is recorded on the subtending phylloclade with an indelible marker.

5.3 Pollen Collection and Storage

Sometimes it may be desirable to cross two different *Hatiora* or *Schlumbergera* species that have asynchronous flowering times. Breeders may be able to synchronize the flowering times of different species by manipulating photoperiod and/or temperature. Another option is to collect and store pollen for future crosses. Pollen of *Hatiora* and *Schlumbergera* can be successfully stored for at least 6 months if handled and stored properly (Boyle, 2001). Newly-opened flowers (≈1-2 days after anthesis) yield the most viable pollen and thus are preferred for pollen collection. Pollen can be collected by holding individual flowers with the anthers projecting into a small container (e.g., a scintillation vial) and dislodging pollen from the anthers using a vortex mixer. After collection, pollen should be spread in a thin layer in a dish and dried at room temperature (≈20°C) and low relative humidity (20-40%) for ≈1 hour. Afterwards, pollen samples are transferred to a container with a humidity control agent

(silica gel, glycerol/water solution, etc.) that will maintain the relative humidity between 10% and 50%.

Boyle (2001) used a two-container system for storing *Schlumbergera* pollen. Pollen was dried as described above and samples (≈100 mg) were transferred to uncapped scintillation vials which in turn were placed inside screw top bottles (138 cm^3 capacity) containing 25 mL glycerol or a glycerol-water mixture. Glycerol is an inexpensive humidity control agent and will maintain a low (≈15%) relative humidity if used alone. Bottles should be sealed with parafilm (to maintain a constant relative humidity) and kept in a refrigerator (3-5°C) or a freezer (-18°C) until use. Repeated warming-cooling cycles will not have a marked effect on pollen viability, so pollen samples can be removed from cold storage several times without affecting the viability of the pollen remaining in storage. Once pollen is removed from cold storage, it should be rehydrated at ≈20°C and high relative humidity (90-100%) for 1 hour before use. Rehydrated pollen can be applied to stigmas with a fine artist's brush. Brushes should be cleaned in 70% ethanol and dried thoroughly before applying a different pollen sample. Interspecific hybrids of several different *Schlumbergera* species have been produced at the University of Massachusetts using stored pollen.

5.4 Fruit Set and Maturation

Mature fruits of holiday cactus and Easter cactus are fleshy berries that are 8-12 mm wide and 10-15 mm long. Each berry contains 25-300 seeds. At maturity, the seeds are dark brown to nearly black in color and are ≈1 mm wide and ≈2 mm long. When plants are grown at 18°C minimum temperature, fruits of Easter cactus ripen about 3 to 4 months after pollination (Boyle et al., 1994) and holiday cactus fruits mature about 6 months after pollination (Boyle et al., 1995). Fruit ripening will be delayed when plants are grown at cooler temperatures.

Immature fruits of *Hatiora* and *Schlumbergera* species are green but mature fruit color varies among species. Mature fruits of *H. rosea*, *H. herminiae*, *S. opuntioides*, *S. orssichiana*, and *S. russelliana* are pale green, greenish-yellow, or white. Mature fruits of *H. gaertneri* are reddish brown while those of *H.* x *graeseri* vary from bright green to reddish brown. Mature fruits of *S. truncata* and *S.* x *buckleyi* are white, bright pink, red, or magenta purple. *Hatiora* and *Schlumbergera* have indehiscent fruits that will remain on plants indefinitely (2+ years) under greenhouse conditions. Some seeds may germinate prior to fruit harvest (= vivipary) if the fruits remain on the mother plants after they have matured. Rodents such as ground squirrels will devour mature fruits of holiday cactus or Easter cactus, so measures should be taken to prevent these pests from entering greenhouses.

Figure 13-5. Flowers of a *Schlumbergera truncata* cultivar that were emasculated prior to pollination. A fine-tip permanent marker has been used to record the pollen source and pollination date on the subtending phylloclade.

5.5 Seed Cleaning and Storage

New cultivars of holiday cactus and Easter cactus originate almost exclusively as seedlings selected from segregating populations (Cobia, 1992; Gräser, 1956; Hansson, 1992). Hence, seed harvesting and processing are important steps in breeding these two crops. Seeds can be extracted and cleaned by slicing the fruit open, squeezing or scooping the seeds into a water-filled container, pouring the seed onto absorbent paper, and scraping away the pericarp, but this method is inefficient and time-consuming. Since relatively small quantities of fruit are harvested for seed, large-scale procedures like those used for extracting seed from the fleshy fruits of *Lycopersicon esculentum* (tomato) or *Cucumis melo* (muskmelon) are not practical. Boyle (1994) devised a vacuum-filtration apparatus for extracting and cleaning seeds of *Hatiora* and *Schlumbergera*. The apparatus is suitable for handling small quantities of fruit like those obtained in commercial breeding programs. The apparatus consists of a vacuum pump with regulator, a trap flask (250 mL capacity), a heavy-walled filtering flask (500 or 1000 mL capacity), and a 10-cm diameter porcelian Büchner funnel. Filtration is performed with a qualitative-grade, coarse-porosity filter paper. Before extraction, a 9-cm circle of filter paper is placed in the funnel, moistened, and tightly sealed against the perforated plate by applying a vacuum for a few seconds. Mature fruit are sliced longitudinally and the seeds are removed with a curved metal spatula or similar tool.

In mature fruits, the pericarp (interior) is juicy and highly mucilaginous. Seeds should be removed from the ovary cavity with a least amount of pericarp tissue. The vacuum is applied, and the seeds are washed onto the filter paper with a plastic wash bottle. Seeds are rinsed thoroughly to remove the mucilaginous pericarp tissue and to reduce clumping. Seeds from one fruit can be extracted and cleaned by vacuum-filtration in ≈30 seconds. One circle of filter paper can accommodate ≈500 seeds. After extraction, the filter paper is placed in a beaker or small cup for drying. Once seeds have dried, they can be scraped off the surface of the filter paper with a metal spatula.

Mature seeds germinate readily and do not require any special treatments, i.e., after-ripening, scarification, or stratification. Germination trays should be kept constantly moist and at warm (22-25°C) temperatures. Seeds will remain viable for many (10+) years if stored in a refrigerator (3-5°C) at low to moderate relative humidity.

6. GENETIC DIVERSITY

The genetic diversity present in progenitor species and cultivars of holiday cactus and Easter cactus has made it possible to develop the modern cultivars with commercially desirable traits. Future breeding efforts will place additional demands on this genetic diversity. The purpose of this section is to consider the main factors affecting genetic diversity in these two crops.

O'Leary and Boyle (1999, 2000) used isozymes to analyze the multigene diversity within germplasm collections of *Hatiora* and *Schlumbergera* clones held at the University of Massachusetts. The *Hatiora* germplasm collection included accessions of *H. gaertneri*, *H. rosea*, *H. salicornioides*, and *H. herminiae* plus older and modern *H.* x *graeseri* cultivars. The isozyme data suggested that the majority of the *H.* x *graeseri* clones in cultivation descend from as few as two *H. gaertneri* clones and one *H. rosea* clone. This situation probably occurred because few clones of *H. gaertneri* and *H. rosea* were available to breeders. For example, since the 1880s, only four different *H. gaertneri* clones and perhaps three different *H. rosea* clones have been collected in Brazil and distributed widely. The F_1 *H.* x *graeseri* clones produced by Alfred Gräser in 1928 are the progenitors of most modern-day Easter cactus cultivars, and the parents of these F_1 hybrids were probably the original clones of *H. gaertneri* and *H. rosea* introduced around 1882 and 1911, respectively. Two *H.* x *graeseri* cultivars bred by Harry Johnson of Johnson Cactus Gardens, Paramount, California (= 'Rainbow' and 'Sutter's Gold') apparently have *H. gaertneri* var. *makoyanum* (also known as 'Cat's Whiskers') in their pedigree. Similarly, isozyme studies of the *Schlumbergera* germplasm collection suggest that only a few clones of *S. truncata* and *S. russelliana* contributed to the development of modern *Schlumbergera* cultivars (O'Leary and Boyle, 2000).

Genetic diversity in crop plants can also be diminished by inadvertent loss or deliberate elimination of old and/or obsolete cultivars. For example, a collection of 40 Easter cactus clones which included field-collected clones of *H. gaertneri* and *H. rosea* plus older and modern cultivars contained greater genetic diversity than a subset of 13 *H.* x *graeseri* clones that comprised the bulk of the Easter cactus clones grown commercially (O'Leary and Boyle, 1999). Many of the uncommon isozyme alleles present in older (obsolete) Easter cactus cultivars were not observed in the 13 modern *H.* x *graeseri* clones. Hence, loss of alleles due to breeding (roguing) or discarding older cultivars can further erode the genetic diversity. These results emphasize the importance of preserving species clones and obsolete cultivars in germplasm collections for use in future breeding programs. This is vitally important for *Schlumbergera* and *Hatiora* because: 1) relatively few species clones have been preserved as living specimens; and 2) the Convention on Biological Diversity enacted in 1992 is likely to limit the collection of additional germplasm from wild populations in southeastern Brazil.

7. GENERATION OF GENETIC VARIANTS

Four methods have been used to generate genetic variants in holiday cactus and Easter cactus: intraspecific hybridization, interspecific hybridization, polyploidy, and mutation breeding. Each method is considered below.

7.1 Intraspecific Hybridization

Most holiday cactus and Easter cactus cultivars are bred by crossing elite clones (commercial cultivars or unreleased clones with breeding potential) and selecting promising seedlings in half- or full-sib families. Elite clones must be erect or semi-erect in habit and have large, showy flowers with good postproduction longevity (Table 13-3). These traits are more likely to occur in the progeny if the parents also manifest these qualities. Most cultivars of holiday cactus and Easter cactus are highly SI (Boyle, 2003) and must be cross-pollinated to set seed. In addition, cultivars are extremely heterozygous for most traits. Thus, a wide range of phenotypes may be observed in half- or full-sib families. Novel single traits or a unique combination of characters in seedlings may result from recombination of pre-existing genes or by spontaneous mutation.

Seedlings become sufficiently large for asexual propagation between 6 and 12 months after germination. Weak seedlings may be rogued prior to this time. The most promising seedlings are cloned for further testing and undesirable seedlings are rogued. Promising seedlings are evaluated in replicated trials that are conducted over several years. Commercial crops of holiday cactus and Easter cactus typically require 8 to 12 months from propagation to finish. Thus, there is usually one trial per year and

it takes several years to determine the commercial potential of a single clone. Clones that have withstood the rigors of several trials may be deemed commercially acceptable and mass-propagated for release.

Hansson (1992) described the selection procedure used for breeding holiday cactus at Gartneriet PKM in Odense, Denmark. Progeny are obtained from controlled crosses between selected parents. In excess of 10,000 seedlings are raised annually. The primary selection criteria are: growth habit; disease resistance; flower color and size; and postproduction longevity. Approximately 80-90% of the seedlings are culled each year so that only one or two superior seedlings remain after 7-8 years. The rationale for the long (7-8 year) trialing period was to increase the likelihood of detecting weak and/or disease-susceptible clones.

7.2 Interspecific Hybridization

Interspecific hybridization has played an important role in development of modern holiday cactus and Easter cactus cultivars. Nearly all commercial cultivars of Easter cactus are *H.* x *graeseri*, and approximately half of the holiday cactus cultivars grown commercially are *S.* x *buckleyi*. The parents of *H.* x *graeseri* (= *H. gaertneri* and *H. rosea*) occur in the same general area in southeastern Brazil (Paraná, Santa Catarina) but exhibit ecological isolation: *H. gaertneri* is found at lower elevations (350-1300 m) whereas *H. rosea* occurs at higher elevations (1000-2000 m) (Barthlott and Taylor, 1995; N.P. Taylor, personal communication). Similarly, *S. truncata* and *S. russelliana* are native to the same geographical location (the Serra dos Orgãos northeast of Rio de Janeiro) but apparently do not hybridize due to ecological isolation, with the former species occurring at 700-1000 m and the latter species at 1400-2100 m (Barthlott and Taylor, 1995; McMillan and Horobin, 1995). Although they exhibit reproductive isolation, *H. gaertneri* and *H. rosea* cross readily and yield fertile offspring. Also, hybridization between *S. truncata* and *S. russelliana* yields highly fertile F_1, F_2, and BC_1 progeny. Isozyme segregation patterns in 2*x* and 4*x H.* x *graeseri* indicate that *H. gaertneri* and *H. rosea* are closely related taxa that have the same chromosome number ($2n = 22$) and share a high degree of chromosome homology (O'Leary and Boyle, 1998; Karle et al., 2002). *Schlumbergera truncata* and *S. russelliana* also have the same chromosome number ($2n = 22$), and I speculate that these two species exhibit few chromosomal differences. Hence, the lack of formidable barriers in producing hybrids between *H. gaertneri* and *H. rosea* or between *S. truncata* and *S. russelliana* and the high fertility of the resulting interspecific hybrids suggest that these species have not genetically diverged to any great extent.

Other than *H.* x *graeseri* and *S.* x *buckleyi*, few interspecific hybrids have been used as commercial cultivars of either crop. *Schlumbergera truncata* can be crossed with *S. orssichiana* (= *S.* x *reginae*) and with *S. opuntioides* (= *S.* x *exotica*). F_1 and BC_1 hybrids of *S.* x *reginae* and *S.* x *exotica* have been produced at the University of Massachusetts to determine their suitability as commercial cultivars (Figure 13-6).

Schlumbergera x *reginae* hybrids demonstrate more commercial potential than *S.* x *exotica* hybrids. Like *S. orssichiana*, *S.* x *reginae* F_1 plants have exceptionally large and attractive flowers but their longevity is shorter than those of commercial *S. truncata* cultivars. Also, *S.* x *reginae* F_1 plants have a pendulous habit and are more susceptible to root or crown rots than commercial cultivars of *S. truncata*. Compared to the F_1s, some *S.* x *reginae* F_2 and BC_1 clones are more erect, less susceptible to diseases, and have better flower longevity (Boyle, unpublished data). More work is needed to introgress the novel characters of *S. orssichiana* and *S. opuntioides* without losing the requisite traits for commercialization (Table 13-3). These results demonstrate one of the challenges of working with interspecific hybrids: eliminating the undesirable trait(s) of the donor species while maintaining the desirable traits of the recurrent parent.

With the exception of *H. gaertneri* and *H. rosea*, only two other *Hatiora* species have been hybridized: *H. salicornioides* and *H. herminiae*. F_1 and F_2 hybrids of *H. salicornioides* and *H. herminiae* are fertile but they exhibit little commercial potential (Boyle, unpublished data). One species that may have promise for crossing with Easter cactus is *H. epiphylloides*. *Hatiora epiphylloides* has yellow flowers that are about 1 cm long. Presently, yellow flower color is not found in Easter cactus, and thus *H. epiphylloides* may be useful for expanding the flower color range. It is not known if *H. epiphylloides* will cross with other *Hatiora* species. *Hatiora epiphylloides*, *H. gaertneri*, and *H. rosea* are in the same subgenus, which suggests that the former species may cross with the latter two. Unfortunately, *H. epiphylloides* is difficult to grow and only a few clones are in cultivation.

7.3 Polyploidy

Most cultivars of holiday cactus and Easter cactus are diploid (2*x*) but a few are polyploid (Parks, 2002; Parks and Boyle, 2003). Several *Schlumbergera* cultivars developed by the firm B.L. Cobia are polyploid, including the triploid (3*x*) 'Gold Charm' and the tetraploid (4*x*) 'Peach Parfait' (Parks and Boyle, 2003). 'Bridgeport', 'Cambridge', 'Santa Cruz', and 'Sanibel' are likely to be hexaploid (6*x*) or octoploid (8*x*) (Cobia, 1992). *Hatiora* 'Amherst', 'Louise', and 'Mira' are tetraploid (Parks, 2002; Boyle, unpublished data).

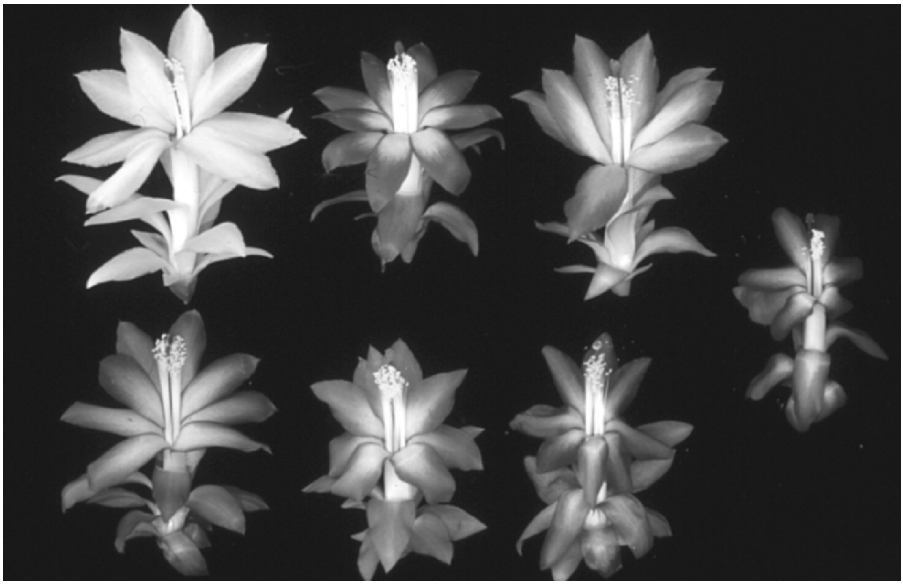

Figure 13-6. Six backcross (BC_1) hybrids of the cross *Schlumbergera orssichiana* x *S. truncata* with *S. truncata* as the recurrent parent. The flower on the far right is *S. truncata* 'Dark Marie' for size comparison.

Karle and Boyle (1999) and Karle et al. (2002) discovered spontaneous mutants of two diploid Easter cactus cultivars ('Evita' and 'Purple Pride') that had a diploid epidermis and tetraploid subepidermis (cytochimeras). Both mutants were SC whereas the original (diploid) clones were SI. Flowers of the 'Evita' cytochimera senesced earlier than those of the original 'Evita' clone, but the original and cytochimera clones of 'Purple Pride' did not differ in flower longevity. Variation in flower longevity for the two cytochimeras was attributed to the stage of floral development in which autogamy commenced. Autogamy began on the day of anthesis in flowers of the 'Evita' cytochimera but not occur until about the fifth day after anthesis in flowers of the 'Purple Pride' cytochimera. The results showed that floral longevity is decreased in SC genotypes that self-pollinate early in floral development but is not affected by the timing of self-pollination in SI (diploid) genotypes. When breeding tetraploid (SC) cultivars of Easter cactus and holiday cactus, seedlings that exhibit the greatest herkogamy (spatial separation between anthers and stigma) should be selected to maximize the flower longevity.

Compared to diploids, polyploids typically have larger and thicker phylloclades and larger flowers but are slower to root, produce fewer phylloclades, and exhibit less branching. In holiday cactus, poor branching and slow growth are more pronounced in 6*x* or 8*x* cultivars compared to the 3*x* or 4*x* clones. Polyploidy probably confers few

advantages in Easter cactus and holiday cactus aside from the larger, more showy flowers.

7.4 Mutation Breeding

Breeders have tried to induce desirable mutations in numerous vegetatively propagated ornamentals by exposing plant material to ionizing radiation or by applying chemical mutagens (Broertjes and van Harten, 1988). Mutation breeding has been used in Easter cactus and holiday cactus in an attempt to generate novel plant types and new flower colors from otherwise commercially acceptable cultivars. Broertjes and van Harten (1988) reported that the optimum dose for irradiating rooted segments of *Hatiora* and *Schlumbergera* is 15 Gy X- or γ-rays at 1 or 2 Gy·min^{-1}. In a cooperative project with Association Euratom (ITAL), Broertjes irradiated "tens of thousands" of Easter cactus and holiday cactus phylloclades to produce mutants with a more compact growth habit, improved branching, and/or novel flower colors. A few mutants were produced but none of them were promising as commercial cultivars. The extent to which mutation breeding has been used by commercial breeders of Easter cactus and holiday cactus is not known.

Easter cactus and holiday cactus appear to be good candidates for mutation breeding because they are vegetatively propagated and highly heterozygous. However, mutation breeding has two major disadvantages: 1) deleterious mutations greatly outnumber beneficial mutations by a ratio of ≈800:1 (Allard, 1960); and 2) most mutations are recessive. Recessive mutations may not be expressed in M_1 plants (= the generation in which a mutagen was applied) unless: 1) the M_1 plant is heterozygous (*Aa*) for a gene exhibiting complete dominance (*Aa* mutates to *aa*); or 2) the trait is controlled by a gene with codominant alleles (A^rA^r mutates to A^rA^w or A^rA^w mutates to A^wA^w). The presence of SI in most *Hatiora* and *Schlumbergera* cultivars means that M_2 progeny cannot be obtained by selfing M_1 plants, so rare recessive mutations occurring at genes homozygous for a dominant allele (*AA* mutates to *Aa*) are unlikely to be expressed in M_2 progeny obtained from crossing different M_1 plants. Also, the probability of crossing two different M_1 plants with the same mutation is extremely remote.

A few holiday cactus cultivars originated as spontaneous mutations on existing cultivars. For example, 'Dark Eva', 'Dark Marie', and 'Dark Madisto' arose as sports of 'Eva', 'Marie', and 'Madisto', respectively (H. de Vries, personal communication). These three sports have darker-colored flowers than their progenitor clones. 'Witte Eva' is a white-flowered sport of 'Eva'.

8. FUTURE CHALLENGES

8.1 Developing New Flower Colors

Betalains are the pigments responsible for the various shades of red, purple, pink, orange, and yellow found in flowers and fruits of cacti (Kobayashi et al., 2000; Piattelli and Imperato, 1969; Strack et al., 1981). The reddish-violet pigments are betacyanins and the yellow pigments are betaxanthins. Betacyanins and betaxanthins are restricted to the order Caryophyllales and are found in all families within this order with the exception of the Caryophyllaceae and Molluginaceae (Gibson and Nobel, 1986).

Numerous studies have been published on the biochemistry of pigments present in cactus flowers and fruit but there are no published reports on the inheritance of flower color in cacti. In Easter cactus, scarlet red flower color is dominant to magenta purple, and segregation ratios for flower color suggest that a single gene controls this trait (Boyle, unpublished data). Flower color in holiday cactus is controlled by numerous genes. White flower color is recessive. Crosses between two salmon orange cultivars or two mallow purple cultivars can yield progeny with pigmented and unpigmented (white) flowers in a 3:1 or 1:0 ratio. As expected, crosses between two white-flowered cultivars usually yield 100% white-flowered progeny, but some crosses do not. Crosses between 'Sanne' and several other white-flowered cultivars yielded a 1:1 ratio of mallow purple and white-flowered seedlings. These results suggest that 'Sanne' is a chimera with an "white" epidermis and subepidermal layer heterozygous for mallow purple and white. Crossing two different clones with salmon orange flowers yields 100% salmon-orange progeny or salmon orange:white-flowered progeny in a 3:1 ratio. Similarly, crossing two different clones with mallow purple flowers can yield all progeny with mallow purple flowers or mallow purple: white-flowered plants in a 3:1 ratio. When two cultivars with scarlet red or true red flowers are crossed, the progeny segregate for scarlet red, true red, salmon orange, or mallow purple pink flowers, but white-flowered progeny have not been observed. Similar results are obtained when cultivars with scarlet red or true red flowers are crossed with cultivars that have mallow purple flowers.

Expanding the flower color range in Easter cactus is highly desirable. Currently, flower color in commercial Easter cactus cultivars is limited to tints and shades of magenta purple and scarlet red. The first whilte-flowered *Hatiora* cultivar ('Sirius') was released in 2002. A few *H.* x *graeseri* cultivars, such as 'Parnell' (coral-orange flowers) and 'Sutters Gold' (golden-orange flowers), may be useful for breeding pastel flower colors. 'Parnell' and 'Sutter's Gold' are popular among amateur collectors but are not grown by commercial flower growers. It might be useful to cross these two cultivars with erect (commercial) clones with the hopes of obtaining commercially acceptable progeny with unique flower colors. Alternatively, interspecific

hybridization might also be a useful approach for expanding the flower color range in Easter cactus. *Hatiora epiphylloides* has 1-cm long yellow flowers but is difficult to grow and rare in cultivation. It is not known if *H. epiphylloides* will cross with *H.* x *graeseri* or its progenitor species, but this cross may yield some interesting flower colors if progeny could be obtained.

8.2 Extending Flower Longevity

Flower longevity partially determines the duration that plants remain aesthetic and therefore is an important component of plant quality. Clones with superior flower longevity should result in improved plant appearance in the retail setting as well as greater consumer satisfaction, thus stimulating repeat purchasing (Langton, 1987).

Scott et al. (1994) evaluated eight Easter cactus cultivars and five holiday cactus cultivars in a growth chamber set at 20 ± 1°C, 75 ± 5% relative humidity, and a 12-hour photoperiod provided by cool-white fluorescent lamps (52 ± 8 $\mu mol \cdot s^{-1} \cdot m^{-2}$ photosynthetic photon flux). These environmental conditions were comparable to those found in a retail setting. Flower longevity ranged from 7 to 12 days for the Easter cactus cultivars and from 4 to 6 days for the holiday cactus cultivars. The results showed that, within both crops, there were significant differences among cultivars in flower longevity. Thus, it is feasible to select Easter cactus and holiday cactus clones with increased flower longevity.

Flower longevity of promising clones should be determined as early as possible in the breeding process. Ideally, plants should be evaluated in a controlled environment with environmental conditions simulating those encountered in a retail setting. Flower longevity should be assessed using several replicates (pots) per clone.

Effectiveness of selection for longer flower life depends on the heritability of this trait. There have been no reports on the heritability of flower longevity in Easter cactus or holiday cactus. Narrow-sense heritability (h^2) estimates [= the ratio of the additive genetic variance (V_A) to the phenotypic variance (V_P)] for flower longevity would indicate the extent to which progeny phenotypes are determined by genes transmitted from their parents (Falconer, 1981). These estimates would be helpful for selecting parents for crosses.

8.3 Improved Disease Resistance

Several diseases affect *Hatiora* and *Schlumbergera* plants and can cause significant economic losses (Table 13-13-4). Incorporation of disease resistance into commercial cultivars would be a worthwhile breeding objective. There have been only two published reports on screening holiday cactus cultivars for disease resistance. Raabe (1989) wounded phylloclades of several holiday cactus cultivars and inoculated them with an isolate of *Bipolaris cactivora* (formerly *Drechslera cactivora*). The cultivars differed in their disease responses, with ‘Maria’ (= ‘Marie’) the most

susceptible, 'Majestic' and 'Rita' the least susceptible, and 'Annette' showing an intermediate response.

Chase (1993a,b) screened 20 holiday cactus cultivars for disease reactions to three fungal pathogens [*B. cactivora*, *Fusarium oxysporum*, and *Phytophthora parasitica*] and a bacterial pathogen (*Erwinia carotovora* subsp. *carotovora*). Three separate greenhouse tests were conducted with each pathogen. There were significant differences among cultivars in disease severity. 'Gold Charm' was highly susceptible to all four pathogens. Most cultivars, however, varied in susceptibility to the four pathogens. For example, disease severity in 'Peach Parfait' was low when inoculated with *F. oxysporum* but was severe when inoculated with *B. cactivora*. 'White Christmas' showed an intermediate level of resistance to *F. oxysporum* and *P. parasitica* but a higher level of resistance to *E. carotovora* and *B. cactivora*. Generally, the level of disease resistance in the 20 cultivars was not high and the three tests often yielded inconsistent results. It is notable that all of the cultivars used in Chase's (1993a,b) study were bred by B.L. Cobia. Assessment of the disease responses of a broader array of *Schlumbergera* clones (modern and obsolete cultivars from several breeders plus unimproved clones) may be a more useful approach for identifying sources of resistance to these pathogens.

Table 13-4. Major diseases of *Hatiora* and *Schlumbergera*. Data complied from Daughtrey et al. (1995).

Disease (pathogen)	Reported host(s)
Bacterial soft rot (*Erwinia carotovora subsp. carotovora)*	*S. truncata*
Bipolaris stem rot and leaf spot *(Bipolaris cactivora)*	*H. gaertneri*
Fusarium stem rot *(Fusarium moniliforme, F. oxysporum)*	*H. gaertneri, S. truncata*
Pythium root and stem rot *(Pythium aphanidermatum)*	*S. truncata*
Phytophthora root and crown rot *(Phytophthora parasitica)*	*S. truncata*

A disease screening experiment employing a diverse array of *Hatiora* genotypes was conducted at the University of Massachusetts (Boyle and Wick, unpublished data). Nineteen different *Hatiora* clones were used to screen for resistance to *B. cactivora*. Two growth chamber experiments and one greenhouse experiment were conducted. Stem pieces (consisting of two attached phylloclades) were placed in petri plates on top of moist filter paper. Basal phylloclades were stuck with a needle and inoculated with 5 µL of spore suspension (≈5 spores·µL^{-1}). Petri dishes were covered

and sealed after inoculation. Phylloclades were checked for disease symptoms and abscission of apical and basal phylloclades one week after inoculation. In the greenhouse experiment, potted plants were sprayed with a spore suspension and disease reactions were recorded one week after inoculation. The growth chamber and greenhouse experiments yielded similar results. Sixteen entries were highly susceptible, one entry was moderate susceptible, and two entries were resistant to the *B. cactivora* isolate (Table 13-5). Resistant entries produced small lesions with little or no sporulation and phylloclade abscission was ≤33%. Susceptible entries developed large lesions with abundant spores, and phylloclade abscission was ≥83%. 'Hatherton Star' was the most resistant *H.* x *graeseri* accession in all three experiments; this clone may be useful for breeding disease-resistant cultivars. *Hatiora salicornioides* 'Logee' also exhibited resistance to *B. cactivora*. Unfortunately, hybridization between *H. salicornioides* and *H. gaertneri* or *H. rosea* has been unsuccessful thus far. These results underscore the importance of using a broad-based array of genotypes in disease screening studies.

Table 13-5. Disease reactions of *Hatiora species* and cultivars to an isolate of *Bipolaris cactivora* (T.H. Boyle and R.L Wick, unpublished data).

Disease reaction	Entry
Highly susceptible	Annika, Cassiopeia, Crimson Giant, Crystal Lakes 32, Evita, Flash, Leo's Pink, Monarch, Peter Pan, Pink Perfection, Rainbow, Rood, Shocking Pink, Sutter's Gold, Thor-Anne, *H. rosea* MD861
Moderately susceptible	Andre
Resistant	Hatherton Star, *H. salicornioides* Logee

8.4 Extending the Flowering Period

In the northern hemisphere, flowering plants of Easter cactus are available from January to June but it would be desirable to extend the marketing period to other times of the year. For example, red-flowered cultivars might be a highly marketable as a Christmas crop. In order to produce budded plants of Easter cactus by early December, cool (8-13°C) temperatures would need to begin by early September and must be maintained for ≈6 weeks to induce flower initiation (Boyle, 1995). From early September to mid-October, however, the average daily temperature exceeds 13°C in many areas of North America, particularly in the southern United States. Therefore, it might be difficult to schedule Easter cactus as a Christmas crop due to the inability to maintain greenhouse temperatures ≤13°C for flower initiation.

An alternative to cooling greenhouses to the desired temperatures would be to select clones capable of setting flower buds at supraoptimal temperatures (>13°C). Research by Boyle (1995) has shown that the latter approach may be feasible. In a

greenhouse experiment, 23 Easter cactus clones were exposed to 18 ± 2°C minimum temperature and an 8-hour (short-day) photoperiod for 6 weeks, then forced into flower at 18 ± 2°C minimum temperature and long days [natural daylengths plus irradiance from high-pressure sodium lamps (30 ± 5 $\mu mol \cdot s^{-1} \cdot m^{-2}$ photosynthetic photon flux)]. The clones differed in the percentage of plants flowering, days to flowering, percentage of apical phylloclades flowering, and number of flower buds per plant. 'Crimson Giant' was superior to the other clones in its ability to flower at 18°C minimum. All of the 'Crimson Giant' plants flowered but ≤75% of the plants of the other 22 clones flowered. Also, 'Crimson Giant' yielded the highest percentage of apical phylloclades flowering and the most flower buds among the 23 clones (Boyle, 1995).

It may be worthwhile to evaluate a more diverse array of *Hatiora* clones (including obsolete and current cultivars plus wild species) to assess their potential for flowering at warm temperatures. Further selection and breeding may yield genotypes that flower more prolifically at 18°C minimum than those grown currently.

8.5 Improved Stress Tolerance

Exogenous ethylene (C_2H_4) or stresses encountered during shipping or retail display such as high temperature, low irradiance, or drought can accelerate flower senescence or induce abscission of flowers and buds of holiday cactus and Easter cactus (Cameron and Reid, 1981; Han and Boyle, 1996). Generally, holiday cactus is more sensitive to exogenous C_2H_4 than Easter cactus. Commercial growers extend the display life of holiday cactus by applying an inhibitor of C_2H_4 action [silver thiosulfate (STS) or 1-methylcyclopropene (MCP)] to budded plants prior to shipping. An alternative approach would be to select genotypes that are less sensitive to C_2H_4 or other stresses that occur in shipping or retail display. Research by Han and Boyle (1996) showed that Easter cactus cultivars differ in their sensitivity to C_2H_4 (Table 13-6). In this experiment, five cultivars were exposed to 0, 0.4, or 1.0 $\mu L \cdot L^{-1}$ C_2H_4 in glass tanks for 48 hours and the plants were transferred to a growth room immediately after C_2H_4 exposure. Percent flower bud abscission was determined five days after C_2H_4 exposure. Bud abscission for `Andre' and `Red Pride' was not significantly affected by the C_2H_4 concentrations used in this study, but bud abscission of `Evita', `Rood', and `Thor-Anne' increased as C_2H_4 concentration increased from 0 to 1.0 $\mu L \cdot L^{-1}$ (Table 13-6). Also, percent bud abscission was similar for all five cultivars when plants were exposed to 0 $\mu L \cdot L^{-1}$ C_2H_4, but cultivar differences were apparent when plants were exposed to either 0.4 or 1.0 $\mu L \cdot L^{-1}$ C_2H_4. Additional experiments by Han and Boyle (unpublished data) have shown that holiday cactus cultivars also differ in their sensitivity to C_2H_4. Identification of clones that are less sensitive to C_2H_4 may thus have practical value for commercial growers and also for breeders aiming to develop cultivars with greater postproduction longevity.

Table 13-6. Effect of C_2H_4 concentration on percent flower bud abscission for five Easter cactus cultivars. Data from Han and Boyle (1996).

	($\mu L \cdot L^{-1}$)	($\mu L \cdot L^{-1}$)	($\mu L \cdot L^{-1}$)	
Cultivar	0	0.4	1.0	Significance
Andre	6.2 ± 1.8	2.6 ± 1.4	9.2 ± 4.2	NS
Evita	4.4 ± 1.5	10.1 ± 2.3	51.9 ± 6.0	$L^{***}Q^{**}$
Red Pride	6.3 ± 1.3	11.5 ± 3.6	13.0 ± 3.9	NS
Rood	3.8 ± 1.4	31.0 ± 5.0	66.4 ± 5.6	L^{***}
Thor-Anne	5.4 ± 1.9	14.3 ± 3.7	38.8 ± 8.4	L^{***}
Significance[a]	NS	***	***	

[a]Heterogeneity chi-square tests for differences among cultivars at each C_2H_4 concentration
NS, **, ***Nonsignificant or significant at $0.001<P<0.01$ or $P \leq 0.001$; L=linear, Q=quadratic

9. FUTURE PROSPECTS

Easter cactus and holiday cactus display a substantial amount of genetic variation for many traits, and this is somewhat suprising given the narrow genetic base that occurs in both crops. There is, however, a need for collecting additional germplasm of *Schlumbergera* and *Hatiora* taxa from their native habitats. The highest priority for acquisition would be the three species in the subgenus *Rhipsalidopsis* (= *H. gaertneri*, *H. rosea*, and *H. epiphylloides*). Interspecific hybridization appears to be a promising approach for broadening the genetic base in holiday cactus. Continued work in hybridizing holiday cactus with *S. orssichiana* and *S. opuntioides* is likely to yield commercially acceptable clones with novel traits. Crossing *H. gaertneri*, *H. rosea*, or *H.* x *graeseri* with other *Hatiora* taxa may be a challenging endeavor but could reap some useful hybrids. Progress in hybridizing distantly-related *Hatiora* or *Schlumbergera* taxa may require the use of in vitro techniques for culturing embryos and/or ovules. For the near future, exploiting the genetic variation in non-commercial *H.* x *graeseri* clones and/or field-collected clones of *H. gaertneri* and *H. rosea* may prove to be useful for developing improved and/or novel clones of Easter cactus.

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Chapter 14

CHRYSANTHEMUM

Dendranthema x grandiflora Tzvelv.

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Abstract: Garden and greenhouse chrysanthemums, *Dendranthema x grandiflora,* have a lengthy association with various world cultures. As a result, flower breeders have created numerous genotypes during the millennia of breeding this crop, which has enabled its establishment in the top ten cuts, potted flowering, and garden crops worldwide. The crop arises from multiple species; its allopolyploid nature (*2n=6x=54)* has complicated progress in crop development due to inbreeding depression, genetic load, and aneuploidy. Consequently, most cultivars are vegetatively propagated, few genes have been characterized, and none have been mapped to chromosomes. Research has focused on seed-propagated hybrids, flowering earliness, winter hardiness, improvement of flower colors/form, plant habit, day neutrality, characterization of self incompatibility, and selection of pseudo-self compatible parents. Future crop ideotypes are proposed to continue transformation of this important crop during the next millennium.

Key words: Allopolyploids, hybrid seed production, inbreeding depression, rapid generation cycling, self incompatibility.

1. INTRODUCTION

The word chrysanthemum is derived from the Greek words ‘chrysos’ (gold) and ‘anthemon’ or ‘anthos’ (flower) (Morton, 1891). Pictorial reliefs from the reign of Thutmose III (ca. 900 B.C.) depict chrysanthemums taken from King Solomon’s royal gardens (Schweinfurth, 1919). “Chu-tzu”, a Chinese book of poetry by Chu Yuan from 300 B.C. mentions chrysanthemum flowers (Ackerson, 1967a). The Chinese cultivated it for pharmaceutical purposes according to Confucius in *Li-Ki,* published in 500 B.C. (Morton, 1891; Bailey, 1914; Herrington, 1917; Arneson,

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1927; Emsweller et al., 1937; Cumming, 1939; Laurie and Poesch, 1939; Woolman, 1953; Randall and Wren 1983). 'Chu' is the Chinese character for chrysanthemum and the Japanese refer to it as 'kiku', although both characters are remarkably similar (Ackerson, 1967a). So popular is this flower in the Orient that the Chinese named a city, Chu-hsien, the Chrysanthemum city (Randall and Wren 1983).

In 385 A.D., Korea bestowed Japan with a gift of chrysanthemum seeds; resultant seedlings had flower colors of blue, red, white, black, and yellow (Ackerson, 1967a). Oddly enough, 'kiku', the character for chrysanthemum existed in the Japanese language before Korea's gift. Cultivation of chrysanthemums became a favored Japanese pastime. Japan instituted several cultural symbols or events in honor of the chrysanthemum, including a festival of happiness (Chrysanthemum Day). A single daisy flower with n=16 petals became the seal and crest of the Chrysanthemum Throne and Emperor of Japan; the imperial Order of the Chrysanthemum became the highest order of chivalry in the country (Emsweller, et al., 1937; Randall and Wren 1983). In addition to their aesthetic value, chrysanthemums are also a food-source, may be fragrant, or used medicinally. Flower petals are eaten in salads or used in teas, whereas new shoots (stems/leaves) are steamed or stir-fried as a vegetable (Woolman, 1953).

The western world was introduced to chrysanthemums via The Netherlands in 1688, where they were cultivated as *Matricaria japonica*, although these apparently died from unknown causes. Chrysanthemums later spread to Great Britain in 1754 and the United States in 1798 (Clark, 1962; Dowrick, 1953; Emsweller, et al., 1937). Indeed the chrysanthemum is an internationally recognized flower and floricultural crop.

While the crop was most likely hybridized in Asia, the first recorded hybridization of chrysanthemums and germination/selection of superior seedlings occurred in 1827 by M. Bernet, although earlier hybridizers undoubtedly crossed chrysanthemums centuries before (Emsweller, et al., 1937). Robert Fortune introduced two 'small-flowered' types (pompon flower types), 'Pompon' and 'Chusan Daisy', to English gardeners in 1843-1846 (Dowrick, 1953; Emsweller, et al., 1937). They were not highly regarded in England, but were sent to France where they were popularized and used widely in hybridization. It is possible that these few genotypes were the progenitors of our modern-day "small-flowered varieties" (Emsweller, et al., 1937). French mum breeders such as Simon Delaux and Auguste Nonin bred many improved cultivars in the late 1800s (Jones, 1958), creating hybrids still popular to this day (Figure 14-1). Some 28 years after being introduced into the United States, Prince's Nursery offered 26 cultivars for sale; in 1835 as many as 50 were listed in Hovey's American Gardener's Magazine and Register (Emsweller, et al., 1937).

American and British National Chrysanthemum Societies have long been recognized for continued popularization of chrysanthemums with gardeners, as well as spawning numerous amateur chrysanthemum breeding programs (Scott, 1957;

Jones, 1958; Clark, 1962). The earliest known chrysanthemum breeder in the U.S. was Robert Kilvington (Philadelphia, Pennsylvania) who exhibited a new cultivar 'William Penn' at the 1841 annual meeting of the Pennsylvania Horticultural Society (Emsweller, et al., 1937). A later meeting of the society in 1846 had a chrysanthemum exhibit, advertising the chrysanthemum as "the coming flower" (Emsweller, et al., 1937). Currently, it is not uncommon to have special chrysanthemum shows in the fall season throughout most of the United Kingdom and North America.

Figure 14-1. Descendants of chrysanthemums derived from the 1800s breeding programs of Monsieurs Simon Delaux and Augusta Nonin displayed at le Tour d'Eiffel, Paris, in 2004.

Noted amateur or private sector breeders of greenhouse chrysanthemums include Charles Totty (Madison, New Jersey, USA), Eugene H. Mitchel (Dreer Co., Philadelphia, Pennsylvania, USA), and Elmer Smith (Adrian, Michigan, USA) (Crook, 1942). Elmer Smith had introduced 445 cultivars by 1928, after 30 years of breeding work (Viehmeyer and Uhlinger, 1955). Garden chrysanthemum breeders in the public and private sectors such as E. M. Byrnes, J.W. Byrnes, F.L. Mulford (U.S. Department of Agriculture), V.R. DePetris (Detroit, Michigan, USA), Alex Cumming Jr. (Bristol, Connecticut, USA), Dr. J.E. Krause (University of Chicago), W.J. Carpenter, T.B. Shackelford (Kansas State University), Dr. L.E. Longley (University of Minnesota), R. Lehman (Mums from Minnesota, Lehman Gardens, Faribault, Minnesota), and Bauer & Steinkemp (Indianapolis, Indiana, USA) bred numerous cultivars (Crook, 1942).

The chrysanthemum craze in the United States is often illustrated by the sale of a large white greenhouse variety to Mr. and Mrs. Alpheus Hardy for US$1,500 in

1888 (Crook, 1942); it was later named 'Mrs. Alpheus Hardy'. By the 1930s as many as 3,000 cultivars existed; most of these were greenhouse chrysanthemums. Numerous publications have recorded the history of this popular flower. Bailey (1914) noted that >100 monographs existed and the number of popular trade articles was exceeded only by that of roses. Crook (1942) noted "chrysanthemum is the flower of the East, as the rose is the flower of the West."

2. CURRENT MARKET STATUS

Chrysanthemums are one of the most important floricultural crops in the cut flower, flowering potted plant, and herbaceous perennial markets of the world. Japan is the leading producing country in the world, with more than two billion stems per year in 1993, primarily for domestic consumption (Horst, 1990). In The Netherlands, chrysanthemums ranked as the second cut flower in Dutch auctions (800 million stems) and fifth in potted plant rankings (Pathfast Publishing, 1994). Other top-producing countries include Columbia (600 million stems), Italy (500 million), and the United States (300 million).

Cut flower, potted plants, and garden chrysanthemums have long ranked in the top ten for sales in the United States. As early as 1939, they were the No. 3 cut flower in the U.S. (Laurie and Poesch, 1939). In 2003, cut pompon chrysanthemums (US$18.181 million wholesale) and flowering potted plant (US$76.093 million wholesale) sales ranked as Nos. 8 and 3, respectively, in the cut flower and potted plant top ten listing (Anderson, 2004; United States Dept. of Agric. National Agricultural Statistics Service, 2004). Garden chrysanthemums are the No. 1 herbaceous perennial in the United States with US$120.424 million (w) in sales (United States Dept. of Agric. National Agricultural Statistics Service, 2004).

3. TAXONOMY AND SPECIES

As many as 200 species were originally contained within the genus *Chrysanthemum*, but the majority have been subdivided into 38 satellite genera of the chrysanthemum complex (Table 14-1; Anderson, 1987). Taxonomic reclassifications of the *Chrysanthemum* complex have happened repeatedly (Heywood, 1976; Humphries, 1976a, b; Kitamura, 1978; Nordenstam, 1976; Tzvelv, et al., 1961). Anderson (1987) theorized that Linnaeus most likely conceived of a *Chrysanthemum* complex (Linnaeus, 1737; 1753). Linnaeus classified species of this complex into five major genera including *Argyranthemum* Webb ex Schultz Bip., *Chrysanthemum sensu stricto* L., *Dendranthema* Des Moul., *Leucanthemum* Miller, and *Tanacetum* L. (Table 1) (Bentham, 1873; Heywood, 1954, 1958, 1976; Hoffman, 1889-1894; Kitamura, 1978; Nordenstam, 1976). Numerous "independent

satellite genera" fill out the remainder of the complex (Table 14-1) (Anderson, 1987; Heywood, 1976a, b; Heywood and Humphries, 1977; Humphries, 1976). Engler and Prantl (1926) originally divided the genus *Chrysanthemum* into eight sections, four sections contained annual species while the remaining sections consisted of perennial species. Cultivated chrysanthemums (*Dendranthema x grandiflora)* and pyrethrum (*Tanacetum cinerariifolium, T. coccineum),* the two most important domesticated species within the genus, were taxonomically classified into Section VI Pyrethrum (Engler and Prantl, 1926). Long stalked capitala with a composite inflorescence is the most common characteristic shared by the 50 species found in this section (Anderson, 1989).

At present, taxonomic classification is based on embryo sac development, cypselar anatomy, plant habit, molecular markers, and phytochemical characteristics (Borgen, 1972; Briquet and Cavillier, 1916-1917; Harling, 1951; Heywood, 1958, 1959; Humphries, 1976a). Cytology is primarily used to characterize species relationships rather than generic ones, since all species in the *Chrysanthemum* complex have a base number of x=9 (Shimotomai and Takemoto, 1940; Tanaka and Watanabe, 1972; Watanabe, 1977a, b). In the strictest sense, therefore, the genus *Chrysanthemum* has two annual species, *C. coronarium* and *C. segetum* (Table 14-1; Anderson, 1987; Boase, et al., 1997). Cultivated greenhouse and garden chrysanthemums, *Dendranthema x grandiflora* Tzvelv. (=*Chrysanthemum x morifolium* Ramat., *C. hortorum*), are members of the Asteraceae Dumort. or formerly the Compositae (Tribe: Anthemideae, Subtribe: Chrysantheminae), the most phylogenetically advanced dicotyledonous family with numerous floricultural crops (Heywood and Humphries, 1977; Anderson, 1987).

Table 14-1. Revised taxonomic designations of genera and commercial species within the chrysanthemum complex (Anderson, 1987; Boase, et al., 1997; Heywood, 1976; Heywood and Humphries, 1977; Humphries, 1976a, b).

Genera	Species	Common name	Previous name
Primary Genera			
Argyranthemum Webb ex Schultz Bip.	*frutescens* Schultz-Bip.	Marguerite daisy	*C. frutescens*
Chrysanthemum L.	*coronarium* Schousboe	Rainbow daisy	
	segetum L.		
Dendranthema Des Moulins	*x grandiflora* Tzvelv.	Chrysanthemum	*C. x morifolium*
	indicum L.		*C. indicum*
	japonicum Makino		*C. japonicum*
	weyrichii (Maxim.) Tzvelv.		*C. weyrichii*
Leucanthemum Miller	*x superbum* Berg. ex Kert.	Shasta daisy	*C. x superbum*
	vulgare Lam.	Oxeye daisy	*C. leucanthemum*
Tanacetum L.	*balsamita* L.	Costmary	*C. balsamita*
	cinerariifolium Schultz-Bip.	Pyrethrum	*C. cinerarifolium*

Genera	Species	Common name	Previous name
	coccineum Willd.	Painted daisy	*C. coccineum*
	macrophyllum Scultz-Bip.	Feverfew	*C. macrophyllum*
	ptarmiciflorum Schultz-Bip.	Dusty Miller	*C. ptarmiciflorum*
	parthenium Schultz-Bip.	Feverfew	*C.partheniume*
	vulgare L.	Tansy	*C. vulgare*
Independent Satellite Genera			
Ajania Polj.			
Arctanthemum Tzvelv.			
Balsamita P. Miller	*major* Desf.	Costmary	*C. balsamita*
Coleostephus Cass.	*myconis* (L.) Cass.		*C. myconis*
Glossopappus G. Kunze	*macrotus* (Durieu) Briq.		
Heteranthemis Schott.	*viscidehirta* Schott	Sticky oxeye	
Hymenostemma Kunze ex Willkomm	*pseudoanthemis* (G. Kunze) Willk.		
Ismelia Cass.	*carinatum*	Painted daisy	*C. carinatum*
Leucanthemella Tzvelv.	*serotinum* Tzvelv.	High daisy	*C. serotinum*
Leucanthemopsis (Giroux.) Heywood	*alpina* (L.) Heywood		*C. alpinum*
Nipponanthemum Kitamura	*nipponicum* (Franch. Ex Maxim.) Kitamura		
Phalacrocarpum (DC.) Willkomm	*oppositifolium*		
Pinardia Cass.	*anisocephala* Cass.		
Prolongoa Boiss.	*pectinata* (L.) Boiss		

Chrysanthemums are native to the northern hemisphere, primarily Europe (the Mediterranean region, centered in Algeria and the Canary Islands) and Asia (China, Korea, and Japan) (Dowrick, 1952b; Hemsley, 1889). From these centers of diversity, numerous species are widespread across Eurasia (Figure 14-2). Most New World species are introduced exotics, with the notable exception of twelve species, including seven *Tanacetum spp., Dendranthema arcticum, D. angustifolium, D. camphoratum,* and *D. cespitosum.* Species found in the Mediterranean region are diploid ($2n=2x=18$), with the exception of *Leucanthemum maximum* ($2n=10x=90$). Oriental species have a much greater ploidy range, from diploid to decaploid (Figure 14-2), which led Dowrick (1952b) to theorize that polyploidy was associated with an increase in latitude. Most diploid species occur in the presumed center of origin, the Mediterranean, while only polyploids exist in the far north such as Siberia and the Arctic, e.g. *D. arcticum* ($2n=8x=72$).

All cultivated chrysanthemums are allohexaploid ($2n=6x=54$) with somatic chromosome numbers ranging from $2n=47$-63 both between and within plants (Dowrick, 1953). *Dendranthema x grandiflora* cultivars are complex interspecific

hybrids, whose ancestry includes ten or more primarily hexaploid species, including *Dendranthema erubescens, D. indicum, D. japonense, D. makinoi, D. ornatum, D. sinense,* and *D. zawadskii* var. *latilobum* (Ackerson, 1967b; Dowrick, 1953). Wild populations occur in China and Japan (Crook, 1942). As with most other wild chrysanthemum species, *D. x grandiflora* has a single daisy flower type with flower colors of white, pink, and lavender, to yellow. However, as early as 910 A.D. a double form 'appeared', either as a spontaneous mutation or the result of directed breeding efforts (Crook, 1942).

Several species have been of interest to breeders for genetic improvement and many have been integrated into the *Dendranthema x grandiflora* gene pool (Table 14-2) (Cumming, 1939). Longley (1949, 1950) used an early-flowering parent, 'Deanna Durbin', derived from *D. zawadskii* to improve winter hardiness and stem strength. *Dendranthema coreanum, D. arcticum* 'Astrid', *D. nipponicum, D. rubellum* (now reclassified *D. zawadskii*), and *D. sibiricum* have also been used widely (Cumming, 1939).

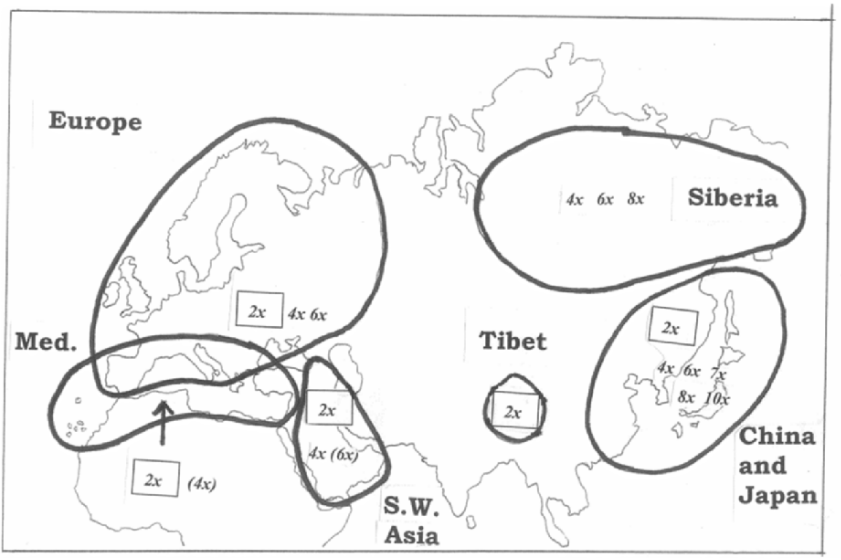

Figure 14-2. Eurasian distribution of chrysanthemum species (Dowrick, 1952b). Most of the diploid species are contained in the center of origin, the Mediterranean, while increasing latitudes are associated with polyploidy.

3.1 Gene Pools

3.1.1 Greenhouse Chrysanthemums

Before 1850, all chrysanthemums were grown out-of-doors (Emsweller, et al., 1937). The hybridization of florist or greenhouse chrysanthemums began after 1850 when culturing mums in greenhouses commenced. Commercial greenhouse chrysanthemum cultivars are 8-14 weeks short day flowering response groups (Crater, 1980). Greenhouse chrysanthemums include both cut flower types and flowering potted plant cultivars, each with specific production protocols (Dole and Wilkins, 2004). Cut and potted mums include the full range of flower colors, flower types, and varying numbers of flowers per flower stalk—ranging from singles (standards) which are disbudded to remove all lateral flower buds (multiple bud removal) to spray types (single bud removal of the terminal flower bud to encourage lateral branching).

Breeding and selection bifurcated greenhouse chrysanthemums from garden types post-1850 (Emsweller, et al., 1937). Selection for both cut flower and potted plant types resulted in numerous series and cultivars which possess longer short day response groups for flower bud development, none to few strap-shaped leaves subtending the terminal floret, stronger but more brittle stems, doubleness (preferred over the singles or semi-doubles to reduce the production of pollen), sterility (a function of Muller's ratchet due to higher frequencies of asexual or clonal propagation to meet high cutting production requirements; and increased post-harvest life (flowering duration) (Anderson and Ascher, 1994; Teynor, et al, 1989b).

Mr. Hosea Waterer largely influenced interest in breeding greenhouse chrysanthemums after he imported ~50 genotypes of cut flowers from Japan into the U.S. (Emsweller, et al., 1937). The expensive cultivar 'Mrs. Alpheus Hardy' appeared shortly thereafter. Other successful breeders during the 1800's in the United States included F. Dorner & Son, E. Fewkes, V.H. Hallock, E.G. Hill, Pitcher & Manda, W.C. Pyfer, and T.H. Spaulding. By 1894 as many as 163 greenhouse cultivars had been bred (Emsweller, et al., 1937). As a result of these breeding efforts and, particularly after the discovery of photoperiodism effects on flower bud initiation and development, numerous public and private sector chrysanthemum breeding programs arose in the early 1900s. Thereafter, greenhouse mums were programmable year-round to flower on specific dates once the response group was characterized and 'artificial' short days were instituted with the use of black cloth.

3.1.2 Garden Chrysanthemums

Garden chrysanthemums are the most popular in North American gardens and, to a lesser extent, in Europe, Japan, and Asia (Crater, 1980). Commercial cultivars

are 6-8 week short day response groups. They are frequently sold in the spring (as small flowering potted plants to be set out-of-doors) and autumn (as mature flowering specimens for containers or direct planting) (Gaston, et al., n.d.; Widmer, 1980). Most early chrysanthemum breeders in Europe and the United States bred both greenhouse and garden types. Some, however, such as Alex Cumming, Jr. (Bristol, Connecticut, USA) focused on garden types during the early 1900s (Emsweller, et al., 1937). Much of this germplasm built the successful private sector breeding program at Yoder Brothers, Inc. (Barberton, Ohio, USA) or resides in plantings at the New York Botanical Garden. The U.S. Department of Agriculture and numerous universities (Minnesota, Nebraska, Kansas, Connecticut) had active chrysanthemum breeding programs during the early 1900s. Now, the only public sector breeding program remaining in North America is at the University of Minnesota (Anderson, 2004).

Hybridization between greenhouse and garden gene pools continues to this day for trait transfer, although as the gene pools diverge hybridizations become increasingly difficult (Viehmeyer and Uhlinger, 1955; Teynor, et al., 1989b). Typical traits for gene pool transfer include flowering earliness (source: garden gene pool), stem strength (source: greenhouse), novel flower colors (striping; source: greenhouse), or flower forms (quilled and carnation-flowered forms were derived from greenhouse germplasm to create garden cultivars, e.g. 'Ak-Sar-Ben', 'Pathfinder', 'Plainsman') (Viehmeyer and Uhlinger, 1955). Crosses in either direction allow for trait transfer but this is usually accompanied by undesirable linked gene(s), necessitating recurrent or congruity backcrosses of the hybrids with inbred or non-inbred parents in the targeted gene pool.

4. FLORAL MORPHOLOGY

The entire flowering shoot or 'spray' of a chrysanthemum is a cyme with multiple inflorescences, the oldest of which terminates the main shoot (Cockshull, 1985). Each inflorescence individually is a raceme with older florets surrounding the outside perimeter. Flowering of the inflorescences subtending the terminal occurs basipetally. Chrysanthemum inflorescences consist of central hermaphrodite disc florets (pistillate + staminate) and/or marginal female ray florets (pistillate) with inferior ovaries (Cockshull, 1985). Collectively, a flower head is known as a capitulum surrounded by an involucre of numerous bracts (Cockshull, 1985). There is a single anatropous ovule per floret for both the disc and ray florets (Anderson, et al., 1990; Cockshull, 1985).

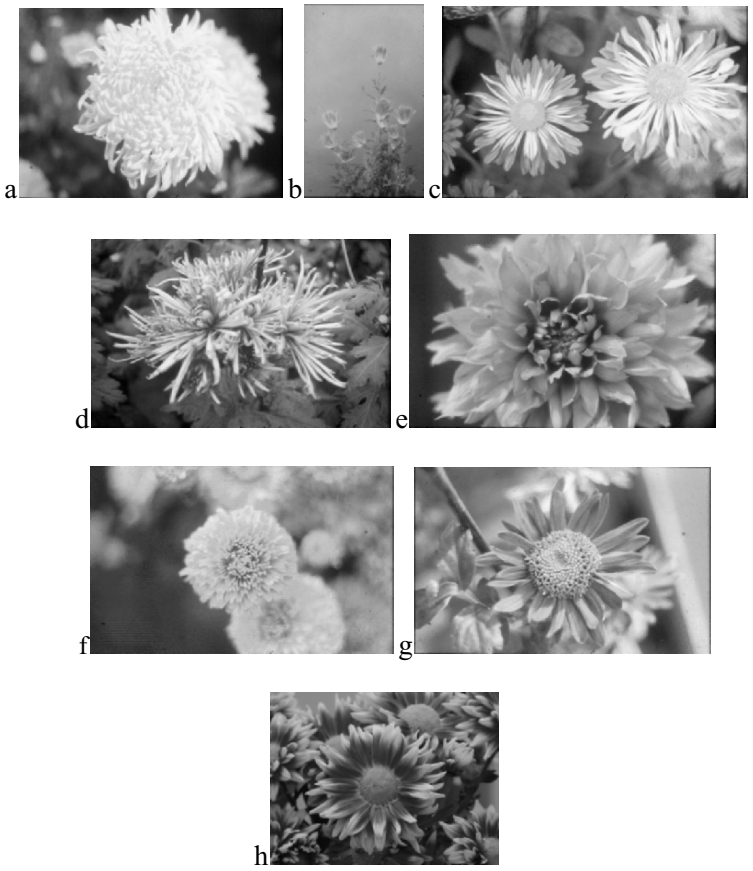

Figure 14-3. Flower types in greenhouse and garden chrysanthemums include modifications to petal number, orientation, and petal types, which produce incurved (a), brush/thistle (b), spoon (c), quill (d), decorative (e), pompon (f), anemone (g), and daisy (h) phenotypes.

Modifications of the basic flower form are common and cultivars are classified by flower type due to ray petal modifications and the relative number of ray petal rows. Most flower forms in existence today were the result of intensive breeding and domestication by the Japanese more than 1,000 years ago (Dowrick, 1953). The most important commercial flower types (Figure 14-3) include incurved (petal tips curved inward), reflexed (opposite of incurved), anemone (center disc floret tubes elongated and colored; also termed 'duet'), pompons (tubular ray florets; no disk florets visible), singles (daisy-type with 1-5 whorls of ray florets; visible disk florets), decoratives (outer ray florets longer than the center ones; disk florets hidden), spiders (ray florets are long and quilled, hooked, and drooping), Fuji (spider-like with shorter ray florets and less drooping), quills (tubular ray florets, not drooping), spoons (quill-like with the ray floret tips flattened like a spoon),

brush/thistle (very fine tubular ray florets), and hairy (hairs on the back of ray florets) (Ackerson, 1957; Bailey, 1914; Everett, 1980; Gortzig and Gillow, 1964; Kofranek, 1980; Langevin, 1992; National Chrysanthemum Society, 1996; Schillinger, 2000; Thistlewaite, 1960). Single versus double flowers is quantitatively inherited (Viehmeyer and Uhlinger, 1955).

Variations in the number of ray versus disc florets are due to the genetic and/or environmental variations, causing considerable differences in seed set potential. Each floret contains a single ovule, producing an achene at the termination of the seed ripening cycle (Anderson, et al., 1990). The variable number of disc and ray florets can significantly impact reproductive studies when seed set data is used to infer self incompatibility status (Anderson, et al., 1988). Researchers must incorporate ovule and seed counts per inflorescence to accurately reflect the impact of the incompatibility system. Excluding ovule counts can lead to inaccurate genetic inferences. Disc and ray florets constitute distinct ovule populations (Anderson, et al., 1988). In diploid *Chrysanthemum spp.,* ray floret numbers are relatively static when the flower type is a single daisy whereas the disc floret numbers are variable (Anderson, et al., 1988). As flower types were domesticated from single daisies to semi-doubles and, ultimately, to complete doubles, the variability shifted from the disc (completely absent in double forms) to ray florets.

5. PLANT HABIT

In addition to taxonomy, floricultural commerce and chrysanthemum societies have additional classifications for chrysanthemums (Boase, et al., 1997; National Chrysanthemum Society, 1996). As many as six plant habits constitute the various commercial greenhouse and garden chrysanthemum products, although some are culturally-derived forms and not under genetic control: specimens, upright (Figure 14-4a; standard, sprays), cushion (Figure 14-4b), charms, lilliputs, large shrubs (Figure 14-4c), and wave (Figure 14-4d). Specimens and most waves are trained to grow in specific forms (Boase, et al., 1997); the former are grown on upright frames for symmetrical growth (Allerton, 1949). Greenhouse chrysanthemums are divided into cut and potted plant types, all with upright (standard/spray) plant habits (Figure 14-4a). Cut flowers are grown upright with either the terminal as the sole flower (all subtending lateral buds removed, known as multiple bud removal) or only the lateral flowers (terminal flower bud in removed, known as single bud removal); standard and spray chrysanthemums are the terms used to denote the former and latter classes, respectively (Dole and Wilkins, 2004). Flowering potted chrysanthemums likewise have standard or spray types with one or multiple plants per container (based on the pot diameter) or may be cushions (Figure 14-4b; potted plants with small flowers), charms (compact plants with very small single flowers), or lilliputs

(dwarf plants with small double flowers) (Jones, 1958; Locke, 1990; Woolman, 1953).

Chrysanthemums grown in hanging baskets for display at public conservatories in the United States and the United Kingdom or for the annual October festivals in honor of 'kiku' (the Japanese character for chrysanthemum or the queen of autumn) in Japan are frequently grown as 'cascade' or 'bonsai' plant habits (Maisano, 1971). Both cascade (Figure 14-4e) and bonsai (Figure 14-4f) chrysanthemums, however, are created using genotypes with an upright plant habit which are 'trained' and manipulated to grow against gravity. First, the upright stems are trained to grow at a 45º angle on wires and then successively bent to create a cascading form during flower bud initiation and development. The cascade and bonsai phenotypes are the result of such cultural manipulations and, thus, have no genetic control for the plant habits.

Until the 1950s, most garden types had an upright plant habit with the flowers at the top of the plant only, due to their derivation from greenhouse types. An upright plant habit out-of-doors required staking to avoid lodging (Anderson, et al., 2001). The result of interspecific hybridizations with related wild species and use of an old cultivar 'Pink Cushion', produced a new garden phenotype in 1955, the 'cushion' plant habit, created by Dr. Richard Widmer at the University of Minnesota chrysanthemum breeding program (Anderson, et al., 2001; Viehmeyer and Whitney, 1955). A cushion habit (Figure 14-4b) infers that the plant is low-growing, forming a symmetrical hemisphere such that, at 100% flowering, the entire outer surface of the plant is covered with flowers and few foliage is visible (Viehmeyer and Whitney, 1955). The first garden chrysanthemum series displaying this new phenotype were the 'Minn' series with all color classes represented in the cultivars 'Minnbronze', 'Minngopher' (U.S. Plant Patent No. 4,327), 'Minnqueen', 'Minnruby', 'Minnwhite', and 'Minnyellow'. Within a few years, both private and public sector breeding programs had incorporated the 'Minn' series cushion habit and were releasing cultivars with this new phenotype. Currently, the cushion habit has the majority market share (Anderson, et al., 2001).

Large shrub chrysanthemums, *D. x hybridum,* with the cushion habit have also been created by interspecific hybridization of *D. weyrichii x* upright *[D. x grandiflora]* (Ascher, et al., 1997). These genotypes reach their maximal plant dimensions in the second year, frequently 1 m high x 1-2 m in width, and are extremely winter-hardy (Figure 14-4c). Several cultivars with this plant habit have been released onto the market under the series names Maxi-Mum™ and My Favorite™.

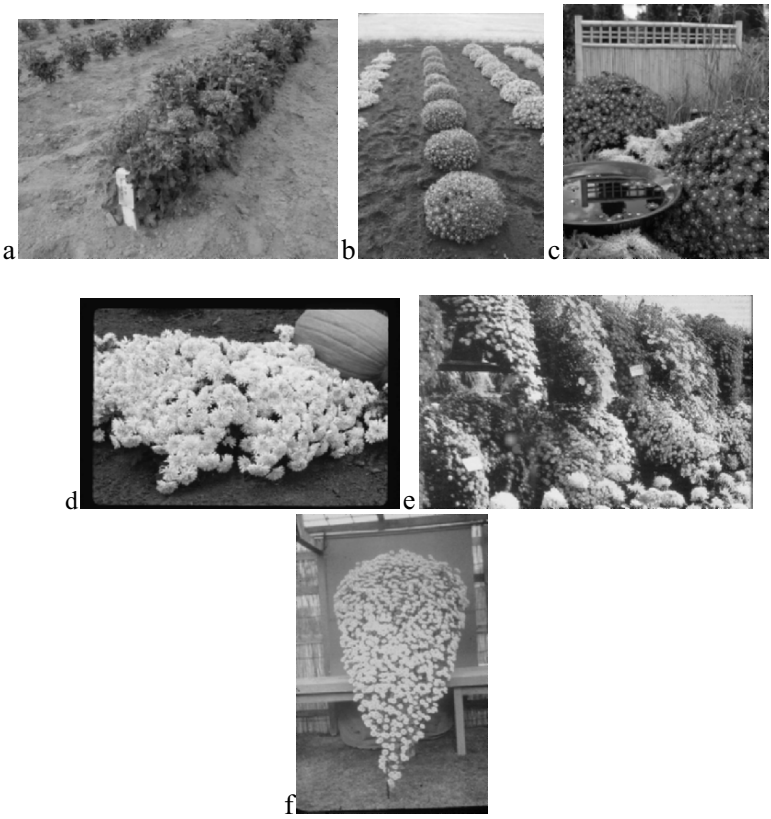

Figure 14-4. Example plant habits bred into commercial product classes of greenhouse and garden chrysanthemums include upright (a), cushion (b), large shrub (c), wave (d), cascade (e) or bonsai (f).

The wave (also known as cascades or prostrate) plant habit is similar in appearance to the cascade, culturally-manipulated phenotype, but differs by being genetically controlled (Chen, et al., 1995). A wave habit is characterized by horizontal growth of the terminal and lateral branches, followed by flowers growing upright (Figure 14-4d). The horizontally-growing branches do not root into the ground, leaving the central crown to overwinter. Interspecific hybrids derived from crossing prostrate *D. weyrichii x* upright *[D. x grandiflora]* produced primarily cushion progeny with large plant diameters and height (Ascher, et al., 1997). Any derived-wave or prostrate progeny often had an obligate vernalization requirement before flowering. Several chrysanthemum breeding programs have this plant habit as a breeding objective (Ascher, et al., 1997; Chen, et al., 1995).

6. FLOWER COLOR

For the majority of flower types, floral pigmentation is located in the ray florets; the notable exception is anemone flowers (Figure 14-3f) with pigmented disc and ray florets. Upper and lower epidermal layers (L1) and the internal cell meristematic layer (L2) are the locations for floral pigments (Langton, 1976; 1980). Two classes of pigments, plastids (carotenoids) and sap-solubles (anthocyanins, anthoxanthins), coupled with cellular pH, copigments, and other compounds, combine to give the pattern and tone of flower colors (Fleming, 1929). Flavonoid pigments, particularly anthocyanins and anthoxanthins, are located only in the cell vacuoles of the L1 layer (Langton, 1980; Stewart and Derman, 1970). Anthocyanins range in color from salmon and scarlet through red and purple to blue. Anthoxanthins (flavones, flavonols) produce shades of pale ivory to deep yellow (Scott-Moncrieff, 1936). Plastid pigments, present in tiny plastid bodies primarily in the L2 cell layers (chromoplasts), range from yellows to oranges due to xanthophylls and carotenoids (carotenes, carotenols) (Kawase and Tsukamoto, 1976). All pigment types may be present as the sole pigment source or produced in concert (Crane and Lawrence, 1952).

High night temperatures can affect pigment production, expression, and the resulting flower coloration (Kosaka, 1932; Rutland, 1968). Studies on 'Fandago' greenhouse chrysanthemum, normally having purple flowers, showed that florets grown at 15 C (nights) were red, at 30 C the color was bright yellow, and pale yellow at 6 C (Stickland, 1974). Particularly in garden chrysanthemums, breeding programs will select against 'pinking' or 'purpling' in seedlings to prevent release of white/cream, yellow, or bronze types which develop anthocyanin pigmentation. An additional trait selected against is fading in bronzes or reds under warm night temperatures ($\geq$30 C). Most anthocyanin compounds may be produced at higher temperatures, but the sugar moieties will not be added on to the molecule as the carbohydrates are required for increased respiration (Post, 1950). Both anthocyanin and carotenoid production are severely hampered or may cease altogether at $\geq$30 C.

The fundamental flower color classes of chrysanthemum consist of (1) white or cream, (2) lavender to purple, pink, (3) bronze, red, orange, and (4) yellow (Figure 14-5) (Anderson, et al., 1988; Hattori and Futsuhara, 1970; Kawase and Tsukamoto, 1974; Miyake and Imai, 1935). White flowers possess flavonols, but may also have other precursors to anthocyanins, which can develop under cool night conditions. Cream colored flowers typically contain anthoxanthins. Lavender, purple, and pink flowers contain anthocyanins and flavonols, differing either with the pigment base compound, the addition of sugars, and/or cell pH differences. Bronze and red flowers contain predominantly both a background carotenoid or xanthophyll pigment with anthocyanin(s) and flavonols superimposed in the epidermal layers to varying degrees. Yellows usually consist of pure carotenoid or xanthophyll compounds in the L1 apical layer, but not in the L2, but may also have cream-

colored anthoxanthins (Langton, 1980). Yellows are homozygous recessive for both the water soluble and plastid pigment genes which means that they will never sport to a color other than yellow (Teynor, et al., 1989a). In most instances, inbred and non-inbred genotypes within each flower color phenotypic class do not exhibit qualitative pigment differences (Anderson, et al., 1988).

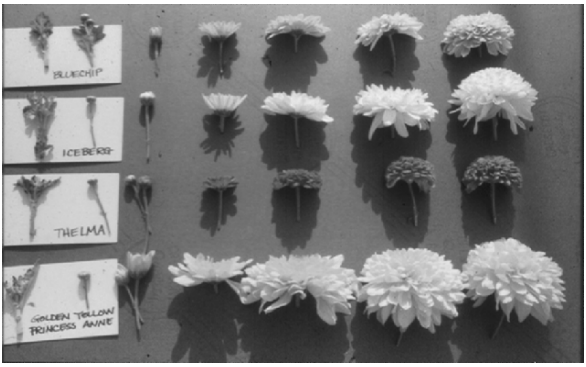

Figure 14-5. The four major flower color classes (white/cream, lavender/purple, red/bronze, and yellow) of cultivated greenhouse and garden chrysanthemums (Anderson, 1985).

Anthocyanin (anthocyanidin) pigments identified in *D. x grandiflora* are chrysanthemin, its derivatives, and cyanin (cyanidin), responsible for pink/purple, orange and red flower colors (Anderson, et al., 1988; Kawase, et al., 1970; Kawase and Tsukamoto, 1976). One flavone and two phenylpropanoids also exist in these color groups (Anderson, et al., 1988). White-flowered cultivars possessed four phenylpropanoids, two of which were caffeic and ferulic acids (Anderson, et al., 1988). Eight carotenoid pigments (including lutein) have been reported in yellow and bronze-red-orange color groups; five of these carotenoids varied quantitatively (Anderson, et al., 1988). Two genes for flower color have been ascribed to *D. sinense, C* and *Y* (Miyake and Imai, 1935). *Chrysanthemum carinatum* has an "anthocyanic-xantheic-xanthin series" in which anthocyanic pigments were found in magentas and crimsons, xanthin in pale yellows, while yellow contained xantheic compounds (Wheldale, 1909). The same four fundamental flower color classes are present in this species as in the cultivated greenhouse and garden *D. x grandiflora*.

Anthocyanin inheritance in orange-flowered 'Vulcan' and its orange progeny may be attributed to a single dominant gene responsible for transposable element expression of red pigments overlaid on carotenoids (Teynor, et al., 1989b). Carotenoid pigment inheritance best fit a disomic inheritance model with one dominant gene, *I-* (inhibitor locus) (Teynor, et al., 1989a). Yellow-flowered genotypes with carotenoid pigments are denoted as *ii*. One family exhibited an excess of carotenoid progeny (deviating from the expected 1:1 ratio) in three

different environments. Segregating progeny from one family deviated from the 1:1 expected ratio with an excess of white (non-carotenoid) genotypes when grown under glasshouse conditions. The opposite (an excess of carotenoid-expressing genotypes) occurred with the identical cloned genotypes under field conditions; this also has been reported with anthocyanin inheritance studies (Teynor, et al., 1989b). Anderson et al. (1988) and Anderson (1985) also reported similar environmental variation in pigmentation for clones. Segregating progenies need to be classified in more than one environment (Teynor, et al., 1989a).

Recent data in the University of Minnesota breeding program have reconfirmed the existence of specific hybrid progeny with an excess of *ii* or *I-* classes, possibly due to a lethal gene tightly-linked to *ii* (Anderson, unpublished data). Teynor et al. (1989a) proposed that such a lethal would convert the expected 1:1 to a 2:1 ratio. Clonal cultivars which have been asexually propagated for numerous generations, e.g. 'Puritan' (Teynor, et al., 1989a), may accumulate recessive alleles at the lethality locus(-i) linked with *ii* on the chromosomes.

7. REPRODUCTIVE BIOLOGY

Chrysanthemums reproduce sexually as outcrossing species and asexually via rhizomes (emergent, non-emergent) in perennial species in the wild. In commercial production they are asexually propagated as terminal stem cuttings (*in vitro, ex vitro*), emergent/non-emergent rhizomes, and division (Anderson, 2004; Dole and Wilkins, 2004; Kofranek, 1980). Self incompatibility (SI) enforces outcrossing and was first reported in cultivated chrysanthemums in 1931 (Niwa, 1931; Mulford, 1937b; Crook, 1942). Self incompatibility occurs at all ploidy levels in species of the chrysanthemum complex: diploid (*Dendranthema boreale*), hexaploid (*D. japonense*), octaploid (*D. ornatum*), and decaploid (*D. shiwogiku*) all possess an active SI system (Table 14-2; Tanaka, 1952). Pyrethrum, *Tanacetum cinerariifolium*, an economically important species (Tables 14-1, 14-2), is also SI (Thorpe, 1940). Genetic analysis of cultivated chrysanthemums revealed the lack of pollen (trinucleate) germination or stigmatic inhibition of pollen tubes and reciprocal crossing differences, indicating the existence of a sporophytic SI system (Drewlow, et al., 1973). There are at least three epistatic *S* loci controlling SI expression (Stephens, et al., 1984; Zagorski, et al., 1983).

Table 14-2. Morphological traits, ploidy levels (2*n*=2*x*=18 to 2*n*=22*x*=198), and crossability of important chrysanthemum species useful for genetic improvement of cultivated *Dendranthema x grandiflora*.

Species	Morphological traits	Ploidy	Crossability with *D. x grandiflora*	References
Chrysanthemum coronarium		*2x*		Gaiser, 1930
C. segetum		*2x*		Gaiser, 1930
Dendranthema alpinum		*4x*		Gaiser, 1930
D. arcticum	Dwarf, winter hardiness	*10x*	+ but no success in transferring hardiness	Gaiser, 1930
D. boreale		*2x*	SI	Watanabe, et al., 1972
D. decaisneanum		*8x*		Shimotomai, 1931
D. x hybridum	large shrub plant habits	*6x*	+ SI or PSC	Ascher, et al., 1997
D. indicum	mini yellow flowers, sprays, early flowering	*4x, 6x*	+	Gaiser, 1930; Taniguchi, 1987
D. japonense		*6x*		Shimotomai, 1934b
D. j. var. octoploid		*8x*	SI	Watanabe, et al., 1972
D. j. var. crassum		*_10x*		Watanabe, et al., 1972
D. japonicum		*4x*		Gaiser, 1930
D. koreanum (=D. coreanum)			+	Cumming, 1939
D. marginatum		*10x*	+ highly fertile hybrids	Emsweller, et al., 1937; Shimotomai, 1931, 1932a
D. makinoi		*2x*	+	Shimotomai, 1934a
D. nipponicum	shrub-like, woody stems, oblong lvs.	*2x*	+ crosses readily	Gaiser, 1930
D. ornatum		*8x*	SI	Shimotomai, 1934b
D. pacificum	dwarf, hairy leaves, apetalous, transposable elements	*10x*		Shimotomai, 1934b; Shimizu, et al., 1998
D. x rubellum 'Clara Curtis'		*8x+1=73*	+	Dowrick, 1952b, 1953
D. shiwogiku		*8x, 10x*	SI	Kawata and Toyoda, 1982
D. sibiricum		*6x*		Shimotomai, 1934b
D. uliginosum	giant daisy,	*2x*		Cumming, 1939

Species	Morphological traits	Ploidy	Crossability with *D. x grandiflora*	References
	plants to 2m tall			
D. wakasaense		*4x*		Dowrick, 1952b, 1953
D. weyrichii	winter hardiness, prostrate plant habit	*6x, 8x*	+ used to create shrub forms	Shimotomai, 1932b
D. yezoense		*10x*		Dowrick, 1952b, 1953
D. yoshinaganthum		*4x*		Kawata and Toyoda, 1982
D. zawadskii		*4x, 6x*		Lee, 1967, 1975
Leucanthemum lacustre	perennial	*22x*		Dowrick, 1952b
L. maximum	large white flowers, waxy leaves	*8x*	~ little; use as male parent	Shimotomai 1934b
Tanacetum cinerariifolium	'painted' daisy flowers, insect resistance	*2x*	+ as male; recessive cytoplasm	Cumming, 1939
T. coccineum	'painted' daisy	*8x*		Magulaev, 1992

Both garden and greenhouse chrysanthemums are highly SI, rarely producing seeds after selfing or outcrossing between genotypes sharing *S* alleles (Anderson and Ascher, 2000; Ronald and Ascher, 1975a). Lack of seed set has plagued chrysanthemum breeders as more species were incorporated into the *D. x grandiflora* genome (Smith, 1913). Emsweller et al. (1937) lamented that one of the greatest challenges to chrysanthemum breeders is the lack of seed set, either due to lack of fertility, inbreeding depression, or SI. Random outcross pollinations among unrelated, noninbred genotypes produce low seed set, ranging from 36% to 71% in one study (Ronald, 1974) and even lower in another—24.5% - 38.5% (Anderson and Ascher, 2000); most seed set is <50%. The primary reason for such low seed set is SI, although inbreeding depression and genetic load also operate in this polyploid crop (Anderson, et al., 1992a, b).

Teynor et al. (1989a, b) found that greenhouse cultivars, which are frequently double (gynoecious florets only) and propagated for multiple asexual generations, were highly sterile. Parental selection for high general combining ability is important to maximize seed set (Mulford, 1937a). It is possible to select for inbred parents with high fertility levels (Anderson and Ascher, 2000).

Pseudo-self compatibility (PSC) exists in garden chrysanthemums, although at a very low frequency (Ronald and Ascher, 1975a). However, end-of-season PSC does not exist (Anderson and Ascher, 1996), although heat treatment can increase self seed set (Ronald and Ascher, 1975a). SI expression and the related temperatures to overcome it are genotype-specific (Ronald and Ascher, 1975a). Drewlow, et al.

(1973) reported that inbreeding decreased the number of heterozygous *S* loci. Zagorski, et al. (1983) found some inbred parents that set higher self than outcross seed set. Greenhouse chrysanthemums rarely express PSC, although F_1 hybrid progenies from crossing greenhouse (SI) x garden (PSC) cultivars segregated with a 1:1 (PSC:SI) ratio (Ronald and Ascher, 1975b), suggesting PSC could be transferred from garden to greenhouse types (Ronald and Ascher, 1975c).

PSC garden chrysanthemums produced progenies with both SI and PSC individuals following self pollination or when crossed with SI plants (Ronald and Ascher, 1975a). Anderson and Ascher (1996) found that inbred parents had a wide range in %PSC (calculated as [mean self seed set / mean outcross seed set] x 100), ranging from 0 to 68.8%, while recombinant inbreds had wider variation (0.2-99.7%), compared with non-inbreds (0.6-25.7%) which had not been previously selected for PSC. Most parents had low PSC expression, with high PSC levels being the least common (Anderson and Ascher, 1996). PSC distributions within inbred populations were primarily continuous in distribution and quantitatively inherited. High PSC levels were not highly heritable; realized heritability (H_R) ranged from H_R =0.05% to H_R =10.19% (Anderson and Ascher, 1996). Since all low PSC x low PSC crosses and self pollinations of low PSC parents produced 43-50% of the inbred progeny with higher PSC levels, many low PSC parents possess unexpressed PSC genes. A PSC threshold with additive gene action operates when low – mid PSC selection occurs, but as soon as high PSC levels are obtained, non-additive gene action is operating (Anderson and Ascher, 1996).

8. CYTOLOGY

Dendranthema indicum, one of the oldest wild species native to Japan, exhibits karyomorphological diversity in morphology and geographical variance for the number of satellites and C-band variation (Taniguchi, 1987). Shimotomai (1934) found that Japanese species populations with higher ploidy levels were typically distributed near coastal areas whereas those with lower ploidy were inland.

Wolff and Peters-van Rijn (1993) examined genetic variation of 15 genotypes in 13 chrysanthemum species using six RAPD primers. They found high levels of genetic variation among species. Several RAPD primers produced different banding patterns in each species, with some RAPD bands being diagnostic for a species. The mean similarity among species was S=0.49, significantly lower than cultivar similarity within cultivated chrysanthemums (Wolff and Peters-van Rijn, 1993). They did not find any relationship between ploidy levels and the corresponding number of RAPD fragments generated.

Bleier (1934) reported that, in general, interspecific hybrids predominantly formed bivalents, although univalents have been observed. In extreme cases, where the parental ploidy levels differed widely, trivalent and quadrivalent formation also

occurred. Aneuploidy and euploidy causes irregular inheritance patterns and segregation ratios differing widely from expectations (Sansome and Philp, 1932). The occurrence of aneuploidy and euploidy in interspecific crosses between chrysanthemum species indicates that hexaploid *D. x grandiflora* would be expected to segregate, at least occasionally, from expected diploid ratios.

In most polyploid chrysanthemum species (ranging from 2*x* to 22*x,* Table 14-2), preferential pairing or a "5B type gene system" (preventing homoeologous pairing in hexaploid wheat) are the strategies whereby a stable, essentially diploid meiotic process occurs for the establishment of a sexually self-maintaining polyploid (Dowrick and El-Bayoumi, 1969; Watanabe, 1977a, b). Multivalent formation may be prevented in chrysanthemums by restriction of pairing initiation to a single site per chromosome. This multivalent suppressor system may have evolved through a gradual reduction in the number of zygomeres and the differentiation in their homology recognition and regulation systems. Genetic stabilization of diploid-like meiosis occurs in all polyploid chrysanthemums (Watanabe, 1977a). Bivalent formation is the norm and multivalents are rare.

Interspecific hybridization between wild species with differing ploidy levels, as a general rule, produce true-breeding (non-segregating) hybrids (Table 14-2). For example, *D. japonense (2n=6x=54) x D. pacificum (2n=10x=90)* produces octaploid hybrids (*2n=8x=72*); hybrids from the cross *D. makinoi* (*2n=2x=18) x D. decaisneanum (2n=8x=72)* were also all octaploid, while *D. makinoi* (*2n=2x=18) x D. japonense (2n=6x=54)* hybrids were septaploid (*2n=7x=63)* (Shimotomai, 1934).

Greenhouse and garden chrysanthemums, *D. x grandiflora,* have somatic chromosome numbers which vary from the expected *2n=6x=54* for this allohexaploid. As many as n=125 aneuploid genotypes have been documented to range from *2n=6x=47-63* (Dowrick, 1953; Shimotomai, 1934). There is a direct relationship between increasing chromosome number and inflorescence diameter (Dowrick, 1953). Fertile hybrids have also been obtained by crossing wild species with *D. x grandiflora*: *D. marginatum (2n=10x=90) x [D. x grandiflora] (2n=6x=54)* created fertile hybrids (*2n=16x=144*) (Table 14-2; Emsweller et al., 1937).

8.1 Sports

At least one third of the commercial cultivars on the market are 'sports' arising from mutation (Wasscher, 1956). Many sports arise from spontaneous mutations, due to background irradiation, while others are artificially induced with X rays (1000r total; 120 KV, 5 mA at a dose rate of 120r/min.) and γ rays (1 K rad total from Co^{60} at a dose rate of 350 rad/min.) exposure, as well as chemical mutagens (Dowrick and El-Bayoumi, 1966). Higher irradiation dosage rates from either

ionizing radiation source are lethal to all tissues. Mitotic or meiotic division errors account for numerous sports which commonly arise in any type of asexual propagule (cuttings, division, tissue culture) (Dowrick and El-Bayoumi, 1966). Sports may be due to point mutations, inversions, deletions, or the gain/loss of chromosomes. Several researchers noted that mutations affecting morphology (particularly flower coloration) coincided with cytological differences; higher chromosome numbers also convey larger flower sizes (Dowrick and El-Bayoumi, 1966; Sampson, et al., 1958). Earlier reports found a similar correlation (Morton, 1891).

Bud sports (chimeras) are common sources of new mutations; often a subtending lateral branch will mutate into a flower color that differs from the original clone (Dowrick and El-Bayoumi, 1966). Mitotic cell division errors (chromosome non-disjunction, lagging and sticky chromosomes ends at anaphase) are highly likely to produce mericlinal chimeras; lateral branches (the result of many cell layers) arising within a mericlinal chimera may have one (mericlinal) or both (periclinal) cell types (Dowrick and El-Bayoumi, 1966). Most chrysanthemum mutants are periclinal chimeras. Sectorial chimeras, mutant flower petals occurring in pie-shaped wedges within a single flower cannot be directly propagated asexually via cuttings but must first be tissue cultured. Root tip chromosome counts do not accurately reflect the chimera cytology. Tissue culturing mutated ray florets can be accomplished with Murashige and Skoog (MS) basal medium plus 0.5 mg/L 1-naphthaleneacetic acid (NAA) and 1 mg/L 6-benzylaminopurine (BAP) (Datta, et al., 2001). Shoot organogenesis begins within 2 wks followed by transfer to rooting medium and transfer out of culture for evaluation.

As early as 1918, ~400 cultivars had originated by sporting (Shamel, 1918). Another noted effect of mutation included striping (‘Queen of England’ sported from red to striped) (Crook, 1942). Emsweller et al. (1937) observed that mutation breeding was an important tool in the development of new cultivars. Crook (1942) postulated that mutation rates were higher in some genotypes than others. Selected mutants must be tested for stability through asexual propagation and production cycles, prior to market release, to ensure that the mutant does not revert to the original clone (Yoder Brothers, Inc., 1967). If they are stable sports, each new mutant genotype can be forced under the exact production protocols for the original clone. Thus, a mutant family or series arises with each new mutant possessing the name of the original clone with a color added on and may differ in chromosome counts, e.g. the Fred Shoesmith Family consists of numerous mutants derived from ‘Fred Shoesmith’ ($2n$=54-58) such as ‘Apricot Fred Shoesmith’ ($2n$=54-58), ‘Yellow Fred Shoesmith’ ($2n$=57-58), or ‘Golden Fred Shoesmith’ ($2n$= 56-58) (Dowrick and El-Bayoumi, 1966). Clones within a mutation family often have a wide range in ploidy (Dowrick, 1953, 1958).

Mitotic division errors (spontaneous mutations) in clonally maintained cultivars were found to be inducible by high (23 C) or low (3.5 C) temperatures, causing a 0-2.3% abnormality at anaphase due to nondisjunction, lagging, or stickiness of

chromosomes (Dowrick, 1953, 1958; Walker, 1955; Dowrick and El-Bayoumi, 1966). These chromosome abnormalities occur primarily in clonal cultivars several asexual generations removed from sexual cycles. In addition, meiotic errors causing unreduced gametes have been reported in a closely related species, *D. atratum* (Dowrick, 1952a).

Yellow flower color, being recessive for both anthocyanin and carotenoid pigment genes, will not produce any new flower color sports. All other flower colors (red, bronze, purple, white, and cream) will produce varying types of new color mutants. Typically any breeding program will endeavor to create and own the rights to as many sports as possible, prior to the release of a new cultivar onto the market. Virtually all commercial, asexually-propagated sports or new seedlings are protected by plant patents (United States) or plant breeder rights (PBR; in Canada, Europe, Africa, Japan, Australia, and New Zealand), prior to release into the commercial market (Vandenberg, 2004).

Molecular markers are useful for cultivar identification (genetic fingerprinting in patent or PBR infringement litigation), as well as identifying genetic variation within a sport family. Wolff and Peters-van Rijn (1993) used random amplified polymorphic DNAs (RAPDs) in a sport family (n=13 genotypes using n=27 primers) and found that the sport family derived from a single cultivar all had identical fragment patterns. In another study, Wolff, et al. (1995) reported that a sport family had the same DNA fragment patterns with RAPDs, ISSRs (inter-simple sequence repeats), or RFLPs (restriction fragment length polymorphisms) techniques. However, when DNA extractions of various cells layers (L1 from epidermal peels, L1 & L2 from florets, L1– L3 from leaves) were analyzed, polymorphisms between cultivars within a sport family could be found (Wolff, 1996).

9. GENETICS & BREEDING

Several gene designations have been published for the species, although none have been mapped to chromosomes. *Y* (yellow plastids) and *C* (colored anthocyanins) were the first genes proposed (Miyake and Imai, 1935). White (*ccY-*), magenta (*C-Y-*), orange-red (*C-yy*), and yellow (*ccyy*) flowers result from interactions between these two genes. *F-* (anthocyanin factor) was introduced as a component of several partially allelic anthocyanin genes, including *C* (Reimann-Philipp and Jordan, 1978). Later, Jordan and Reimann-Philipp (1983) proposed a dominant gene *I-* (inhibitor) for elimination of carotenoid production in either the L_1 or L_2 cell layers of chrysanthemum flowers. *I,* in conjunction with an anthocyanin production gene, *A,* would account for flower color. The literature is unclear whether the first- and latter-named flower color genes are, in fact, different. It also remains controversial whether the inheritance of flower color and other traits is

under disomic, tetrasomic, or hexasomic control (Reimann-Philipp and Jordan, 1978; Teynor, et al., 1989a).

Genes controlling traits other than floral characteristics include *S* for strong growth habit (Reimann-Philipp and Jordan, 1978) and *Ph* for resistance to *Puccinia horiana* (de Jong and Rademaker, 1986). It should be noted, however, that *S* is also the universal gene designation for the self incompatibility locus, which exist in chrysanthemums (Ascher, 1976). Diploid plants taller than 50 cm possess the dominant allelic form for strong growth habit (*S*-), while those <50 cm are *ss.* The *Ph* gene acts similarly, with *Ph*- genotypes being resistant and *phph* diploids being susceptible to *P. horiana.* Numerous genes have been proposed for flower size, type, and petal orientation and are discussed subsequently (see Floral Traits section; Crook, 1942). The lack of genetic information for other economically important traits provides an open field for future research.

The existence of transposable elements has been reported in two hexaploid species of chrysanthemums, *Dendranthema x grandiflora* and *D. pacificum* (Anderson and Ascher, 2004; Shimizu, et al., 1998; Teynor, et al., 1989b). Embryo-rescued seedling cotyledons expressed transposable elements with epidermal cells producing anthocyanin pigment(s) (Anderson and Ascher, 2004). Polymerase chain reactions (PCRs) demonstrated that wild *D. pacificum* genotypes possessed conserved sequences of the reverse transcriptase domain of *Ty-1-copia* group with the 'AFLNG' motif derived from *copia* of *Drosophila* (Shimizu, et al., 1998). None of the retrotransposon sequences in three clones were identical; one clone closely resembled retrotransposons in rice.

Due to recent escalations in heating costs for greenhouse production, breeding programs have been striving to decrease the short day response period. Effort has also been devoted to the development of seed-propagated cultivars to minimize the costs unique to vegetative propagation, i.e. stock plant maintenance of disease-free material, etc. (Strefeler, et al., 1996; Meynet, 1978). In the past decade, the University of Minnesota garden chrysanthemum breeding program has been pursuing studies on the reproductive biology and creation of acceptable, uniform seed-propagated cultivars by developing inbred lines homozygous for important phenotypic traits (Anderson, et al., 1995; Anderson, 2004). Whether seed-propagated hybrids will replace a portion or all of the vegetative market has not been determined.

9.1 Rapid Generation Cycling

Most hybridizing in private and public sector breeding programs has been conducted in greenhouse conditions. Viehmeyer and Uhlinger (1955) developed a 'water culture' method to accommodate situations of limited greenhouse space. This technique was accidentally discovered after cut flower stems for use as pollen sources were allowed to senesce in a vase and produced seed. In 1951, the water

culture method was re-tested with cut stems placed in jars of water, placed in lab conditions (ambient temperatures and light conditions), and proved successful in seed production. Subsequent trials demonstrated that seed could be ripened in darkness, seed viability and % seed set were equal to *in situ* ripening, and seed maturation occurred 2-4 wks. earlier (Viehmeyer and Uhlinger, 1955).

Inbred line development has been compounded by SI and a long generation time of 6-8 months (minimum). Embryo rescue is a technique that has improved seed germination, prevented the loss of embryos resulting from wide intra- and inter-specific crosses, and reduced generation times (Anderson, et al., 1990; Watanabe, 1977b), since *in situ* seed-ripening procedures are 1-2 months in duration (Scott, 1957). Before tissue culture media components had been developed for embryo rescue, attempts at rescuing crosses of cultivated chrysanthemums frequently were unsuccessful (Fan, 1965). Recently, however, intra- and inter-specific crosses have been embryo rescued with success (Kaneko, 1957; Watanabe, 1977a).

Figure 14-6. Laboratory seed ripening of pollinated inflorescences suspended (floating) in a floral preservative solution to facilitate ease of pollination, maximize seed set, and hasten embryogenesis (Anderson, et al., 1990).

Cut flower preservatives may be used as nutritive and bactericide sources in ripening seeds *ex situ*. Anderson, et al. (1990) successfully used 200 ppm 8-HQC (8-hydroxy-quinoline citrate) plus 1% sucrose at 150 μmol m^{-2} sec^{-1} (16 hr photoperiod supplied by cool white fluorescent lamps) and 29°C (Figure 14-6) to open chrysanthemum flower buds 50-60 mm dia. in size (Marousky, 1971) and ripen seed, a technique termed laboratory seed ripening. Since 8-HQC is no longer commercially available, Anderson's lab uses commercial floral preservatives (prepared at the recommended rate) as substitutes. This, coupled with embryo rescue at the heart stage as early as 2 d. post-pollination, allowed for an average generation time of ~100 d (compared with 200-550 d *in situ*). Embryogenesis occurred significantly faster with laboratory seed ripening than *in situ* ripening (Figure 14-7). Embryo rescue proceeds with seed coat removal and placement of embryos on MS medium with 1% sucrose and no plant growth regulators

(Anderson, et al., 1990). Culture vessels are placed in the dark for germination, followed by a 16 hr photoperiod (92 μmol m^{-2} sec^{-1}) at room temperature (29°C constant). The combination of laboratory seed ripening and embryo rescue techniques are both used in rapid generation cycling to enhance seed production by reducing source/sink interactions, promote rapid embryo germination, and significantly decrease generation times.

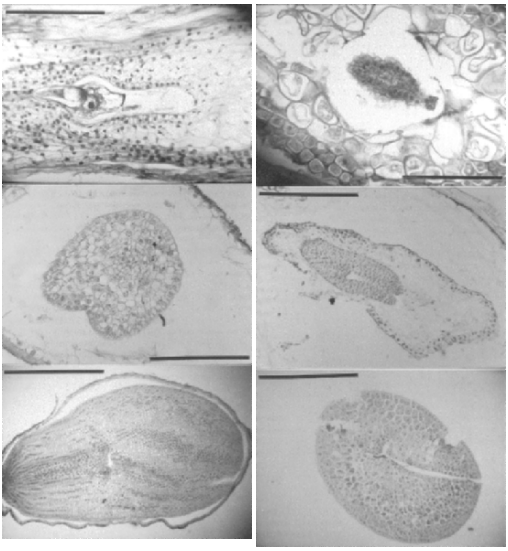

Figure 14 -7. Embryogenesis stages of embryo-rescued seedlings using the rapid generation cycling technique (Anderson, et al., 1990). Embryo sac (top left) with an unfertilized egg cell (arow) at day 0; (top right) globular embryo at day 1 (arrow denotes hypophysis); (center left) heart stage at day 2; (center right) torpedo on day 4; (bottom left) cotyledonary at day 6 with an apical dome defined and vascular traces appearing in the cotyledons, and (bottom right) a mature seed occurring at day 25. Bars = 0.1 μm.

9.2 Inbreeding, Inbreeding Depression, & Genetic Load

Marshall (1973) proposed that, for cultivated allohexaploid chrysanthemums, inbreeding may be relatively ineffective due to fixed heterozygosity. If this were the case, chrysanthemums would follow the pattern of other polyploid species (with disomic inheritance) that have fixed heterozygosity in the multiple genomes, e.g. homosporous ferns (Haufler and Soltis, 1986), *Equisetum* (Soltis, 1986), *Triticum, Avena, Nicotiana,* and *Gossypium* (Bingham, 1979). Inferences about the possible occurrence of inbreeding depression in polyploid chrysanthemum species appear in experiments designed to analyze the inheritance of other traits. In the first report to

document SI, wild and cultivated chrysanthemum species were selfed to produce n=1-3 inbred generations in five years (Niwa, 1931). Selfing resulted in decreased height, seed set, germination, and flowering, when compared to outcross pollinations. This led to the initial conclusion that these species were SI and that inbreeding depression could exist. Subsequent studies (Mulford, 1937b; Tsukamoto, et al., 1964; Kawase and Tsukamoto, 1966) also compared the performance of F_1 and I_1 (first inbred generation) progeny for fertility or flower characters. While there was a general reduction in the average I_1 performance, the standard errors were large enough to encompass the ranges observed for the F_1.

The use of rapid generation cycling techniques (Figure 6) accelerated the rate of progress in chrysanthemum breeding programs and inbreeding by decreasing the generation time (Anderson, et al., 1990). In one report, it was used to further inbred line development with eight generations of inbreeding (Anderson and Ascher, 2000). Use of these techniques allowed for the creation and evaluation of three inbred generations in <1 year using multiple plant descent. Sixty-six non-inbred or inbred parents selected for PSC were used to create 1-3 inbred generations, depending on the level(s) of inbreeding depression. By the end of the second inbred generation, all noninbred parent-derived populations were extinct due to SI or inbreeding depression (Anderson, et al., 1992a, b). Inbreeding level (generation) on seed germination and survivorship (yield potential) were negatively correlated and highly significant. Seed germination and yield potential were highly correlated, which suggests that lethality due to inbreeding is not independent between life cycle stages. High levels of inbreeding (F=0.995) did not eliminate the expression of inbreeding depression, which was attributable to both dominance and epistasis (Anderson, et al., 1992a). Fertility (seed set, pollen stainability) was also negatively affected by continued inbreeding (Anderson and Ascher, 2000). Anderson, et al. (1993) demonstrated that the best means of circumventing significant inbreeding depression in early and/or advanced inbred generations was by using recombinant inbreeding, whereby inbreeding and outcrossing is juxtaposed every 2-3 generations (Bailey, 1971; Campbell, 1988).

9.3 Hybrid Seed Production

Public and private sector breeding programs have historically emphasized continued development and release of asexually propagated cultivars. The reasons for this are varied, including the ease of obtaining natural or irradiation-induced sport families which are readily adaptable to current production protocols, long generation time, SI, sterilities associated with clonal cultivars, source/sink interactions in herbaceous perennials, and polyploidy (Anderson, et al., 1988). Thus, less attention has been given to investigating the potential alternative of hybrid seed production. While asexual propagation provides homogeneity, it has the distinct disadvantages of virus buildup in stock plants and the high cost of plant

material (production, shipping, etc.), particularly if it is certified virus-free (Langton, 1987). Hybrid seed is predominantly virus-free and comparatively cheaper than cuttings to produce.

Hybrid seed cultivars are not new to the floricultural crop arena. They have been primarily applied to annuals (e.g. *Ageratum, Impatiens, Petunia, Tagetes*) and less commonly to biennials or perennials (e.g. *Begonia x tuberhybrida, Cyclamen, Freesia, Streptocarpus)* (Wellensiek, 1959; Sparnaaij, 1968; Schmidt and Erickson, 1981; Reimann-Philipp, 1983). In recent decades, renewed interest has arisen among flower breeders to develop hybrids in crops that have been asexually propagated, e.g. *Pelargonium x hortorum* (Craig, 1976; White and Quatchak, 1985), *P. peltatum* (Langton, 1987), and *Gerbera jamesonii* (Meynet, 1978). Such has also been the case for chrysanthemum (Satory, 1986). This trend is likely to continue, as releases of seed-propagated flower crops escalates (Anderson, 2004; Anderson, et al., 1995).

Breeding programs aimed at hybrid seed production can face formidable problems: SI, new incompatibility specificities, PSC, ploidy, and severe inbreeding depression (Frankel and Galun, 1977; Langton, 1987). While chrysanthemums possess all of these attributes, such barriers have not been insurmountable in other polyploid crops commercially available as hybrids. In other species, the problems are not as severe and it has been postulated that *Kalanchoe* may also join the ranks as a as seed-propagated floriculture crop (Royle, 1982).

Both the private (T. Sakata Seed Company, Yokohama, Japan; Bodgers Seeds., Gilroy, California, USA) and public (Hiroshima and Kyoto Universities, Japan; University of Minnesota, USA; Federal Research Center, Ahrensburg, Germany) sector breeding programs (past and present) have initiated the research and development of hybrid chrysanthemum seed production (Anderson and Ascher, 1996, 2000). Initial studies concentrated on characterizing the SI system and creation of homozygous inbred lines. Use of techniques, such as rapid generation cycling, enabled faster derivation of nearly homozygous inbreds.

The private sector has released several F_1 and F_2 cultivars, e.g. 'Autumn Glory', 'Petit Point', 'Korean Eriso', 'Super Jet', 'Korean Jewel', 'Golden Dream', and 'Fanfare' (Anderson, et al., 1988, 1995; Park Seed Co., 1985), although none are grown extensively or on a commercial basis. Standard comparison trials of these hybrids by the University of Minnesota breeding program (45ºN lat.) demonstrated that these seed lines lacked uniformity for important phenotypic traits, particularly flower color, quality, and shape; plant habit; flowering time (Figure 14-8a; Anderson, et al., 1988, 1995). These hybrids, presumably produced from crossing inbred parents, lack uniformity for phenotypically important traits (flower color and type, flowering earliness, and plant habit) and environmental stability (flowering time).

Realizing the potential contribution F_1 hybrid seed-propagated cultivars could have for commercial production, the University of Minnesota breeding program has

devoted 15-20 years on this objective (Anderson, et al., 1995). Genetic information and inheritance studies are needed before such hybrids may be commercialized. However, significant progress has been realized with the use of inbred parents to create hybrids, which are uniform for flower color, flowering time, and plant habit (Figure 14-8b). Such seed-propagated hybrids, however, will most likely never reach the level of clonal uniformity in asexually-propagated cultivars.

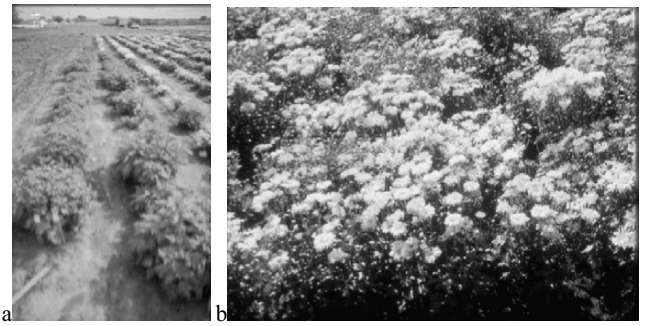

Figure 14-8. Commercial F_1 or F_2 hybrid seed-propagated commercial cultivars (a) which do not flower at 45ºN. lat. (St. Paul, Minnesota, U.S.A.) and flowering, uniform hybrids (b) selected for northern latitudes.

9.4 Floral Traits—Size, Type, Petal Orientation

Several research programs have reported on the inheritance of various floral traits, from flower size to color and form. Typical problems noted throughout many experiments are accurate classification of phenotypes (many appear to be more quantitative than qualitative in nature) and small progeny sizes (Crook, 1942), which can limit the reliability of goodness of fit tests (Chi-square, χ^2) for genetic classes.

Flower size was hypothesized to be controlled by four unnamed genes (Crook, 1942). Four size classes were identified, ranging from 'baby' (<0.75" dia.) to 'small' (0.75-1.75"), 'medium' (1.75-2.75"), and 'large' (>2.75"). Test crosses between progeny derived from crossing two parents of similar flower size, 'Mrs. Tricker' x 'Crimson Splendor', showed high levels of agreement between observed and expected ratios although the progeny sizes were small (ranging from n=3 – 79).

Flower type is based on the number of ray petal rows (single to fully double) as well as petal types (plain to quill, spoon, etc.) (Figure 14-3). Both categories are not mutually exclusive for most combinations. Additionally, petal orientation is another floral trait, which may range from flat to incurved (petal tips curved towards the center of the flower) or reflexed (petal tips curved away from the floral center). Incurved petalage was first termed "petals which reverse over the eye" (Crook, 1942).

The number of ray petal rows is classified into single (1 row), duplex (2), triplex (3), quadriplex (4), pentaplex (5), and so forth. When the number of ray petal rows

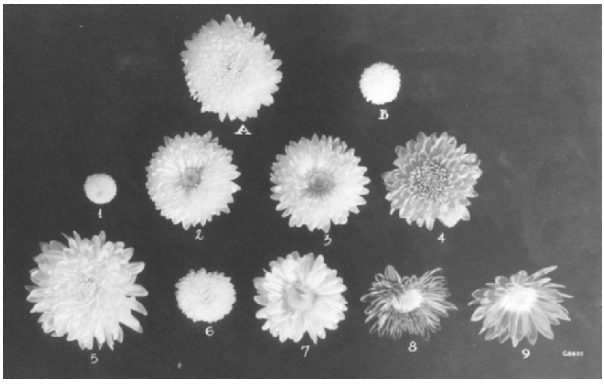

Figure 14-9. Example inheritance of floral forms and petal number in the cross between 'Yellow Doty' (A) x 'Varsity' (B); the F_1 hybrid progeny (1-9) display the parental and additional flower types (Crook, 1942).

is equivalent to replacing 50% of the disc floret rows (ranging from 3-10 rows of ray petals) it is termed 'semi-double'; if the number of ray petal rows reaches 100%, the fully double or 'super double' state is achieved (with >10 rows of ray petals and zero rows of disc florets). Crook (1942) proposed that four dominant genes (all designated as *T* with superscript numerical designators to distinguish between varying genes for this trait) conferred single flowers (T^1- T^2- T^3- T^4-), while any three sets of genes were dominant led to semi-doubleness and the presence of one or no dominants led to super-doubleness. Crosses between unrelated parents which differ in floral morphology create F_1 hybrid progeny with parental types and other flower forms (Figure 14-9).

Incurved flower petals were hypothesized to be controlled by two dominant genes, designated as *O* and *P* (Crook, 1942). Genotypes must have at least one dominant allele at each loci to exhibit the 'incurved' phenotype. Quilled petal inheritance studies are incomplete, since inadequate numbers of progeny were routine (Crook, 1942). However, positive assortative mating of parents is required to obtain a majority of progeny with quilled petals.

Disc floret morphology may also vary, ranging from essentially colorless upon pollen dehiscence (wild type in most chrysanthemum species) to colored florets with morphological modifications. For instance, the 'anemone' flower type possesses such modifications. One species, *Leucanthemum frutescens,* has anemone flowers as a diagnostic key (Heywood, 1976; Heywood and Humphries, 1977).

Promiscuous ray petals or 'petals in the eye' occurring in the disc florets are problematic. The U.S. national flower judging standards manual (Pi Alpha Xi, 1998) notes this to be a significant grower-related fault in commercial cut chrysanthemums and, to a lesser extent, in flowering potted types. Genotypes with promiscuous rays can be created via mutation breeding; the expression of this trait

may be stable or unstable when the genotype is grown in varying environmental conditions (Crook, 1942). Two dominant genes, *R* and *S,* were postulated to control this trait (Crook, 1942).

9.5 Flowering Requirements—Earliness, Day Neutrality, Heat Delay Insensitivity

Flower initiation and development are controlled by short day photoperiods that naturally occur by shortening day lengths in late summer and early fall (Post, 1949). Both garden and greenhouse chrysanthemums are short day (SD) plants for flowering (Cathey and Borthwick, 1957, 1961, 1964). Flower bud development (FBD) of SD chrysanthemums is reversibly controlled by red (~660 nm) and far red (730 nm) light. Continuous or intermittent exposure to red light, with the use of either incandescent, high pressure sodium, or fluorescent light sources in the middle of a long dark period (night), inhibits flower bud initiation (FBI) and development (FBD) (Borthwick and Cathey, 1962).

Garden and greenhouse chrysanthemums are categorized into SD response groups, the number of weeks from the start of the SD treatment to anthesis (Anderson and Ascher, 2001). Vegetative growth has a critical photoperiod of $\geq$13.5 hr while reproductive development (flowering) requires $\leq$12 hr (Cockshull, 1985). Genotypes are categorized into response groups of early, mid, and late depending on the number of weeks of SD required for FBI and FBD. Garden chrysanthemum response groups range from six to eight weeks while greenhouse types are 6.5-11 wks for flowering potted plants and 8-15 wks for cut flowers (van Zanten, North America, 1999; Yoder Brothers, Inc., 2000). Early flowering types (6-8 wk response group) are facultative SD for FBI but qualitative (obligate) SD plants for FBD (Cockshull and Kofranek, 1992). Later flowering response groups (>8 wks SD) are obligate SD plants for both FBI and FBD.

Early, mid, and late flowering response groups will initiate flower buds under non-inductive long day conditions (morphologically termed ‘crown buds’ with subtending strap-shaped leaves), but only early response groups will also undergo FBD (Langton, 1977). Crown buds have a flattened appearance due to arrested floret development, as well as the lack of subtending axillary meristems (Anderson and Ascher, 2001).

Long day leaf number (LDLN), defined as the mean number of leaves initiated by the terminal meristem prior to commencing FBI under a long day photoperiod, is used as a quantitative measurement of vegetative growth and juvenility in seedlings (Cockshull, 1976; Cockshull and Kofranek, 1985). FBI, but not short day response group (based on FBD), is linked with LDLN (Anderson and Ascher, 2001; Langton, 1981). Broad sense heritability for LDLN ranges from $h^2 = 0.79$, with a 95% C.I. of 0.76-0.82 (Anderson and Ascher, 2001), to $h^2 = 0.83$ (Langton and Dixon, 1984).

Mean stem lengths of the terminal shoot are significantly longer with SD genotypes, in comparison with day neutrals (Anderson and Ascher, 2001). Stem length has a broad sense heritability of $h^2 = 0.91$, with a 95% C.I. of 0.90-0.92 (Anderson and Ascher, 2001). The number of nodes with axillary branching is unrelated to photoperiodic response and has a broad sense heritability of $h^2 = 0.75$ (95% C.I. of 0.74-0.76) (Anderson and Ascher, 2001).

Chrysanthemum breeders in the U.S. during the early 1900s focused on early flowering genotypes—particularly those reaching anthesis prior to the first frost date (Mulford 1935). Since a number of environmental factors can influence occurrence of FBI and the rate of FBD extensive testing over years and locations is necessary to ensure stability of cultivars prior to their release. Mulford (1938) found three exceptional garden genotypes that did not vary in flowering time from year-to-year. Decades of selection for early flowering garden chrysanthemum genotypes resulted in FBI and FBD occurring under natural long day photoperiods of the summer. When these genotypes were tested in the critical photoperiod experiments, many were day neutral (Anderson and Ascher, 2001).

True day neutral chrysanthemums have been selected which will undergo both FBI and FBD under any combination of photoperiods within the normal temperature range (10-12C nights) for commercial production of the crop (Kawata and Toyoda, 1982; Anderson and Ascher, 2001). Day neutral genotypes will undergo autonomous FBI and FBD for all flower buds in the inflorescence (primaries, secondaries, tertiaries, etc.) under any photoperiod or combination of light quality (Cathey and Borthwick, 1970; Kawata and Toyoda, 1982; Langton, 1977; Schwabe, 1953). It is not necessary to test for day neutrality under all combination of photoperiods. Rather, a continuous (24 hr) far-red + red light treatment during FBI and FBD may be used to select day neutral progeny in which all of the first six flower buds (ranging from the first or terminal flower bud to the fifth subtending lateral) reach anthesis (Anderson and Ascher, 2001). This environment may be supplemented with high night temperatures to simultaneously select for heat delay insensitivity.

A variety of day neutral genotypes have been selected including ‘Dr. Longley’ and Mn. Sel’n. 83-267-3 (Figure 14-10) from the University of Minnesota breeding program (Anderson and Ascher, 2001; Seeley, 1966)—both of which flower under any photoperiod ranging from 8 – 24 hrs in duration; ‘Jeongwoon’ and ‘Mezame’ from Japanese breeding programs (Kim and Lee, 1998; Langton, 1978), and numerous others (Harada and Nitsch, 1959; Okada, 1957; Satory, 1986; Seeley, 1966). Mn. Sel’n. 83-267-3 is both day neutral and heat delay insensitive (Anderson, et al., 1989). An ideotype for selecting day neutral, heat-delay insensitive genotypes was proposed by Anderson and Ascher (2001) and subsequently tested (Anderson and Ascher, 2004).

Day neutrality offers commercial greenhouse growers advantages when producing garden chrysanthemum for spring sales (flowering in pots), potted and cut

greenhouse types. For example, the institution of black cloth for SD photoperiods during periods of the year when natural photoperiods exceed the requirement for FBI and FBD in SD genotypes would be eliminated (Anderson, 1991; Anderson and Ascher, 2001; Ascher, 1986). Day neutral cultivars, as well as those with short juvenility periods, require exogenous applications of ethylene using Ethephon (Florel, Union Carbide Co., Research Triangle Park, North Carolina, USA) to inhibit FBI and/or FBD in stock plants (Cockshull, et al., 1979; Strefeler, et al., 1996; Stuart, et al., 1988). Home gardeners could also benefit from growing day-neutral cultivars as this trait would offer the opportunity for continuous flowering during the growing season.

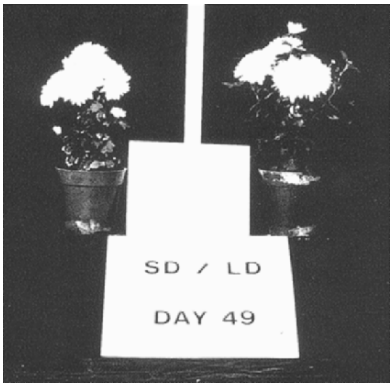

Figure 14-10. Flowering of day-neutral garden chrysanthemum Mn. Sel'n. 83-267-3 under short days and long days (red light) photoperiods (Anderson and Ascher, 2001).

High night temperatures (≥22C) during FBI and/or FBD, termed heat delay, can delay flowering and produce abnormal inflorescence development (Cathey, 1954; Cockshull, 1979; Crater, 1980; Whealey, et al., 1987). Likewise, prior to FBI, high night temperatures may also increase LDLN (Cockshull, 1979). Heat delay most frequently occurs during greenhouse production when black cloth is pulled over the crop for FBI and FBD in SD cultivars. Example cultivars sensitive to heat delay are: 'Delano', 'Yellow Mandalay', and 'Sunny Mandalay' (Yoder Brothers, Inc., 2000). Heat delay insensitivity has been bred into numerous greenhouse cultivars and may also be accompanied by a corollary low temperature (10 C nights) delay (de Jong, 1978).

9.6 Winter Hardiness

Winter hardiness is an essential trait for herbaceous perennials in northern temperate regions circumboreally (Still, et al., 1988; Griesbach and Berberich, 1995). Early cultivated forms were not winter hardy in northern temperate regions, as noted by Morrison (1923): "chrysanthemums are of little value as hardy plants in

the extreme North...". Winter hardiness or cold tolerance continues to be an important trait for perennial garden chrysanthemums (Anderson and Gesick 2004) that are frequently sold as "hardy" mums (Holley, 1945; Wulster and Lacey, 1985). This is a frequent misnomer as many cultivars are not perennial (winter hardy) north of the 40th parallel, necessitating repurchase each year as herbaceous annuals (Anderson and Gesick, 2004; Holley, 1945; Wulster and Lacey, 1985).

In the early 1900s, garden chrysanthemum breeding programs commenced with breeding for increased winter hardiness (Hieke, 1976; Askew and Chaput, 1987; Griesbach and Berberich, 1995; Widmer, 1958; Wildung, 1979). Dr. A.C. Hildreth (Cheyenne Horticulture Field Station, USA) planted n=2,000 cultivars of chrysanthemums at the field station in 1932 and selected n=20 hardy genotypes that survived the winter (Viehmeyer and Uhlinger, 1955). These became the parental gene pool of winter hardiness in U.S. breeding programs (North Platte Station, Nebraska; University of Minnesota, USA) and included subsequent releases ('Red Chief'). Crosses between 'hardy' and 'tender' parents demonstrated that hardiness is not dominant (Viehmeyer and Uhlinger, 1955). F_1 hybrids were primarily in the midparent range, although a few genotypes approach that of the hardy parent. Viehmeyer and Uhlinger (1955) theorized that genotypes with shallow rhizomes were more likely to receive winter damage than deep ones although genetic variation existed in shallow rhizome genotypes to occasionally survive through the winter.

The U.S. Department of Agriculture's program breeding program released n=12 genotypes in 1937 with promising hardiness (Mulford, 1937b, 1938). Other public sector breeding programs—particularly the University of Minnesota followed with continued release of winter hardy cultivars beginning with 'Duluth' in 1939 (Widmer, 1997). Holley (1945) found that ~-12.2C killed chrysanthemum crowns (roots, rhizomes). Controlled freezing studies in the laboratory found that –10 to –15C injured acclimated rhizomes (Widmer, 1958). Yang (1995) also found that free proline content and electrolyte leakages were correlated with winter hardiness.

More recent studies have shown that a breeding program may implement a non-destructive method to select among first-year seedlings by counting the number of emergent rhizomes (Anderson and Gesick, 2004). Non-winter-hardy genotypes had significantly fewer emergent rhizomes (Figure 14-11a) than winter-hardy types (Figure 14-11b) (Anderson and Gesick, 2004). This selection method, however, requires a corollary 'test' winter with adequate cold temperatures and snowfall. The recent resurgence in el Niño throughout central North America has necessitated supplementing selection for high emergent rhizome genotypes with laboratory freezing tests of the entire crown to replace the 'test' winter (Kim and Anderson, 2005).

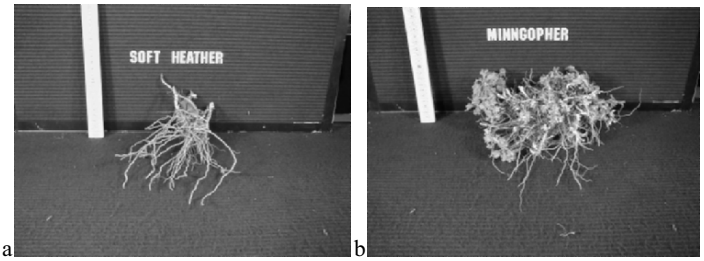

Figure 14-11. Phenotypes of (a) non-winter-hardy 'Soft Heather' and (b) winter-hardy 'Minngopher' garden chrysanthemum crowns recorded at the end of the first growing season (Anderson and Gesick, 2004).

Two different laboratory freezing tests were evaluated for their effectiveness in determining cold tolerance (Kim and Anderson, 2005). Acclimated crowns of hardy and non-hardy garden chrysanthemum genotypes were used in Omega Block (using detached, emergent rhizomes) and chamber (using entire, intact crowns with emergent, non-emergent rhizomes) freezing test methods. Comparative winter survival in the field was monitored over locations and years. Cold tolerance was assessed at 0°C to -12°C with varying ramp and soak time periods. The chamber freezing method was the most powerful to discern LT_{50} values (lethal temperature at which 50% of the samples were killed) (Figure 14-12). Cold tolerant genotypes included 'Duluth' and Mn. Sel'n. 98-89-7 (LT_{50} = -12°C). Three genotypes had intermediate cold tolerance (LT_{50}=-10°C) and one genotype was not cold tolerant (LT_{50} = -6°C). Cold-tolerant genotypes also had significantly higher regrowth ratings for rhizomes at 1cm and 3cm depths (Kim and Anderson, 2005).

10. MOLECULAR BIOLOGY

The highly interspecific nature of cultivated chrysanthemums and the tight nature of SI, coupled with the repeated introduction of new species and germplasm into breeding programs, have resulted in high levels of genetic diversity in the gene pool (Wolff and Peters-van Rijn, 1993). Genetic variation in *D. x grandiflora* is similar to other cultivated crops with similar mating systems and breeding programs (Carlson, et al., 1991; Reiter, et al., 1992; van Heusden and Bachmann, 1992).

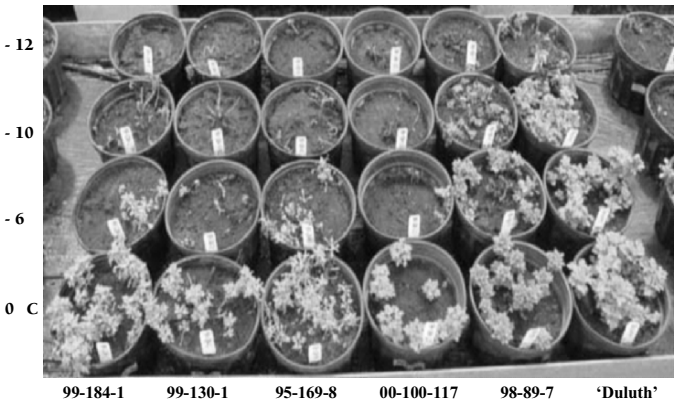

Figure 14-12. A comparison of regrowth results of chrysanthemum crowns, *Dendranthema ×grandiflora*, after exposure to laboratory freezing tests of 0ºC, -6ºC, -10ºC, and -12ºC (rows). Clones of genotypes are depicted in each row (Kim and Anderson, 2005).

Molecular techniques have been used with chrysanthemums for a wide range of purposes from detecting genetic diversity (RAPDs; Wolff and Peters-van Rijn, 1993), RFLP probe and primer development (Wolff, et al., 1993; 1994), sport and chimera characterization (Wolff, 1996), transformation (Young, et al., 1998), to genetic fingerprinting (Wolff, et al., 1995). Wolff and Peters-van Rijn (1993) used RAPDs to study clonal stability in a sport family (n=13 genotypes using n=27 primers), cultivar variation (using n=18 cultivars from three breeding programs and n=8 primers), and species variability (n=13 species, n=15 genotypes, and n=6 primers). Intercultivar variation was high and as few as two primers could be used to distinguish between cultivars. The sport family derived from a single cultivar all had identical fragment patterns. Wolff, et al. (1995) found similar results within sport families when regardless of which molecular fingerprinting method was used, i.e. from RAPDs, inter-SSR PCR (simple sequence repeat polymerase chain reaction), hybridization-based DNA fingerprinting, or RFLPs (restriction fragment length polymorphisms). Thus, due to the high levels of polymorphism and clonal stability in chrysanthemum, RAPDs were used for cultivar identification or fingerprinting.

Regeneration and transformation systems have been developed to successfully transform cultivated chrysanthemums. Young, et al. (1998) transformed two cultivars using *Agrobacterium tumefaciens* LBA4404 with three vectors, pBI121, pCMAsCP121-123, and pTOK233; other researchers have noted cultivar-strain specificity to *A. tumefaciens* (Bush & Pueppke, 1991). *Agrobacterium* readily works with chrysanthemums since the species is also readily infected with crown gall

(Miller, et al., 1975). The most effective shoot regeneration medium consisted of Murashige-Skoog basal salts with 2.0 mg/L NAA and 0.5 mg/L BA (benzyl adenine) (Young, et al., 1998). A 20 mg/L kanamyacin concentration resulted in the highest tissue formation rates. De Jong, et al. (1994) reported stable expression of the GUS reporter gene in chrysanthemums.

While most molecular techniques are useful and regeneration/transformation systems are easily developed, they are limited in value due to the wide range of genetic variability present in this interspecific, polyploid crop. Future research may be useful in focusing on marker-assisted selection and the development of molecular maps, since classic genetic maps are nonexistent (Wolff, et al., 1994).

11. IDEOTYPE BREEDING

Plant ideotypes are predictable plant growth models for a crop in a defined environment (Donald, 1968). In chrysanthemum breeding programs, ideotypes may be used to select plants with the suite of traits for the defined environment and market class. Langton and Cockshull (1976) developed an ideotype for cut spray chrysanthemums. Ascher (1986) also presented an ideotype for F_1 hybrid (seed-propagated) chrysanthemums. More recently, Anderson and Ascher (2001) proposed an ideotype for breeding and selecting day-neutral, heat-delay insensitive genotypes. Other ideotypes can be created, depending on the breeding objectives.

Chrysanthemum breeding programs have a variety of breeding objectives, depending on the product being created. Laurie and Poesch (1939) forwarded traits such as flower color, size, form, doubleness, foliage texture, flowering time, and plant habit to be important characteristics. Cumming (1939) also added winter hardiness, heat tolerance, long flowering periods, insect resistance, frost tolerance, dwarf types (for edging borders and roc gardens), and fragrance. Both garden and greenhouse (cut, potted) types require strong stems to prevent lodging (Smith and Laurie, 1928). Resistance for flower color fading in color groups with anthocyanins (reds, bronzes, purples, lavenders) is also a desirable trait (Crook, 1942). More recently, the lack of purpling in white or cream-colored flowers is a necessary trait for selection.

Historically, breeders have used positive assortative mating to obtain the highest frequency of hybrids with the desired traits (Crook, 1942). For instance, to obtain progeny that flower within a specific short day response group a breeder should use parents that flower within or as close to the desired response group as possible (Smith and Laurie, 1928). Private and public sector chrysanthemum breeding programs should incorporate as many desired traits as possible into their ideotypes for simultaneous selection in the target environments (Anderson and Ascher, 2001).

12. FUTURE DIRECTIONS

Visionary chrysanthemum breeding must focus beyond the immediate product potential of this crop to proactively create a continually expanding array of phenotypes (Anderson, 2004). Breeders should spend sufficient time collecting new germplasm and observing new phenotypes in wild germplasm. Numerous species have not been collected from the wild (particularly in western China) or the genetic variation sufficiently sampled and placed in repositories for future use. The U.S. Department of Agriculture, Agricultural Research Service (USDA-ARS) which operates the Ornamental Plant Germplasm Center (OPGC) at Ohio State University, has identified chrysanthemums as a primary genus for preservation (http://opgc.osu.edu/). The USDA-ARS OPGC also has a Chrysanthemum Working Group of public and private sector professionals to direct the directive to collect and preserve chrysanthemum germplasm. Such focused efforts in this area will assure that future generations of chrysanthemum breeders will have access to wild species populations and genetic variation to use in the creation of new flower colors, forms, and plant habits.

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Chapter 15

CRAPEMYRTLE
Lagerstroemia indica

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Abstract: The crapemyrtle has become a mainstay in mild-climate landscapes because of its ease of production and cultivation, long-lasting mid summer bloom, range of plant habits from miniature potted plant to large tree, and diversity of landscape uses. In addition to its place in the landscape, the introduction of dwarf and miniature cultivars has helped to establish crapemyrtle as a potential floriculture, bedding, or container plant that can be grown in a range of hardiness zones. Important traits for both production and landscape use include flower color and bloom time, plant habit and form, cold hardiness, and disease and pest resistance. Breeding efforts in crapemyrtle should focus on elucidating the inheritance of some of these traits, as well as broadening the genetic base of cultivated *Lagerstroemia* through interspecific hybridization or direct gene transfer.

Key words: Lythraceae, interspecific hybridization, disease resistance, breeding, production, biotechnology.

1. INTRODUCTION

The genus *Lagerstroemia* L. contains species endemic primarily to southeastern Asia, but which are cultivated, and in some cases naturalized, in temperate and tropical regions worldwide. Although some species are grown commercially for timber, wood, or tannins (Egolf and Andrick, 1978), or for medicinal purposes (Hayashi et al., 2002), *Lagerstroemia*, or crapemyrtle, is best known for its value as a landscape tree or shrub, and more recently, as a container or bedding plant.

The crapemyrtle has been cultivated in Southeast Asia for many centuries, and was introduced to Europe in the mid 1600s. It was planted in Kew Gardens in

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England in the mid 1700s, and was introduced to the southeastern U.S. soon afterward (Egolf and Andrick, 1978). It is thought that George Washington helped to import seeds of *L. indica* for planting at Mt. Vernon (Egolf and Andrick, 1978), although there are no records of surviving crapemyrtle plants at the estate at that time. Distribution of crapemyrtle by commercial nurseries on the east and west coasts of the U.S. ensured that this plant had become a fairly common landscape fixture in mild-climate gardens by the early 1900s.

In addition to ease of production and cultivation and mid- to late- summer bloom, one reason for the continued popularity of the crapemyrtle in temperate and tropical landscapes is the diversity of habit, color, and size offered by the hundreds of named cultivars. Thanks to breeding and selection efforts by D. Egolf, O. Spring, A. Desmartis, A. Einert, J.B. Fitzpatrick, Carl Whitcomb, David Chopin, S. McFadden, D. Inamota, and others, crapemyrtle cultivars are available in sizes ranging from miniature shrubs or potted plants to trees, with flower colors to complement any landscape setting. They can be used as specimen plants; mass planted in parks, estates, or along streets and highways; as screens; as bedding or foundation plants; or as outdoor container plants. This diversity of form and function, along with often outstanding fall color and striking exfoliating bark, make it little wonder that crapemyrtle has been nicknamed the “lilac of the south”.

2. GERMPLASM

The genus *Lagerstroemia* L. is a member of the loosestrife family (Lythraceae), comprising at least 53 species (Furtado and Montien, 1969; Tobe and Raven, 1990; Table 1). The genus was named after a colleague of Carl Linnaeus, Magnus von Lagerstroem (1696 - 1759) of the Swedish East Indies Company. The majority of the species in the genus are tropical, with only a few species that grow well in temperate regions. The center of origin for *Lagerstroemia* appears to be Southeast Asia and Australia (Egolf and Andrick, 1978), although commercial trade and naturalization of the genus in some areas has obscured the boundaries of its original native range.

A checklist of *Lagerstroemia* cultivars (Egolf and Andrick, 1978) updated in 2000 by R. Dix (www.usna.usda.gov) indicates that there are 217 valid named cultivars of crapemyrtle, although at most half of that number are widely cultivated commercially in the U.S., Europe, Asia, or Australia. Most of these cultivars are selections or hybrids of *L. indica*, although other species, including *L. speciosa*, *L. fauriei*, *L. subcostata*, *L. limii*, and *L. ovalifolia*, are also represented in these cultivars. The introduction of one species in particular, *L. fauriei*, has had an especially profound impact on cultivated crapemyrtle germplasm, as it provides a source of resistance to powdery mildew. In 1956, a plant exploration trip to Japan by John Creech resulted in the introduction and distribution in the U.S. of seeds of *L.*

fauriei by the USDA (Creech, 1985; PI 237884). This rare and possibly threatened species is reportedly found only on the island of Yakushima, Japan (Creech, 1985; Ohwi, 1965). The first named interspecific hybrid cultivar between the two species was 'Basham's Party Pink', a chance seedling discovered in Texas and introduced in 1965. Hybridizations made at the National Arboretum in 1964 between *L. indica* and *L. fauriei* resulted in the introduction in 1978 of the mildew resistant cultivars 'Natchez' and 'Muskogee', the first intentionally developed interspecific hybrid cultivars using *L. fauriei* (Egolf, 1981a). Since then, 22 additional mildew resistant *L. indica* × *L. fauriei* hybrids have been released by the U.S. National Arboretum (Egolf, 1981b, 1986a, 1986b, 1987a, 1987b, 1990a, 1990b; Pooler and Dix, 1999).

Several of the traits that make crapemyrtle such a successful plant in production are also associated with potential invasiveness (Reichard, 1997). *Lagerstroemia* seeds germinate readily without pretreatment, and, under favorable conditions, plants can reach sexual maturity in a year. In addition, clonal propagation is accomplished easily from softwood, hardwood, or root cuttings, sometimes with no need for rooting hormones. However, *Lagerstroemia* has not become invasive in the U.S. or other introduced areas. It requires full sun to thrive, and despite abundant seed set, has not yet been reported to be capable of displacing native species or changing undisturbed ecosystems.

3. COMMERCIAL TRAITS

As mentioned previously, the crapemyrtle has several landscape attributes that contribute to its popularity worldwide, including its most notable trait of long-lasting bloom in mid- to late- summer in shades of pinks, lavenders, magenta, and pure white. However, several other traits contribute to the commercial success of crapemyrtle cultivars, including plant habit, disease and pest resistance, ornamental bark characteristics, cold hardiness, and ease of propagation and production.

3.1 Flower Characteristics

Flower color is frequently used to define or describe crapemyrtle cultivars, yet little research has been conducted to elucidate the inheritance and genetics of flower color in crapemyrtle. An examination of anthocyanin pigments in *L. indica*, *L. fauriei*, and *L.* × *amabilis* (Egolf and Santamour, 1975) revealed the presence of the 3-glucosides of delphinidin, petunidin, and malvidin, which contribute to purple and blue flower color (Harborne, 1965). However, the anthocyanins commonly associated with true deep red pigments, pelargonidin and cyanidin (Harborne, 1965), were not present, leading the authors to conclude that developing crapemyrtle cultivars with true red flowers, such as scarlet or crimson (e.g. RHS 41-44A), may not be possible. Interspecific hybridizations with species that could contain different

pigments may serve to broaden or intensify the range of flower color in crapemyrtle. Three-species hybrids made by D. Egolf in the late 1980s involving *L. indica*, *L. fauriei*, and *L. limii*, resulted in two red-flowering cultivars, 'Arapaho' and 'Cheyenne'. In addition, mutation breeding using chemical mutagens such as EMS (Whitcomb, 1985; 1998) may also lead to novel flower color.

Controlled hybridizations using white-flowering selections of *L. indica* × *L. fauriei* hybrids revealed that flower color appears to be controlled by several genes or modifiers (M. Pooler and R. Dix, unpublished data), as pale pink and lavender flowers frequently appear on seedlings from white-flowering parents.

Another important flower trait is the duration of summer bloom and related ability of a cultivar to rebloom. In USDA hardiness Zone 7, bloom can start in mid June and continue until mid to late September, with peak bloom generally occurring in early August. However, a range of blooming periods has been observed among cultivars (Fare et al., 1985; Knox and Norcini, 1992), and among open-pollinated seedlings from a single tree of *L. speciosa* (Jamaludheen et al., 1995). Thus, it appears that the variability to select for this trait is present in crapemyrtle germplasm. In addition to using the inherent variability in bloom duration to breed or select for this trait, it may also be possible to create reblooming seedless cultivars, either through mutation breeding (Whitcomb et al., 1984; Whitcomb, 1985) or through the creation of sterile triploid plants.

Crapemyrtle flowers are contained in inflorescences or panicles, which vary among cultivars in size and shape (Fig. 15-1). Panicles on some cultivars can be up to 20 inches long, while those on some of the miniature cultivars bear only a few or even a single flower. In addition, flowers can be tightly clustered on panicles or looser and more branching, which also affects the floral display.

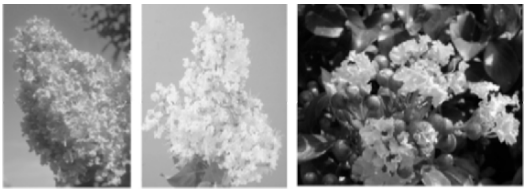

Figure 15-1. Diversity of crapemyrtle inflorescences. Left image is 'Hopi', showing a medium-sized, tight inflorescence; middle image is 'Natchez', showing a larger, looser, more branching inflorescence; and right image is 'Chickasaw', showing an inflorescence with few flowers.

3.2 Plant Habit

Crapemyrtles have traditionally been described as small trees or shrubs of varying habits, and only recently have also been associated with dwarf or potted

plants. Byers (1997) organized cultivars into four size classes based on mature plant height, consisting of cultivars over 20 feet (e.g. 'Natchez'), where habit can range from a broad vase-shape to an upright tree; 10-20 feet (e.g. 'Osage'), where habits range from globose to upright shrubs; 5-10 feet (e.g. 'Acoma'), where habits range from low spreading to compact upright shrubs; and less than 5 feet (e.g. 'Pocomoke'), where habits range from a compact mound to an open globose shrub (Fig. 15-2). Interest in breeding and selection of plants in this last class has increased in the past 30 years due to the new marketing niches that these types of cultivars offer, including use as a potted plant (Guidry and Einert, 1975a; Knox, 1996; Frangi and D'Angelo, 2000; Fig. 2), and, with judicious pruning, as a bedding plant (Einert et al., 1979).

The first dwarf crapemyrtles were introduced in 1960 by Otto Spring in Okmulgee, OK (Egolf and Andrick, 1978), and this germplasm has contributed to programs at Arkansas (Einert et al., 1979; Einert and Watts, 1973), the U.S. National Arboretum (Egolf and Andrick, 1978), and Monrovia Nursery Company (Egolf and Andrick, 1978), among others. The performance and origin of the germplasm of other dwarf selections is not clear. For example, 'Crape Myrtlettes', a seed mix derived from germplasm from Japan and sold by the Park Seed Company, Greenwood, SC, are purported to reach a mature height of 3-5 feet. This germplasm gave rise to compact weeping cultivars released by D. Chopin, Baton Rouge, LA, which are described as reaching 20 inches at maturity (Chopin, 1978), and are marketed primarily as container plants. Programs to develop dwarf selections are also being pursued in Italy (Frangi and D'Angelo, 2000) and Japan (Katsuo, 1992).

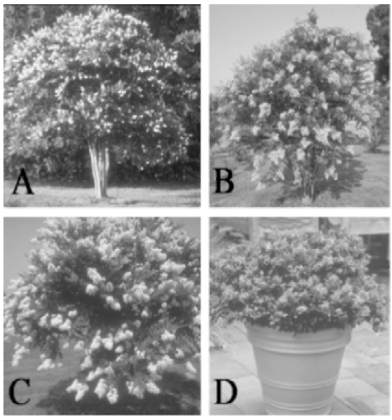

Figure 15-2. Examples of the diversity of crapemyrtle habits. (A) 'Natchez', a large, upright tree-type; (B) 'Osage', a medium globose shrub; (C) 'Acoma', a smaller spreading, semi-pendulous shrub; (D) 'Pocomoke', a dwarf type suitable for growing in containers.

The ability to grow and flower under artificial light is an important trait for pot culture of crapemyrtle. It is unclear how much variation exists among dwarf cultivars in flower forcing ability. Development of flower buds, from the enlargement of the floral meristem to the formation of pollen, can take from one to three months (Motoki et al., 1972) and depends on temperature and daylength (Goi and Tanaka, 1976).

Although not formally documented, some of the extremely compact miniature crapemyrtles have a tendency to send out sports or reversions (Byers, 1997; M. Pooler, personal observation). These sports generally have larger leaves and flowers, longer internodes, and set more seed than their dwarf counterparts. Most sports can be pruned off successfully and often do not reappear; however, the cause of this anomalous growth, whether environmental, chromosomal, physiological, or epigenetic, must be elucidated.

3.3 Cold Hardiness

Cold hardiness is one of the most sought-after traits pursued by crapemyrtle researchers and growers alike. Increasing the cold hardiness of crapemyrtle could have a significant impact on the nursery industry, as it would extend the range for both production and sale of cultivars. Such an improvement appears unlikely at present, as no species or taxa of *Lagerstroemia* has been identified that is reliably top-hardy in a climate colder than USDA hardiness Zone 7b. Exceptions can be found, especially in protected environments (Egolf and Andrick, 1978). Most cultivars are root hardy to at least Zone 6, which allows rapidly growing selections to function as perennials in these climates, as crapemyrtles bloom on new (spring) growth. However, flower production is dependent on both temperature and daylength, so even if a plant survives the winter cold, its overall floral performance may still be limited in northern regions. Comparative studies in the field (Laiche, 1991) and in the laboratory (M. Dirr, personal communication) indicate that variation among cultivars for susceptibility to cold damage exists, although it is complicated by environmental factors, including the rate of cooling (Haynes et al., 1992), pruning (Haynes et al., 1991), and the conditions under which the plant is grown (Lindstrom and Dirr, 1994). Without the introduction of new germplasm, either through wide hybridizations, mutation breeding, or direct gene transfer, it appears unlikely that significant improvements will be made to extend the cold hardiness of crapemyrtle beyond its current boundaries.

Although cold hardiness *per se* is unlikely to change, growing crapemyrtles in colder climates has become feasible recently with the introduction of dwarf and miniature cultivars that can be grown in containers. These cultivars can be grown outside in containers as patio plants during the spring, summer, and fall, then moved into a cool protected area for winter dormancy.

3.4 Disease and Pest Resistance

There are few disease and pest problems that will actually kill a crapemyrtle plant, although several can cause disfiguration or sufficient stress to hinder the performance of the plant. Two fungal diseases, powdery mildew caused by *Erysiphe lagerstroemiae* E. West and Cercospora leaf spot caused by *Cercospora lythracearum* Heald and F.A. Wolf, are considered to be the major disease problems of crapemyrtle (Byers, 1997). The crapemyrtle aphid (*Tinocallis kahawaluokalani*), the flea beetle (*Altica* species) and the Japanese beetle (*Popilla japonica*) are probably the most serious insect problems (Mizell and Knox, 1993; Pettis et al., 2004), although damage from Florida wax scale (*Ceroplastes floridensis*), borers, and ambrosia beetles (*Xylosandrus crassiusculus*) has been reported (Byers, 1997).

Traditionally, powdery mildew was one of the most serious disease problems of crapemyrtle, causing an unsightly whitish powder on the surface of leaves, stems, and flowers, and leading to distorted and stunted growth. However, with the availability of diverse cultivars of mildew resistant *L. indica* × *L. fauriei* hybrids, problems with this disease are readily overcome by planting disease-resistant germplasm. Although some degree of resistance to powdery mildew has been observed in cultivars of *L. indica* (Egolf, 1967, 1970; Hagan et al., 1998), the most reliably disease resistant germplasm can be found in cultivars that contain *L. fauriei* germplasm (Hagan et al., 1998; Egolf and Andrick, 1978).

Cercospora leaf spot is characterized by the appearance of brown lesions on the leaves, followed by leaf discoloration and defoliation beginning at the bottom of the plant. As with powdery mildew resistance, resistance to Cercospora leaf spot appears to be associated with *L. fauriei* germplasm (Hagan et al., 1998), although the inheritance of this trait has yet to be determined. As a group, *L. indica* and *L. indica* × *fauriei* were much more susceptible to leaf spot than one cultivar of *L. fauriei* (‘Fantasy’) although several hybrid cultivars, notably ‘Apalachee’, ‘Basham’s Party Pink’, ‘Caddo’, ‘Tonto’, ‘Tuscarora’, and ‘Tuskegee’, also showed tolerance (Hagan et al., 1998).

The host-specific crapemyrtle aphid causes damage both by its feeding and by the deposition of honeydew, which serves as a substrate for sooty mold. Colonization of a cultivar of crapemyrtle by aphids is positively correlated with mature plant size and the presence of *L. fauriei* germplasm (Mizell and Knox, 1993). Hence, breeding for resistance to crapemyrtle aphid may be at odds with selection for resistance to powdery mildew and Cercospora leaf spot. Management of crapemyrtle aphid can also be accomplished by the use of pesticides (Doughty et al., 1992) or possibly through biological control (Mizell and Knox, 1995).

Other arthropod pests that can have a severe impact on nursery production of crapemyrtle are the flea beetle and the Japanese beetle. Recent comparative studies of 54 crapemyrtle cultivars showed that as a group, the cultivars that contained *L. fauriei* germplasm were more resistant to flea beetle damage than the straight *L.*

indica cultivars, although there were variations depending on location of the trial, production methods, and type of feeding trial (Pettis et al., 2004). Resistance to feeding by Japanese beetles was also found in several cultivars, but with no detectable correlation between resistance and genetic background of the cultivar (Pettis et al., 2004).

4. PRODUCTION

Crapemyrtle are relatively easy to produce commercially as landscape plants. Most cultivars propagate readily from cuttings, and the fibrous, shallow root system allows almost year-round transplanting in warmer climates. Little pruning or staking is required, and a liner can be produced in one season.

Crapemyrtles have historically been produced for landscape use, and have only recently been investigated as a floriculture or container crop. While further research is needed on cultivar evaluation, production, and marketing, the potential of crapemyrtle as a floricultural crop is clear (Frangi and D'Angelo, 2000; Guidry and Einert, 1975a).

4.1 Propagation

Although some variation exists among cultivars, propagation of crapemyrtle can be accomplished using a variety of methods. For the most easily propagated cultivars, terminal or subterminal softwood cuttings, preferably taken before flowering, with or without rooting hormone, stuck under intermittent mist, will root within three weeks (Byers, 1997; Einert, 1974). Hardwood cuttings, taken in the fall after defoliation, and stored in a cool location during winter, can be stuck directly in the field in spring with established 12-24" plants by fall (Byers, 1997; Sallee, 1988). Hardwood cuttings taken in the spring may not perform as well, but achieve the highest rooting percentage using an IBA/NAA rooting compound and sticking cuttings in sand (Einert, 1974). Root cuttings can be made during any season, and will result in numerous shoots, which can then be rooted as softwood cuttings (Egolf and Andrick, 1978). Micropropagation through tissue culture, using axillary buds on modified woody plant medium with 4-10 μM BA, is also straightforward (Zhang and Davies, 1986; Shim and Mi, 1994). Propagation by seed, although not desirable for production of cultivar material, is obviously required for breeding. Seed can be sown without stratification directly into flats, and will germinate within two weeks. With proper fertilizer, light, and temperature, many seedlings will flower during the first growing season.

4.2 Culture

Crapemyrtles require full sun for optimal bloom, but tolerate a range of soil types and urban stresses. Once established, they require little maintenance other than occasional and light pruning to remove dead or twiggy branches, suckers, or old flower clusters. Most growers agree that extensive pruning of crapemyrtle is unwarranted if the proper cultivar for the site is used (Byers, 1997).

The rate of fertilizer used on container-grown crapemyrtle can have an effect on post-transplant flowering, with higher levels of fertilizer (120-300 ppm applied nitrogen) causing delayed flowering compared to lower levels (15-60 ppm applied nitrogen; Cabrera and Devereaux, 1999). In addition, studies suggest that high levels of nitrogen applied to container-grown plants can lower shoot and root dry mass, leaf area, and canopy width (Cabrera and Devereaux, 1998).

Crapemyrtles require full sun and high summer temperatures to achieve optimum bloom, so forced greenhouse production of crapemyrtle can be difficult. Dwarf plants grown under short days (11 hours of natural light) did not flower, while those exposed to similar conditions but with a two-hour dark interruption did bloom (Goi and Tanaka, 1976). Studies by the same authors indicate that plants grown at or below 20C, even under long days, did not flower consistently. A production protocol for potted dwarf crapemyrtles has been suggested (Guidry and Einert, 1977; Stimart, 1986), consisting of propagation by softwood cuttings (4 weeks), pinching (chemically or physically) to encourage branching (4 weeks), and flower forcing (12 weeks). High temperatures (>20C), high light intensity, and long days (16 hours) appear critical for successful greenhouse production of potted crapemyrtle (Guidry and Einert, 1975b; Goi and Tanaka, 1976; Stimart, 1986).

Smaller varieties of crapemyrtles perform well as outdoor potted plants as long as they receive full sun, adequate water, and winter protection from prolonged freezing temperatures. Due to high light requirements, tendency to drop spent flowers and seeds, and winter dormancy, crapemyrtles do not work well for extended periods as indoor plants.

5. BREEDING

Several crapemyrtle breeding and/or selection programs have figured prominently in the development of improved cultivars during the past half century, including those of Andre Desmartis, Bergerac, France; A.E. Einert, University of Arkansas, Fayetteville, AR, USA; J.B. Fitzpatrick, Sherman, TX, USA; Daiji Inamota, Nara Prefecture, Japan; and Otto Spring, Okmulgee, OK, USA. However, some of the greatest strides and most lasting contributions to crapemyrtle improvement came from the late Donald Egolf of the U.S. National Arboretum, Washington, D.C. In addition to releasing 6 *L. indica* hybrids and 20 outstanding

disease tolerant *L. indica* × *L. fauriei* cultivars, many of which have become standards in the nursery industry, he performed the crosses and selection of some of the new miniature crapemyrtle cultivars that have only recently been released from the National Arboretum (Pooler and Dix, 1997). As David Byers, a well-known grower of crapemyrtle in Huntsville, AL, said, "Our story of crapemyrtle development would be very short were it not for Dr. Donald R. Egolf." Mike Dirr, professor at the University of Georgia, summed up Don Egolf's legacy and impact in the statement "His best days in a sense are yet to come" (Byers, 1997).

5.1 Flowers and Pollination

Flowers of *L. indica*, which are borne in 5-20 inch panicles, are 1-2 inches in diameter, homostylic, and perfect. The 6 petals are crinkled and have a long claw. Dimorphic stamens are inserted near the base of the floral tube, and consist of 6 longer stamens with thicker filaments and often red anthers in the antesepalous whorl and numerous shorter stamens with thinner filaments and yellow anthers in the antepetalous whorl (Graham et al., 1987; Tobe and Raven, 1990; Fig. 15-3). For controlled hybridizations, flowers should be emasculated by removing the petals and anthers 24-48 hours before the stigma would be exposed naturally. Anthers collected at this time will dehisce readily when placed in a dry environment, and can be stored in gelatin capsules in the refrigerator for the duration of the pollination season, and possibly longer. Pollen dimorphism has been described in *L. indica*, with the 6 larger reddish anthers from the antesepalous whorl yielding thin-walled pollen grains, and the numerous smaller yellow anthers from the antepetalous whorl yielding thick-walled pollen grains (Graham et al., 1987; Kim et al., 1994). It has been suggested by some authors (Pacini and Bellani, 1986; Harris, 1914) that pollen from the antesepalous whorl is more fertile, while the moister pollen from the antepetalous whorl provides a pollen reward for the insect pollinators.

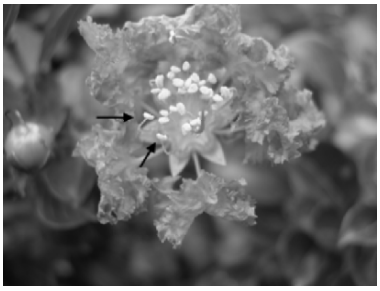

Figure 15-3. Close-up of a flower of 'Pocomoke' crapemyrtle showing flower morphology, including dimorphic stamens. Anthers on the longer, outer stamens (arrows) contain drier, more fertile pollen, while the pollen on the inner stamens is moister and less fertile.

5.2 Seed

The seed capsule, which matures 2-4 months after pollination, depending on environmental conditions, is globose, lignified, and surrounded by the calyx. Numerous winged seeds are borne in locules, and, for seed collection, should be harvested when the seed capsule begins to turn brown or soon after ripening, as it will dehisce when fully ripe. Seed from the same fruit can differ in respect to size and shape, and have been associated with differences in germination in *L. parviflora* (Shukla and Ramakrishnan, 1981).

5.3 Species

Cross species compatibility within the genus *Lagerstroemia* has not been examined. The basic chromosome number for the family Lythraceae is thought to be x=8 (Tobe et al., 1986; Raven, 1974). Data compiled for seven *Lagerstroemia* species (Ali, 1977; Tobe et al., 1986) suggest a range of chromosome numbers of 2n=44, 46, 48, or 50, with the majority of species studied (including *L. indica*) reported as 2n=48 (Ali, 1977; Table 15-1). Although self-incompatibility in *L. indica* has been suggested (Sareen and Kaur, 1991), results from decades of breeding records at the U.S. National Arboretum indicate that viable seed is readily obtained from self pollinations of *L. indica* and *L. indica* x *L. fauriei* hybrids (M. Pooler, unpublished). Based on the ease with which species from different sections hybridize [e.g. *L. indica* L. (section Sibia) and *L. limii* or *L. subcostata* (section Adambea) (Furtado and Montien, 1969)], it appears that interspecific hybridizations in the genus may be relatively easy to accomplish. Although interspecific *Lagerstroemia* hybrids can be sterile (Ali, 1977), they may serve as a means to introduce new traits for disease and insect resistance, tolerance to environmental stresses, plant habit, ease of production, and ornamental characteristics to broaden the genetic base of ornamental *Lagerstroemia*.

Table 15-1. Lagerstroemia species, taxonomic section and subsection, origin, reference (compiled from Furtado and Montien, 1969), and chromosome number (when known).

Species	Section and Subsection	Native Range	Reference	Chromosome Number
L reginae Roxb. (=L. flos-reginae Retz; =L. hirsuta (Lam.) Willd.)	*Adambea* subsect. *Adambea*	India, New Guinea, Burma	(1795) Pl. Corom. 1, 46, t. 65	2n=48 [1, 2]
L. costa-draconis Furtado & Montien	*Adambea* subsect. *Adambea*	Burma, Thailand, Cambodia	(1969) Gard. Bull. Singapore 24: 270	

Species	Section and Subsection	Native Range	Reference	Chromosome Number
L. hypoleuca Kurz	*Adambea* subsect. *Adambea*	India	(1872) Jour. Asiat. Soc. Beng. 41: 307	
L. macrocarpa Kurz	*Adambea* subsect. *Adambea*	Burma, Thailand, Indochina	(1873) Jour. Asiat. Soc. Beng. 42: 234	
L. speciosa (L.) Pers.	*Adambea* subsect. *Adambea*	Burma, Thailand, Indochina, Mala., Philippines	(1807) Synops. 2: 72	2n=48 [3,4] 2n=50 [5,6]
L. duperreana Pierre ex Gagnep. (=L. thorelli Gagnep)	*Adambea* subsect. *Banglamea*	Thailand, Indochina	(1918) Lec. Nat. Syst. 3: 358	2n=48 [2,5]
L. fordii Oliv. & Koehne	*Adambea* subsect. *Banglamea*	China	(1903) Engl. Pflanzenr. 4=17, 216: 262	
L. gagnepainii Furtado & Montien	*Adambea* subsect. *Banglamea*	China	(1969) Gard. Bull. Singapore 24: 287	
L. minuticarpa Debberm. ex. P.C. Kanjilal	*Adambea* subsect. *Banglamea*	India	(1934) Assam Forest Rec. Bot. 1: 9	
L. fauriei Koehne (=L. subcostata var. fauriei Koehne)	*Adambea* subsect. *Microcarpidium*	Japan	(1907) Engl. Jahrb. 41: 102; (1987) J. Fac. Sci. Univ. Tokyo, 14: 98	
L. glabra Koehne (=L. stenopetala Chun; =L. subcostata var. glabra Koehne)	*Adambea* subsect. *Microcarpidium*	China	(1907) Engl. Jahrb. 41: 102	
L. limii Merr. (= L. chekiangensis Cheng)	*Adambea* subsect. *Microcarpidium*	China	(1925) Philipp. J. Sci. 27: 165	
L. micrantha Merr.	*Adambea* subsect. *Microcarpidium*	Indochina	(1940) J. Arnold Arbor. 21: 378	
L. subcostata Koehne (=L. unguiculosa Koehne)	*Adambea* subsect. *Microcarpidium*	China, Japan, Philippines	(1883) Engl. Bot. Jahrb. 4: 20	
L. yangii Chun	*Adambea* subsect. *Microcarpidium*	China	(1948) Sunyatsenia 7:7	
L. alatulata Furtado & Montien	*Sibia* subsect. *Pterocalymma*	Philippines	(1969) Gard. Bull. Singapore 24: 242	
L. aruensis Furtado & Montien	*Sibia* subsect. *Pterocalymma*	Moluccan Islands	(1969) Gard. Bull. Singapore 24: 240	
L. borneensis Furtado &	*Sibia* subsect. *Pterocalymma*	Borneo	(1969) Gard. Bull. Singapore 24: 234	

Species	Section and Subsection	Native Range	Reference	Chromosome Number
Montien				
L. crassifolia Furtado & Montien	*Sibia* subsect. *Pterocalymma*	Borneo	(1969) Gard. Bull. Singapore 24: 232	
L. crispa Pierre ex Gagnep.	*Sibia* subsect. *Pterocalymma*	Indochina	(1914) Lec. Nat. Syst. 3: 357	
L. cristatata Furtado & Montien	*Sibia* subsect. *Pterocalymma*	New Guinea	(1969) Gard. Bull. Singapore 24: 236	
L. inopinata Furtado & Montien	*Sibia* subsect. *Pterocalymma*	Philippines	(1969) Gard. Bull. Singapore 24: 238	
L. koehneana K. Schumann	*Sibia* subsect. *Pterocalymma*	New Guinea	(1889) K. Schum. & Hollr. Fl. Kais. Wilh. Land. 85	
L. moluccana Furtado & Montien	*Sibia* subsect. *Pterocalymma*	Moluccan Islands	(1969) Gard. Bull. Singapore 24: 244	
L. ovalifolia Teijsm & Binnend	*Sibia* subsect. *Pterocalymma*	Malaysia, Indonesia, Thailand, Indochina, New Guinea	(1840) Kruid, Arch. 3: 410	
L. paniculata (Turcz.) S. Vidal (=L. calycina Koehne)	*Sibia* subsect. *Pterocalymma*	Philippines	(1885) Phan. Cuming. Philipp 39:115	
L. piriformis Koehne (=L. batitinan Vidal; =L. hexaptera Miq.)	*Sibia* subsect. *Pterocalymma*	Philippines	(1883) Engl. Bot. Jahrb. 4: 23	
L. pterosepala Furtado & Montien	*Sibia* subsect. *Pterocalymma*	Philippines	(1969) Gard. Bull. Singapore 24: 230	
L. pustulata Furtado & Montien	*Sibia* subsect. *Pterocalymma*	Borneo	(1969) Gard. Bull. Singapore 24: 222	
L. quinquevalvis Koehne	*Sibia* subsect. *Pterocalymma*	Indochina	(1903) Engl. Pflanzenr., 4=17,216: 268	
L. subangulata (Craib) Furtado & Montien	*Sibia* subsect. *Pterocalymma*	Thailand	(1969) Gard. Bull. Singapore 24: 208	
L. subsessilifolia Koehne	*Sibia* subsect. *Pterocalymma*	Australia	(1903) Engl. Pflanzenr., 4=17, 216: 267	
L. undulata	*Sibia* subsect.	Thailand	(1908) Engl. Jahrb.	

Species	Section and Subsection	Native Range	Reference	Chromosome Number
Koehne	*Pterocalymma*		42: 52	
L. venusta Wall.	*Sibia* subsect. *Pterocalymma*	China, Burma, Thailand, Indochina	(1879) Flora Brit. India 2: 576	
L. villosa Wall. ex Kurz	*Sibia* subsect. *Pterocalymma*	China, Burma, Thailand	(1873) J. Asiat. Soc. Bengal 42: 234	
L. indica L.	*Sibia* subsect. *Sibia*	China, Indochina, Japan	(1759) Amoen. Acad. 4: 137	2n=48 [2] 2n=50 [5]
L. microcarpa Wight (=L. thomsonii Koehne)	*Sibia* subsect. *Sibia*	India	(1838) Ic. Pl. I, t. 69	
L. parviflora Roxb. (= L. lanceolata Wall.)	*Sibia* subsect. *Sibia*	India, Burma	(1795) Pl. Corom. 1, 47, t.66	2n=48 [2]
L. archeriana Bailey (=L. engleriana Koehne)	*Trichocarpidium* subsect. *Trichocarpidium*	Australia, New Guinea	(1883) Synops. Queensl. Flora I: 196 & 809	
L. dielsiana Mansfeld	*Trichocarpidium* subsect. *Trichocarpidium*	New Guinea	(1927) Engl. Jahrb. 61: 24	
L. petiolaris Pierre ex. Laness.	*Trichocarpidium* subsect. *Trichocarpidium*	Indochina	(1883) Pl. Util. Col Franc: 321	
L. tomentosa Presl	*Trichocarpidium* subsect. *Trichocarpidium*	Burma, Indochina, China, Thailand	(1844) Bot. Bemerk. 142	
L. anisoptera Kohene	*Trichocarpidium* subsect. *Trichosepalum*	Thailand, Malaysia	(1883) Engl. Jahrb. 4: 407	
L. balansae Koehne	*Trichocarpidium* subsect. *Trichosepalum*	Thailand, China, Indochina	(1897) Engl. Jahrb. 23, 57: 35	
L. calyculata Kurz. (=L. angustifolia Peirre ex. Gagnep)	*Trichocarpidium* subsect. *Trichosepalum*	Burma, Thailand, Indochina	(1872) Journ. Asiat. Soc. Beng. 41: 307	
L. cochinchinensis Pierre ex Lanessan	*Trichocarpidium* subsect. *Trichosepalum*	Indochina, Thailand	(1886) Plant. Util. 321	
L. collinsae Craib	*Trichocarpidium* subsect. *Trichosepalum*	Thailand	(1914) Kew Bull. 282	
L. floribunda Jack (= L. turbinata Koehne)	*Trichocarpidium* subsect. *Trichosepalum*	Thailand, Malaysia	(1820) Malay. Misc. 1: 38	2n=48 [1]

Species	Section and Subsection	Native Range	Reference	Chromosome Number
L. langkawiensis Furtado & Montien	*Trichocarpidium* subsect. *Trichosepalum*	Malaysia	(1969) Gard. Bull. Singapore 24: 327	
L. lecomtei Gagnep.	*Trichocarpidium* subsect. *Trichosepalum*	Indochina	(1918) Lec. Nat. Syst. 3: 360	
L. loudonii Teijsm. & Binn. (=L. rottleri C.B. Clarke)	*Trichocarpidium* subsect. *Trichosepalum*	Thailand, Indochina	(1863) Nat. Tijdschr. Nederl. Ind. 25: 425	
L. noei Craib.	*Trichocarpidium* subsect. *Trichosepalum*	Thailand, Indochina	(1930) Kew Bull. 327	
L. siamica Gagnep.	*Trichocarpidium* subsect. *Trichosepalum*	Thailand, Burma, Malaysia	(1918) Lec. Nat. Syst. 3: 361	
L. spireana Gagnep.	*Trichocarpidium* subsect. *Trichosepalum*	Indochina, Thailand	(1918) Lec. Nat. Syst. 3: 362	

[1] Nanda, 1962
[2]Ali, 1977
[3]Singhal and Gill, 1984
[4]Guha, 1972
[5]Bowden, 1945
[6]Mehra, 1976

6. FUTURE PROSPECTS

Although crapemyrtle has been cultivated for centuries, it has only been in the past several decades that the horticultural versatility of this crop and the associated marketing opportunities have been recognized. Crapemyrtle can function not only in its traditional role of landscape tree or shrub, but also as a potted plant, a bedding plant, a bonsai plant, or even a seed-propagated annual or perennial. The intended use of the plant will dictate the specific breeding objectives; however, broadening the genetic base of ornamental *Lagerstroemia*, whether through interspecific hybridizations, mutation breeding, or biotechnology, could result in novel combinations of genes for production-associated, ornamental, or disease and pest tolerance traits. Unlike many woody ornamental genera, *Lagerstroemia* can go from seed to flowering plant in one year, thereby making breeding methodologies such as backcrossing an effective approach to incorporating traits from other *Lagerstroemia* species. Even broadening the genetic base of *L. fauriei* used in hybrid cultivars

could result in gains, as most of the hybrid cultivars today rely on a single accession of *L. fauriei*, PI 237884 (Pooler, 2003).

The role that biotechnology can play in ornamental horticulture has been addressed in the first volume of this monograph. Although plant regeneration from embryos or somatic tissue has not been reported yet in crapemyrtle, there is reason to believe that with the right combination of growth regulators and explant material, a transformation system could be established. Transgene constructs that affect such traits as insect resistance, flower color, and plant stature have been identified in other systems. In addition, transgenes that influence other more complex traits such as cold hardiness could also have an impact on crapemyrtle breeding and landscape distribution. Regardless of the possibilities afforded through biotechnology, crapemyrtle, because of its ease of production, diversity of landscape forms and function, and bloom time and duration, will likely be a mainstay in southern landscapes in the 21st century and beyond.

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Chapter 16

CYCLAMEN

Cyclamen persicum Mill.

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Abstract: In the genus *Cyclamen*, only *C. persicum* has been used as the major commercial plants. Cyclamen began to be a popular commercial plant in the nineteenth century. Although wild *C. persicum* plants usually flower in winter to spring, production time of commercial flowering cyclamen plants depends on the demands and weather conditions in each country. Cyclamen are usually propagated by seeds. However, most genotypes show inbreeding depression. Seed dormancy in cyclamen is not a serious problem, but temperature and light conditions affect seed germination. Cyclamen production occurs throughout the Temperate Zone and is a big market. The trends and demands for cyclamen production vary depending on the country and/or the time of year. Floral characteristics are important for the production. The petal size and shape of commercial plants vary. Today, various commercial genotypes with white, purple, scarlet or yellow petals are available. Although diploid and tetraploid cultivars are available, the world trends of commercial potted plants are toward diploid F1 cultivars. Development of the researches on cyclamen traits and breeding techniques creates a great possibility in cyclamen breeding. Some modes of inheritance of petal coloration and pigmentation have been clarified; research succeeded in ploidy breeding, and interspecific hybridization between cyclamen and other *Cyclamen* spp. Some *in vitro* propagation techniques have been established, and transgenic plants have been obtained. The breeders must plan a dependable breeding program and develop tactics for the breeding and production of new plants. Marketing research, dependable breeding objective, fundamental knowledge on breeding, and effective breeding technique are needed to establish the program and tactics.

Key words: F_1 cultivars, haploidization, interspecific hybridization, gene transformation, petal pigmentation, polyploidization.

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1. INTRODUCTION

Cyclamen (*Cyclamen persicum* Mill.), with elegant flowers and attractive leaves, is cultivated throughout the Temperate Zone. Many people have enjoyed the charming plants as potted and/or garden plants. The cyclamen is one of the most famous and important commercial ornamental plants in many countries.

1.1 The Genus *Cyclamen*

The genus *Cyclamen* consists of 22 species (Grey-Wilson, 2002), and is distributed in and near the Mediterranean region. The present known species are as follows: *C. africanum*, *C. balearicum*, *C. colchicum, C. coum*, *C. creticum*, *C. cilicium*, *C. cyprium*, *C. graecum*, *C. hederifolium*, *C. intaminatum*, *C. libanoticum*, *C. mirabile*, *C. parviflorum*, *C. persicum*, *C. pseudibericum*, *C. purpurascens*, *C. repandum*, *C. rohlfsianum*, *C. somalanse*, and *C. trochopteranthum*. Anderberg (1994) and Grey-Wilson (2002) made subgenus classification in the genus, whereas the classifications did not coincide in some points between the reports. Anderberg et al. (2000) made a molecular phylogenetic study of the genus *Cyclamen* by DNA sequence, and suggested four monophyletic subgroups from the sequence data and morphological data in the genus. Molecular study will help to clarify relation among species and evolution in the genus in the future.

Every plant in the genus has a tuber and nodding flowers. Flowering time varies with the species, even with different species cultivated in the same place. Some species have horticulturally interesting characteristics, for example strong fragrance in *C. purpurascens*. It is difficult to produce interspecific hybrid seeds in most of the crosses. I have never found a confirmed report of natural production of interspecific hybrids in the genus. Interspecific hybrids from the crosses between commercial cyclamen (*C. persicum*) and some wild species were, however, produced through ovule (or ovary) culture (Ishizaka and Uematsu, 1990, 1992, 1995a; Ishizaka, 1996; Ewald, 1996; Sibusawa and Ogawa, 1997).

1.2 Species origin and the Center of Diversity

To date, only *C. persicum* has been used as the major commercial plant in the genus *Cyclamen*. The word “cyclamen” usually stands for the commercial plants of *C. persicum*. Although some other species, for example *C. hederifolium*, are used as garden plants, they have only very limited importance (Geier et al., 1990). Moreover, it is too difficult to get hybrid seeds in interspecific crosses between commercial cyclamen and other species as mentioned above. Commercial cyclamen has been bred through intraspecific variations including natural mutations and varietal crosses within *C. persicum* for a long time.

Grey-Wilson (2002) suggested that wild *C. persicum* (Figure 16-1) is native to the extreme eastern Mediterranean region, whereas the species is distributed in north African regions as well as the east Mediterranean. Previously, commercial *C. persicum* was often called by the name of the Persian cyclamen. However, Doorenbos (1950) reported that this species was not distributed in Iran.

Figure 16 -1. Wild *Cyclamen persicum* in pot culture.

1.3 Domestication

According to a report by Doorenbos (1950), *C. persicum* had already appeared in France in 1665, and the Persian cyclamen was firmly established in the Netherlands in 1739. Thus, *C. persicum* was introduced from the eastern-Mediterranean region into western Europe at least in the seventeenth century, but the exact year of the first introduction is obscure.

Cyclamen began to be a popular commercial plant in the nineteenth century. Doorenbos (1950) indicated three reasons for the development of cyclamen in the nineteenth century as follows: 1) Improvement of cultivation methods, 2) Improvement of economic circumstances (the period of great prosperity), 3) Success of the breeding. The cultivation period was reduced from 3-4 years to 15-18 months by the latter part of the century (Grey-Wilson, 2002). The reduction of cultivation period was established by eliminating the resting period in summer, and this is regarded as the most important factor that made cyclamen popular (Doorenbos, 1950). Today's cyclamen breeding program using crosses or spontaneous mutation within diploid or tetraploid *C. persicum* should be limited. Many desirable characteristics, especially new flower colors and disease resistance, are not available in *C. persicum*. Therefore, new breeding techniques such as interspecific hybridization and gene transformation should be the key to make cyclamen breeding program rapidly progress, and more research will be desired.

2. SPECIFIC TRAITS AFFECTING COMMERCIAL PRODUCTION

For commercial production of plants, crop time is an important factor affected by genotypes. Propagation method of the plants is also an important factor. Although cyclamen has a tuber, daughter tubers are not formed. In addition, division or splitting of the tuber is difficult, and it is not easy to use the tuber as a storage organ. Therefore, cyclamen is usually propagated by seeds.

2.1 Crop Time

Wild *C. persicum* plants usually flower in winter to spring. In my laboratory, the plants bloom from December to April. Production time of commercial flowering plants depends on the demands and weather conditions in each country. Although flowering plants in the Netherlands were produced from November to December until the 1960s (Grey-Wilson, 2002), the plants are now available at store not only in the winter but also in other seasons. In Japan, the major production season in the 1980s was December, but we now also can also buy the plants in the middle or latter part of autumn. Such variation in production time has occurred in many countries in addition to the Netherlands and Japan. Today, the production time is extended from autumn to spring in many countries, and the plants are sold in the summer in some areas.

For satisfying the demands, various production schedules in cyclamen plants have been developed in each production region. Reducing the cultivation period in cyclamen is an effective way to reduce production costs as well as to adjust the production according to demand. Widmer et al. (1991) developed the production schedule for producing flowering plants for Christmas from seeds sown in May in the USA, whereas the traditional schedules required 15 to 16 months from seeding to bloom. Well-controlled environmental conditions, fertilization, watering and regulation of flowering by chemicals like gibberellic acid are required for shortening the cultivation period. Selection and breeding of suitable cultivars for the schedule are also very important. Most production periods in commercial cyclamen today are 8 to13 months, depending on the cultivar and production region.

2.2 Seed Yield and Reproductive Barriers

It is not difficult for many genotypes of cyclamen to produce more than 500 seeds per plant, but the potential for seed production varies with the genotype, age and size. It is natural that high-quality plants can produce a larger number of high-quality seeds. Cultivation temperature also influences seed production. Although higher temperatures (more than 20 ˚C) hastened the maturation of seeds, the number of seeds and seed set percentage were reduced (Takamura et al., 1996).

Emasculation and artificial pollination are used by breeders for the cross breeding of cyclamen cultivars and the production of F1 hybrid seeds, while there are many cultivars propagated open-pollination (pollination without emasculation and covering flowers). In our laboratory, the flower bud is emasculated about seven days before anthesis, and the emasculated flower is pollinated at the expected day of anthesis. Although pollination on the same day of emasculation may reduce the labor required for crossing, this method reduces the number of seeds in my experience. The emasculation method in my laboratory is not difficult and a popular method in Japan. The petals, with anthers, are removed from a bud as shown in Figure 16-2.

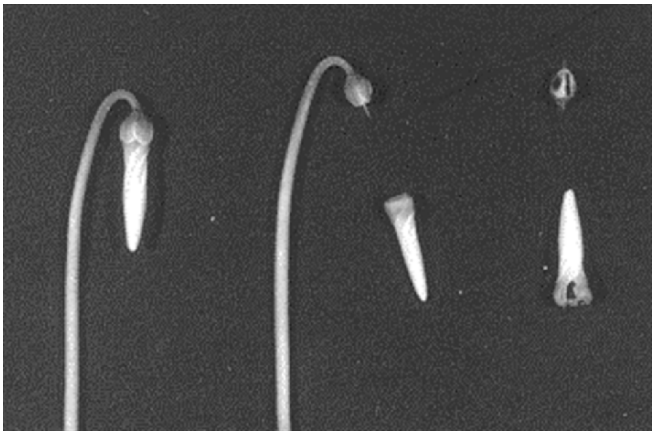

Figure 16 -2. A method of emasculating cyclamen. Anthers are removed by pulling petal parts from a flower bud.

Wellensiek (1959) reported inbreeding depression in cyclamen. In my experience, most cyclamen genotypes show inbreeding depression, though the degree varies with genotype. Inbreeding depression is a severe barriers for producing pure lines in cyclamen. There are diploid and tetraploid cultivars in cyclamen. Takamura and Miyajima (1996a) have reported that the cross compatibility in the reciprocal crosses between diploid and tetraploid cultivars was very low. Thus, breeding between diploid and tetraploid cyclamen is difficult, and cyclamen has been bred within each ploidy level.

2.3 Seed Germination

Seed germination is crucial for the production of seed-propagated plants. Temperature might be the most important factor affecting germination in cyclamen. Suitable temperatures for germination of cyclamen were reported to be in the range of 14 to 22 ˚C (Massante, 1963; Sumitomo and Kosugi, 1963; Neveur et al., 1986; Dottenweich and Röber, 1988; Corbineau et al., 1989), but the exact optimal temperature varies depending on the cultivar (Dottenweich and Röber, 1988). Corbineau et al. (1989) reported the germination percentage at 15 ˚C was higher than that at 20 ˚C, while Sumitomo and Kosugi (1963) reported the inverse result. These differences may be caused by differences of cultivars used in the two reports. However, seed germination at 15 ˚C was quicker than that at 20 ˚C in both reports.

Light is also an important factor for seed germination in cyclamen. Neveur et al. (1986) reported that continuous irradiation with white light inhibited seed germination, even with very low irradiance at an optimal 15 ˚C, and that higher irradiance became a stronger inhibitor. Germination inhibition by continuous irradiation with white light was observed at 10 and 20 ˚C as well as that at 15˚C (Corbineau et al., 1989). Thus, darkness is suitable for the optimum germination in many genotypes of cyclamen.

Gibberellin treatments stimulated the seed germination in cyclamen in previous reports (Anderson and Widmer, 1975; Neveur et al., 1986). Anderson and Widmer (1975) reported that gibberellin solutions accelerated seed germination. Neveur et al. (1986) showed 10^{-5} to 10^{-3} M GA3 reduced inhibitory effects on seed germination, such as light and supra-optimal temperature. Thus, the gibberellin treatments are effective for seed germination of cyclamen, especially in non-ideal germination conditions. However, gibberellin treatment is probably unnecessary in commercial propagation using seeds with enough germinability under the optimal condition for the germination.

Sumitomo and Kosugi (1963) reported that the days from the seed harvesting to the sowing affected germinating duration in cyclamen seeds. The slow and irregular germination of seeds sown within several days after harvesting is often observed in many genotypes of cyclamen, though the degree varies with the genotypes. However, Anderson and Widmer (1975) reported that seed dormancy was probably not a factor affecting germination of cyclamen seeds older than six months. Seed dormancy in cyclamen is rarely a serious problem in commercial production.

3. COMMERCIAL PRODUCTION AND CULTIVARS

Geier et al. (1990) commented that the most important cyclamen production region has been Europe, particularly Germany and the Netherlands, but production occurs throughout the temperate zone. Cyclamen is the most important and popular

potted plants in Japan and very popular in USA. In each area, many cultivars have been developed for specific climate conditions and market demands.

3.1 Commercial Use and Marketing

Cyclamen production is a big market. Europe, Japan and USA may be important regions for the market. More than five million potted plants are sold in USA, and about 17 million potted plants are produced in Japan annually. Grey-Wilson (2002) reported that many millions of potted cyclamen plants are sold on the European continent every year.

Potted cyclamen plants are not so expensive for personal use in Europe and USA. This may be due to development of mass production methods, including reducing labor costs and cultivation period. However, potted cyclamen in Japan are expensive as compared with Europe and USA, because Japanese consumers demand high quality plants, produced by special intensive labor of producers. The market reflects the life-style, custom and culture in the area. The use of cyclamen varies with country. In Europe and USA, the plants are sold for the Christmas season. Some consumers in Japan buy the potted plants as a special gift, a year-end present based on a Japanese custom. Cyclamen plants are mainly used as potted plants in almost all area. The plants are also used for garden flowers in some Mediterranean-climate regions. The use for cyclamen as garden flowers will become more important part of cyclamen production. There has been a rapid increase in the production of garden cyclamen even in Japan with hot summer season for these two or three years. The use as cut flower is not important part of cyclamen production (Grey-Wilson, 2002), but not rare in Europe, especially in Germany. Some cultivars for cut flower production were bred in Europe, but cut-flower cyclamen production is not popular in USA and Japan.

Thus, the trends and demands for cyclamen production vary depending on the country and/or the time of year. Cyclamen breeders and growers must satisfy these demands. Breeding of suitable cultivars for the demands and cultivation in optimal conditions for the cultivars are keys to success in the market. Stimulating the market, by advertisement of new products and/or use is also an effective tactics for the success.

3.2 Commercial Genotypes and History of Improvement

The pot size in which commercial cyclamen is grown to maturity varies, ranging from 6 to more than 20 cm. The plants that produced in larger pots than 20 cm are not popular. The petal size of commercial genotypes also varies. Tanaka (1994) classified four types according to flower size; 1) very large-flowered type (>7 cm in length), 2) large-flowered type (5–6 cm), 3) medium-flowered type (4–5 cm), and 4) small-flowered type (<4 cm). Concerning flower color, commercial genotypes

with white, purple, scarlet or yellow petals are available now, and the intensity of the colors varies from pale to strong. Many genotypes have a reddish or purplish "eye" in the basal part of the petals, and some cultivars express Picotee or stripes in the petals. There are some unique characteristics in the flower shape in addition to the common flat-type; fringed-, crested-, and double-flowered types are available. The commercial cyclamen displays various flower types combined with these characteristics mentioned above (Figure 16-3). Thus, many flower types are available in commercial cyclamen today.

Figure 16-3. Some examples of *Cyclamen* flower types.

The main focus on cyclamen breeding has been improvement of floral characteristics, though cyclamen has also attractive leaves. The size of flower in wild *C. persicum* is small, and major genotypes of commercial cyclamen, until the middle part of the nineteenth century, were small-flowered types. In regard to flower size, many cyclamen breeders may regard the year of 1870 as an important year in the history of cyclamen breeding. A very large-flowered "Giganteum" cyclamen was presented in this year (Doorenbos, 1950). After that, large-flowered cultivars became popular. Many large-flowered commercial cultivars as well as middle- or small-flowered cultivars are available today.

Flower colors in almost all wild *C. persicum* plants are purple, white and neutral tints including pale purplish-pink. Doorenbos (1950) reported that cyclamen with petal color ranging from lilac, rose, purple and crimson had already existed in England around 1870. In 1894, a cultivar 'Salmon Queen' with salmon-colored petals was introduced (Doorenbos, 1950). The introduction was a turning point in the improvement of flower colors in cyclamen. Many genotypes with salmon-colored petals were bred after this introduction. An important breakthrough also came in 1982 when a yellow-flowered individual was found in an inbred population

of diploid white-flowered cyclamen (Miyajima et al., 1991). The yellow-flowered cyclamen was regarded as an invaluable genetic resource for the breeding of cyclamen with new colors (Miyajima et al., 1991). Today, several yellow-flowered cultivars including those with "eye" (Figure 16-4) are available. Thus, flower colors in cyclamen are red, purple, pink, white, yellow and some their neutral tints (Figure 16-5). The color intensity also varies, but no deep-yellow flower is available.

Figure 16 -4. Yellow-flowered cyclamen with eye 'Yellow Girl' (Photo Courtesy: Kage).

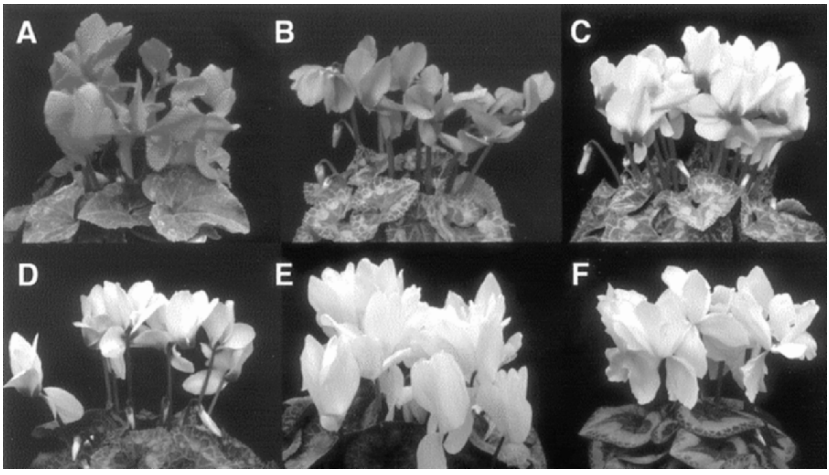

Figure 16-5. Typical petal colors in cyclamen. A, 'Bonfire'; B to D, original strains bred by author; E, 'Golden Boy'; F, 'Pure White'.

Some unique characteristics in flower shape, fringed or crested petals, were found by the end of nineteenth century. Doorenbos (1950) reported that fringed-flowered "Pabilio" and "Rokoko" ("Rococo") type cyclamen were presented in the end of the nineteenth and the early parts of the twentieth century, respectively. A remarkable old cultivar 'Viktoria' ('Victoria') and 'Victoria'-type cyclamen plants (Figure 16-6) with fringed white petals and a crimson margin and "eye", have been popular for a long time. On the other hand, crested-flowered cyclamen, popular about 1900, is not as popular today.

Figure 16 -6. Fringed flowers of *Cyclamen* 'Victoria'.

Leaf characteristics have been also improved. The color, size, and pattern of the leaf blade are important. It is also important that the leaf characteristics are suited to the flower and plant form. Many types of leaves are available in cyclamen. "Rex" type, of which periphery of leaf blade is silver like *Begonia rex-cultorum*, is one of them.

Although diploid and tetraploid cultivars are available, the trends of commercial potted plants are toward diploid F1 cultivars. However, the major cultivars around 1960 were tetraploids, including aneuploids, as Legro (1959) reported. In general, tetraploids have some advantages like larger flowers and/or leaves, and these advantages might be why tetraploid cultivars were more often used around 1960. However, some typical disadvantages in tetraploid, such as complex inheritance of phenotypes, are serious problems. The disadvantages changed the trends of cultivars. Since Wellensiek (1961) indicated the difficulty of cross breeding in tetraploid cyclamen, the diploid cultivars have been increasing. The fact that some diploid large-flowered cultivars existed might be also one of the driving forces behind the change to diploids.

When most popular cyclamen cultivars were tetraploids, F1 cultivars were not popular. However, demands for uniform and vigorous plants and development of diploid cultivars seemed to make F1 cultivars more popular. Clones, pure lines, or F1 hybrids are required to produce uniform products. Production of cyclamen clones is not easy because no genotype propagates vegetatively and commercial micropropagation in many genotypes is still difficult. It is also difficult to breed vigorous pure lines, because of inbreeding depression. Therefore, F1 hybrid cultivars are valuable for satisfying the demands. Today's trend for cyclamen production is diploid F1 hybrids with medium or small flowers, and many F1 cultivars have been bred.

4. BREEDING

Until now, a large number of cyclamen cultivars have been bred. Knowledge on some cyclamen traits, genetics and well-developed breeding technique were useful and helpful for the breeding program. Thus, development of the researches on cyclamen traits and breeding techniques creates a great possibility in cyclamen breeding.

4.1 Traits and Genes Identified

Petal color is an important characteristic in cyclamen. The main pigments in cyclamen petals are flavonoids. Anthocyanins in cyclamen petals cause purple-, pink- and red-flowered cyclamen cultivars. Some peonidin glycosides and/or malvidin glycosides are identified as main pigments in petals of the cyanic cultivars (Van Bragt, 1962; Miyajima et al., 1990; Webby and Boase, 1999). A dominant gene, named *W*, causes anthocyanin synthesis in cyclamen petals, and the white-flowered plants are homozygous recessive *ww* (Wellensiek et al., 1950; Wellensiek, 1952; Seyffert, 1955a,b; Van Bragt 1962). Some white-flowered cultivars have flavonol glycosides as primary pigment in the petals and almost no anthocyanin (Van Bragt, 1962; Takamura et al., 1995), but flavonol glycosides are also detected in some cyanic cultivars. Seyffert (1955a,b) and Van Bragt (1962) reported that a gene, named *F*, control flavonol synthesis. Yellow petal color in cyclamen is caused by accumulation of a chalcone glycoside in the petals (Miyajima et al., 1991). A homozygous recessive genotype *chch* causes the lack or defectiveness of chalcone isomerase for the chalcone accumulation and determines yellow-flowered phenotypes in cyclamen (Takamura et al., 1995, 2000). Some other genes or traits in pigmentation of cyclamen petals were also reported (Wellensiek et al., 1950; Wellensiek, 1952; Seyffert, 1955a,b; Van Bragt 1962). However, knowledge on color expression and the inheritance in cyclamen petals is not enough for establishing systematic breeding programs for improving flower color. More

genetic, chemical and physiological investigations on flower color expression in cyclamen are expected to progress the breeding programs.

In yellow-flowered cyclamen, an interesting trait in color expression and pigmentation is observed: The chalcone glycoside, which is the main pigment in the petals of yellow-flowered cyclamen, is contained not only in the petals but also some vegetative organs (Takamura et al., 1993; 1995). Even in seedlings, the vegetative organs in yellow-flowered cyclamen are yellowish as compared to those in most cultivars without yellow flowers. Therefore, plants predicted to have yellow flowers can be selected in the seedling stage in breeding programs. This rapid detection is useful for breeding because the yellow-flowered characteristic is recessive, as mentioned above. If such rapid detection of other desirable characteristics is possible in other cultivars, it will be very useful for breeding programs. However, few correlations between characteristics in mature plants and seedlings are available.

Although no triploid cyclamen cultivar is available, triploid individuals were obtained by reciprocal crosses between the diploids and tetraploids (Wellensiek, 1955; Legro, 1959). However, many F1 progenies of the reciprocal crosses in these reports and all the F1 progenies in our report (Takamura and Miyajima, 1996a) were tetraploid. The fertilization between an unreduced gamete from a diploid and a normal reduced gamete from a tetraploid is the main mechanism of the tetraploid formation (Wellensiek, 1955; Takamura and Miyajima, 1999, 2002). Unreduced male gametes in cyclamen are easily found because the pollen is larger than reduced pollen. On the other hand, abortion of zygotic embryos in the crosses (Takamura and Miyajima, 1996b) might lead to no or few triploid production. It is possible to rescue the embryos before abortion, and many plants obtained by the embryo rescue are triploids (Takamura and Miyajima, 1996d; Takamura and Yoshimura, 2001).

4.2 Breeding Techniques

Almost all cyclamen cultivars have been bred by cross breeding within *C. persicum* or using spontaneous mutation in the species, because vegetative propagation and interspecific hybridization were difficult. The breeding programs using only crosses and spontaneous mutation have limited possibilities for modifying phenotypes in cyclamen, but these programs have been successfully produced many cultivars, including F1 hybrids. Therefore, research on new breeding techniques is desirable and brings great possibilities to cyclamen breeding programs.

4.3 Tissue Culture

Vegetative propagation, including micropropagation, in itself is not a breeding technique in a narrow sense. However, the vegetative propagation technique has great advantages in plant breeding programs, because it enables cloning of the bred

plants. Although Nakayama (1979, 1980) reported vegetative propagation of cyclamen by notching, effective and reliable vegetative propagation *in vivo* is not easy. Therefore, establishment of tissue culture techniques in cyclamen has been sought.

Since Mayer (1956) reported the *in vitro* propagation of cyclamen through organogenesis from tuber explants, many researchers have studied tissue culture of cyclamen. However, tuber explants with high regeneration ability contained systemic microorganisms, which had prevented tissue culture techniques from developing for a long time. Consequently, the explants with few or no microorganisms, such as sections of etiolated petiole (Ando and Murasaki, 1983) and aseptic seedling tissue (Wainwright and Harwood, 1985), were found for tissue culture, but some other tissues, such as leaf blade, are also still used in some cases.

Two main methods are available for plant regeneration of cyclamen by tissue culture, plant regeneration through adventitious organogenesis or somatic embryogenesis. The usual protocol for plant regeneration through adventitious organogenesis is as follows: the explant forms adventitious shoots on medium with cytokinin or a low concentration of auxin, and then the shoots are rooted on medium with auxin or without plant growth regulators. Somatic embryogenesis in cyclamen consists of two culture steps in usual: 1) induction of embryogenic callus in medium with auxin or with auxin and low concentration of cytokinin, 2) formation of embryoids from the callus in medium without plant growth regulator (Figure 16-7). Plant regeneration through adventitious organogenesis is possible in many genotypes of cyclamen, though the procedure is laborious and the multiplication is not efficient. On the other hand, many clones can be obtained by somatic embryogenesis, but the genotypes with the potential of somatic embryogenesis are limited.

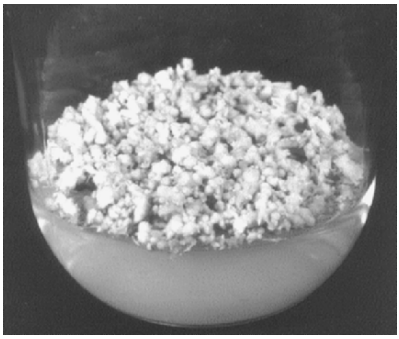

Figure 16 -7. Somatic cyclamen embryos grown *in vitro*.

Micropropagation of cyclamen through both adventitious organogenesis and somatic embryogenesis has common problems; high cost of plant production, mutation, abnormal morphogenesis, genetic difference in regeneration ability, etc. Consequently, only a few cultivars are produced by tissue culture. However, tissue culture is useful for new breeding techniques, such as in vitro chromosome doubling, anther culture, embryo rescue, and gene transformation. It is sure that the new techniques will progress cyclamen breeding programs, as will be seen later.

4.3.1 Interspecific Hybridization

In many plants, interspecific hybridization is an effective method for varying phenotypes. Wild *Cyclamen* species, except *C. persicum*, have desirable characteristics for improving commercial cyclamen cultivars, including different flowering time from that in *C. persicum*, cold or heat tolerance, disease resistance, and strong and sweet fragrance. Therefore, interspecific hybridization between *C. persicum* and other *Cyclamen* species should be useful for cyclamen breeding. It was, however, difficult to produce progenies in interspecific hybridization because of cross-incompatibility (Legro, 1959).

For the difficult interspecific hybridization, Ishizaka and Uematsu (1990, 1992, 1995a) broke a path: they adopted ovule culture as an embryo rescue technique for hybrids between commercial cyclamen cultivars and some other *Cyclamen* species. This was a large step in cyclamen breeding. The production of hybrids by embryo rescue in some other cross combinations between *C. persicum* and its wild relatives (Table 16-1), followed their success. Hybrids between diploid commercial cyclamen and *C. purpurascens* had fragrance like *C. purpurascens* (Ishizaka and Uematsu 1995a; Ewald, 1996), and interspecific hybridization between commercial cyclamen and *C. graecum* produced hybrids with disease resistance like *C. graecum* (Ishizaka, 1996). Thus, progenies with desirable characteristics, which have never shown in *C. persicum*, were produced by interspecific hybridization. These successes will contribute to the progress of cyclamen breeding because breeding of commercial cyclamen had been made only within *C. persicum*. However, breeding programs using interspecific hybridization has some problems, including sterility in the hybrids. Meiotic abnormalities in the hybrids, such as lack of sufficient chromosome pairing and formation of micronuclei, cause the sterility (Ishizaka, 1994; Ishizaka, 1997; Ewald et al., 2000). To overcome this problem in a breeding program, amphidiploidization should be effective (Ishizaka and Uematsu, 1994, 1995b) and is mentioned later.

Table 16-1. Reports of interspecific hybrids between *C. persicum* and other *Cyclamen species* obtained by embryo rescue *in vitro.*

Seed parent (female)	Pollen parent (male)	Citation(s)
C. persicum	*C. africanum*	Takamura et al. (2002)
	C. graecum	Ishizaka (1996)
	C. hederifolium	Ishizaka and Uematsu (1992)
	C. libanoticum	Sibusawa and Ogawa (1997)
	C. purpurascens	Ishizaka and Uematsu (1995a), Ewald (1996)
	C. repandum	Ishizaka and Uematsu (1990)
	C. rohlfsianum	Sibusawa and Ogawa (1997)

4.3.2 Polyploidization and Haploid Production

Polyploidization, including mitotic and meiotic methods, is an useful breeding technique. Both mitotic and meiotic polyploidization in cyclamen are possible, as *in vivo* and *in vitro* chromosome doubling by using colchicine treatments (Ishizaka and Uematsu, 1994, 1995b; Takamura and Miyajima, 1996c; Takamura et al., 1998) and tetraploid production by using unreduced pollen in 4*x* x 2*x* cyclamen crosses (Takamura and Miyajima, 1994, 1996a) has been reported. For overcoming the sterility of interspecific hybrids, amphidiploid production is effective. Ishizaka and Uematsu (1994, 1995b) induced amphidiploids of interspecific hybrids obtained by commercial diploid cyclamen *C. hederifolium* or *C. purpurascens* by colchicine treatment. The amphidiploids could produce progenies by selfing, indicating that breeding programs by using interspecific hybridization could progress. These colchicine treatments were applied to hybrid ovules *in vitro* or mature plants *in vivo*. Treatment through *in vitro* organogenesis was applied to induce tetraploid yellow-flowered cyclamen from the diploids (Takamura and Miyajima, 1996c; Takamura et al., 1998). The induced tetraploid yellow-flowered plants had larger and deeper yellow petals than the diploid relatives, suggesting that polyploidization could be one of the methods for improving flower characteristics. Meiotic polyploidization by using unreduced pollen was also used for breeding of tetraploid yellow-flowered cyclamen (Takamura and Miyajima, 1994).

Thus, polyploidization has great potential to develop the breeding of cyclamen, and some new forms have been produced in breeding programs including the polyploidization. However, polyploids, except amphidiploids, have the disadvantages like complex inheritance and the difficulties in producing uniform plants as mentioned above. Therefore, the development of an efficient vegetative propagation protocol is desirable for overcoming these problems.

Haploid production is also useful for breeding programs in cyclamen, because establishment of pure lines in cyclamen is difficult. Dihaploid must be homozygous, and these plants should be suitable for producing uniform plants as pure lines or parents of F1 cultivars. Ishizaka and Uematsu (1993) have already produced pollen-derived haploids in cyclamen through anther culture, but the efficiency of haploid

production depended on the genotypes of mother plants. Although improving the abilities of haploid production is required to introduce the anther culture technique into cyclamen breeding (Ishizaka and Uematsu, 1993), it is sure that the haploid production technique has a great possibility to improve breeding programs.

4.3.3 Gene Transformation

Gene transformation is a forefront plant breeding technique. Many attempts have been made to apply this technique to improve ornamental plants. However, only minimal information on the transformation systems in cyclamen is available now. Aida et al. (1999) documented on the transformation of cyclamen and they obtained transgenic plants by an *Agrobacterium tumefaciens*–mediated transformation. The gene transformations were made through organogenesis from explants of the etiolated petiole sections of aseptic cyclamen seedlings. The success should be a great step for progressing cyclamen breeding.

Gene transformation and cell fusion are breeding techniques that can introduce a specific characteristic into a plant without using crosses. That is, these techniques can be applied to induce specific genes of other organisms without cross-compatibility. Specifically, a single gene can be introduced into the genome of target plants in gene transformation. Therefore, repeated back crossing is not necessary in breeding program using gene transformation. Thus, the transformation has great advantages as compared to other breeding techniques and should open new possibilities in cyclamen breeding program.

5. BREEDING DIRECTIVES FOR FUTURE RESEARCH

Breeders should create plants that attract the market's attention and satisfy consumer's requests, and never bore or disappoint the markets and consumers. That is, the markets and consumers always require the breeders to create novel and original plants. Growers also require the breeders to produce valuable plants that are convenient for cultivation. The breeders must plan a dependable breeding program and develop tactics for the breeding and production of new plants. Marketing research, dependable breeding objective, fundamental knowledge on breeding, and effective breeding technique are needed to establish the program and tactics. Consequently, these factors and breeding directives control each other.

Demands of market influence breeding objectives directly. However, the demands of cyclamen production vary depending on the country and times, as mentioned above. Although today's trend for cyclamen is diploid F1 hybrids with medium or small pastel-colored flowers, no one can assure that the F1 hybrids will be the trend ten years from now. Therefore, breeders need to pay attention to and

cope with movement of the demands. However, there are some common objectives in cyclamen breeding such as varying petal color and introducing new valuable characteristics like resistance to disease or strong fragrance.

Today's cyclamen breeding program using crosses or spontaneous mutation within diploid or tetraploid *C. persicum* should be limited. Many desirable characteristics, especially new flower colors and disease resistance, are not available in *C. persicum*. Therefore, new breeding techniques such as interspecific hybridization and gene transformation should be the key to make cyclamen breeding program rapidly progress, and more research will be desired.

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Chapter 17

HIBISCUS

Hibiscus rosa-sinensis

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Abstract: Approximately 300 tropical and sub-tropical *Hibiscus species* exist as small trees, shrubs, and herbs. Commercial species have a wide variety of uses, including fibres, food, medicinal, and ornamental. The most popular ornamental species is *H. rosa-sinensis,* used both as an indoor and outdoor potted and landscape plant, respectively. A variety of flower forms, flower colors, vigour, and growth forms exist. This species is a complex hybrid group arising from several *Hibiscus* species, including *H. liliflorus, H. fragilis, H. boryanus, H. arnottianus, H. kokio, H. storckii,* and *H. denisonii.* A wide range in polyploidy (including aneuploidy), polymorphism, and heterozygosity exists within the cultivars of *H. rosa-sinensis*. Thus, stable cultivar phenotypes are maintained and propagated vegetatively. While little genetic information and trait heritability has been established for this crop, plant breeding continues to produce new variants for commercial production. Future breeding and genetic studies are needed to further transform this crop.

Key words: Self compatibility, polyploidy, aneuploidy.

1. INTRODUCTION

Hibiscus L. is the type genus of the tribe Hibisceae of the family Malvaceae (Borssum-Waalkes 1966). The genus contains about 300 species that grow in tropical and sub-tropical regions throughout the world. They take the form of small trees, shrubs and herbs. Many of the species yield bast fibres, notably, *H. cannabinus* L. (Anderson and Pharis 2003), *H. sabdariffa* L., *H. surattensis* L., *H. planifolius* Sweet, *H. flococcus* Mast. and *H. macrophyllus* Roxb. are locally used for fibre in Asia. In West Africa, *H. lunarifolius* Willd., *H. rostellatus* Guill and Perr, *H. squamosus* Hochr. and *H. tiliaceus* L., a small tree of tropical shores

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throughout the world, yield bast fibres for making ropes and mats throughout the Pacific, in Malaysia and in the Sundarbans and Adaman Islands (Cobley 1976).

Some species are of value as sources of food and medicines. For example, *H. cannabinus* and *H. sabdariffa* leaves are consumed as green vegetables while the swollen calyces of *H. sabdariffa* are eaten raw in salads or used in making marmalades. The dried calyces of *H. sabdariffa* are used for making an infusion beverage locally called "zobo" in Nigeria. Leaves of *H. acetosella* Welw. and *H. surattensis* are used as medicines to cure some stomach ailments (Wilson and Menzel 1964; Ugborogho and Shofoyeke 1983).

Several species of the genus are used as ornamentals. *Hibiscus rosa-sinensis* L. has long been cultivated in China, India, Japan and the Pacific islands, and is now one of the most widely planted ornamental shrubs throughout the tropics. It is a very important potted plant crop in Europe, United States of America and other temperate region countries, where the temperature does not fall below 12^{o}C. (Hughes and Hughes 2003). The East-African *H. schizopetalus* Hook. is also grown as an ornamental and its hybrids with *H. rosa-sinensis* do occur. *H. acetosella* Welw. is widely planted in Nigeria and other West African countries. *H. mutabilis* Linn. (changeable rose) has long been cultivated in China: In one form of *H. mutabilis*, the double flowers are white in the morning and turn red by evening (Wilson and Menzel 1964; Purseglove 1974).

In the Eastern United States of America, the rose mallows have been developed as ornamentals since colonial times. They produce abundant, large, brightly coloured flowers that make them more noticeable. Rose mallow species are herbaceous perennials and include *H. coccineus* (Medicus) Walter, *H. grandiflorus* Michx, *H. lasiocarpus* Cav., *H. moscheutos* Linn. and *H. militaris* Cav. Seeds of *H. moscheutos* have been reported to be used medicinally (Winters, 1970).

Borssum – Waalkes (1966) provided a key for the separation of the genera in the tribe Hibisceae. The genus *Hibiscus* was described as follows:

> "Ovary with more than one ovule per cell; capsule usually with more than one seed per cell. Calyx not (or rarely) splitting on one side during anthesis, 5-lobed to 5-parted, not adnate to the corolla, persisting after flowering".

The five antesepalous stamens are represented by teeth at the top of the pollen tube (Willis, 1988). The flowers are actinomorphic, bisexual; while the calyx, corolla, carpel are pentamerous. Stamens are monadelphous.

Hibiscus rosa-sinensis, otherwise called Rose of China or shoe flower, had been grown in China, India, Japan and the Pacific Islands long before the European discovery era. The woody shrub has showy flowers and is now cultivated as an ornamental throughout the tropics and sub-tropics (Kimbrough, 1997). The plants

exhibit many variations in form and flower colour. The flowers are of different sizes, depending on the variety, and may be single or double. Up to 75 forms of the species have been recorded (Sharma and Sharma 1962). Cytologically the species exhibits high polyploidy and aneuploidy (Singh and Khoshoo 1989) and is usually propagated vegetatively.

2. HISTORY AND DOMESTICATION

There is no known record of the history of the domestication of *H. rosa-sinensis.* In one view it is believed that the species is a native to China and was first cultivated by the Chinese for its showy flowers (Kimbrough 1997). No extant wild populations of the species have however, been reported from that country. Another view holds that the species is a native of the South-Indian Ocean islands of which Madagascar, the Mascarene Islands, and the Rodriguez Islands are a part (Borssum – Waalkes 1966). This view is strengthened by the fact that three *Hibiscus* species genetically compatible with *H. rosa-sinensis* are native to this area (Singh and Khoshoo 1989). It is thought that the species was collected and domesticated on these islands by far-easterner during their westward migration.

An attempt has been made by Singh and Khoshoo (1989) to study the evolution of *H. rosa-sinensis.* They have shown that the species is a complex produced by the hybridization of mainly two groups of species. One of these groups is native to the South-Indian Ocean Islands and the East African Coast and consists of *H. schizopetalus* (East-African Coast), *H. liliiflorus* Cav. (Mauritius and Rodriguez Islands), *H. fragilis* and *H. boryanus* Hook and Arn. (Reunion Islands). The other group from the Pacific islands, consists of *H. arnottianus* Gray, which is synonymous with *H. wimeae* Heller according to Roe (1961), *H. kokio* Hillebrand (both from the Hawaiian islands), *H. storckii* Seeman from Fiji, a primitive form of *H. rosa-sinensis* (Skovsted 1941) and *H. denisonii.* The species are fully intercompatible and hybridize freely without regard to the chromosome number of the parents. The authors, therefore, believe that these two groups of species each represent one species, and that their hybridizations have given rise to the present-day diversity in *H. rosa-sinensis.* The cultivars are highly polymorphic and highly heterozygous and produce a wide range of growth habits, vigour, and flowers colour and form. These wide ranges are believed to be the result of introgressive segregation and the interaction of new gene combinations produced during recombination (Singh and Khoshoo 1989).

Cytologically, the species contains a polyploid-aneuploid range as well as varying base numbers (x = 7, 11, 12, 15, 16, 17, 18, 19, 20) (Darlington and Ammal 1945). *Hibiscus rosa-sinensis* is in the group of species with x=18 as a base number. However, there are twenty-seven different aneuploid chromosome numbers within *H. rosa-sinensis,* starting with ‘Queen’ ($2n$ = 46) to ‘Giant Rose’ ($2n$ = 144). At

meiosis, the most common associations are bivalents and univalents. Despite this, there is generally high pollen fertility. This possibly results from the polyploidy, where there are many copies of the genic material and cytological abnormalities are well-tolerated, so that chromosome deficient pollen grains are still fertile (Singh and Khoshoo 1989).

The level of polyploidy has risen to between 4x and 25x probably due to fertilizations involving *2n* or unreduced gametes (Sharma and Sharma 1962). This very heterogeneous *H. rosa-sinensis* complex has been stabilized and perpetuated by efficient vegetative reproduction. Sharma and Sharma (1962) observed that in *H. rosa-sinensis,* as in other species that reproduce vegetatively because of the rarity of seed set, sexual reproduction does not contribute to evolution. Evolution is rather accomplished by the origination of new forms through the participation of nuclei with variant chromosome numbers in the formation of daughter shoots (Skovsted 1935; Sharma and Sharma 1962) and mutations which have been extensively observed in the species. Such evolved varieties include *H. rosa-sinensis* var. *cooperi* Nichols, *H. rosa-sinensis* var. *carneoplenus* Hort. and *H. rosa-sinensis* var. *rubroplenus* (Singh and Khoshoo 1989).

3. CENTRES OF DIVERSITY

Hibiscus rosa-sinensis has been grown all over the tropics and sub-tropics but by far the greatest diversity of the species is found in India, China, South-East Asia as well as the South-Indian Ocean islands. These are the areas where the primitive forms of the species were first domesticated (Borssum-Waalkes 1966; Singh and Koshoo 1989; Kimbrough 1997). These centres may still habour the primitive forms of the species. Skovsted (1941) theorized that *H. storckii* Seemann, found on the island of Fiji, is a primitive form of *H. rosa-sinensis.*

Secondary centers of diversity can be found in the Canary Islands, Madiera and Mascarene Islands. Both the Canary Islands and Madiera were centers for the acclimatization of tropical and sub-tropical plants, including ornamentals, before final introduction to Europe. The Mascarene Islands served as a stopover along the trade routes from the Orient to Europe around the cape of South Africa.

A centre of diversity of special note is the Hawaiian islands. Here *Hibiscus* hybridization, which was first accomplished in 1872 by Governor Archibald Cleghorn of Oahu, had enthused numerous amateur hybridizers to produce thousands of complex hybrids whose parentage is now impossible to trace (Roe 1961). Hawaiian *Hibiscus* includes species which closely resemble *H. rosa-sinensis* in vegetative and floral characteristics, and can easily cross with each other and with varieties of *H. rosa-sinensis* thereby contributing genomes to the *H. rosa-sinensis* complex (Singh and Khoshoo 1989).

The East African Coast, Mauritius and Rodridguez islands are the homes of *H. schizopetalus, H. lilliiflorus, H. fragilis* and *H. boryanus,* respectively, which have also contributed genomes to the *H. rosa-sinensis* complex. The "Butterfly" variety of *Hibiscus* is reported to have resulted from a cross between *H. schizopetalus* and *H. liliiflorus* at the Singapore Botanic Garden (Singh and Khoshoo 1989).

4. REPRODUCTIVE BARRIERS

Hibiscus species are largely self-compatible (Allard 1960). The flowers are large, borne singly on the stalk, bisexual, and perfect. Outer whorls consists of six to ten epicalyx segments and a fused calyx which is five-parted at the top and completely surrounds the inner whorls in the bud. The corolla consists of five petals which are relatively large and brightly coloured being of all shades from white to pink, to orange, to red, depending on the variety (Kimbrough 1997). The stamens are formed into a staminal tube, which encloses the style and is five-pointed at the top; a mass of short filaments arises towards the top third of the tube bearing the monadelphous anthers. From the top of the staminal tube emerges the stigma, which consists of five very short style branches bearing a patch of stigma at the tip (Roe 1961; Borssum-Waalkes 1966). The petals, the staminal column and stigma may be variously modified, especially in double flowered varieties.

On the day before anthesis, the growth of the corolla suddenly spurts and it breaks through the calyx. The exposed portion of the corolla may be from a half to two-thirds of the flower bud; the petals are less tightly packed but nevertheless do not open. It is at this stage that emasculation of the flower bud is done (Akpan 1988). Using fine forceps, the petals are carefully opened with as little damage as possible. The anthers are then carefully picked-off from the staminal column. Studies have shown that *Hibiscus* flowers are very sensitive to damage (Ugborgho and Shofoyeke 1983), and when all petals and anthers are removed the flowers are incompetent to take part in successful crosses (Akpan 1988). Due to these reasons, only the anthers are removed (Akpan 1991).

Very early on the day of anthesis, as soon as the flower opens, pollen grains from a freshly opened flower of the male-parent plant are dusted onto the stigma of the emasculated flower of the female-parent plants. A tag bearing the particulars of the cross is placed on the pollinated emasculated flower and the cross is then observed. If the capsule grows to maturity, the hybrid seeds are harvested and sown. Hybrid *Hibiscus* are very easy to detect at the seedling stage because they tend to blend the genotypic and phenotypic characteristics of both parents thereby becoming distinguishable, provided the parents differ (Akpan and Hossain 1996).

It is very important to make the crosses very early on the day of anthesis especially if the emasculated flower was not covered-up. This avoids the action of insect pollinators, which are attracted to the brightly coloured, large flowers.

Under normal tropical conditions, seed set in *H. rosa-sinensis* is very rare (Sharman and Sharma 1962). There have been no reports as to whether this failure to set seeds is due to incompatibility, failure of the pollen to germinate on the stigma, failure of the pollen tube to grow in the style, failure of the zygote to develop, or failure of the endosperm to develop. Sanyal (1958) showed that hybrids were not produced between *H. cannabinus* and *H. sabdariffa* because of the failure of the endosperm to develop. Menzel and Martin (1970, 1971) overcame this type of problem in *Hibiscus* hybrids by dissecting out and culturing the embryo in an appropriately constituted medium.

H. rosa-sinensis is reported to have a fairly high pollen fertility of over 60% (Singh and Khoshoo, 1989). There are no reports on the viability of the ova but it is probable that these are viable because female gametogenesis is not as easily upset by chromosomal and genic disharmonies as male gametogenesis (Allard 1960). Seed set in *H. rosa-sinensis* is very rare (Sharma and Sharma 1962). The causes of this occurrence in the species has not been reported. However, in the related genus *Gossypium,* Brown and Menzel (1952) found that hybrids between some wild species aborted between early embryology, after meiosis has taken place. Silow (1941) attributed this behaviour to general genic disharmony in the hybrids. Other studies of interspecific hybrids in cotton revealed that hybrid inviability was controlled by simple genetic mechanisms (Stephen 1950; Gerstel 1954).

Even though reports of successful seed-set in *H. rosa-sinensis* are sporadic (Sharma and Sharma 1962), greater success may be attained if the genetic history of the varieties of the species is known and used in the breeding programme. Allard (1960) held that if a variety, as an F_1 hybrid, is backcrossed as the seed parent to one of its parental species (or variety), then there is a good prospect of success. He maintains that this is so because female gametogenesis is not as easily upset by chromosomal and genic disharmonies as male gametogenesis. Thompson (1930) pointed out that the cross would be more successful if the female parent has the higher chromosome number than the male parent.

5. TRAITS AND GENES IDENTIFIED

Due to the complex nature of the species, there is almost no genic information on the species. Singh and Khoshoo (1989) have reported that the only genic information available is that pink flower colour is dominant over red flower colour.

H. rosa-sinensis is rich in traits of horticultural interest, a sample of which are as follows:

(i) The plant is a woody shrub between 1.0m and 4.0m in height depending on the variety, it can be densely or sparsely branched. The branches may be stiff and upright or flexible and pendulous. They may be short or long. Leaves can be broad or narrow, variegated or

plain green. The flowers may be grouped into recognizable racemous inflorescences or may be borne freely and singly.

(ii) There is a wide range of flower variants in the *H. rosa-sinensis* complex. The flowers have a variety of colours and forms, ranging from shades of white (Fig. 17-1a), pink (Fig. 17-1b), red (Figs. 17-1c, d), to yellow (Fig. 17-1e). Flower types may be single or double and large or petite in size. The staminal column is parted in some double-flowered varieties and may be modified into petals thereby obscuring the five-merous nature of the corolla.

(iii) Most *H. rosa-sinensis* varieties can tolerate low to medium drought levels. Most varieties flower profusely with irrigation but revert to mostly vegetative state in extended periods of drought. During the drought period there is a decrease in growth and diminution in the sizes of the leaves and flowers (Akpan, unpublished data).

Figure 17-1. Flower forms and colours in *Hibiscus rosa-sinensis*: (a) white single, (b) pink double, (c) red single, (d) red double, (e) orange double.

6. COMMERCIAL PRODUCTION

Hibiscus rosa-sinensis is easily propagated vegetatively from cuttings (Dole and Wilkins 1999). No special care is required for successful vegetative propagation other than healthy, live cuttings rooted in irrigated soil media.

Planting of cuttings should be done in the rainy season or when there is adequate irrigation in the dry season. The cuttings will not grow well in the dry season without irrigation.

H. rosa-sinensis can be commercialized in various forms. (Fig. 17-2a, b, c, d). It can be used to form hedges where profusely branches, small-leaved varieties are ideal. The species can be planted singly on lawns, or along pathways. The ideal types here depend on the setting but generally branched types with lax branches, or profusely branched types with short stiff branches are preferred. *H. rosa-sinensis* is excellent in pots (Fig. 17-3) (Clatfelter 1997) and is popularly grown as such in northern European and American climates (Dole and Wilkins 1999).

Brightly coloured flowers, white, pink, red or magenta are generally preferred. The plants can be potted or planted in beds. Slow growing types with lax branches, small/variegated leaves and double, brightly coloured flowers are preferred.

Figure 17 -2. Usage of *Hibiscus rosa-sinensis* as a (a) hedge plant, (b) decorative lawn plant, (c) bedding plant, or (d) lawn plant [*var. cooperi*].

Figure 17-3. Hibiscus rosa-sinensis is a popular indoor flowering potted plant in northern European and American climates. It also is excellent as an outdoor container plant.

7. CROP IDEOTYPE

The ideal *H. rosa-sinensis* as a landscape plant should be ~ 2m tall, well branched, with a hemispherical form. Leaves should be luxuriant and counter balance the brightly coloured flowers. The flowers should be profusely produced more or less throughout the year. The plant should be perennial in warmer climates and slow-growing, or amendable to pruning, not growing into a tall rambling plant within a few years. The plant should not be susceptible to fungal, insect and nematode attacks.

The ideal potted plant should be able to establish quickly, growing vigorously with strong roots (Clatfelter 1997). A potted hibiscus plant should be able to grow back quickly after pruning. It should be able to tolerate regular application of fertilizer and frequent irrigation (Forsling 2004). The plant should be able to grow and bloom in well-lit, warm environments (Dole and Wilkins 1999; Lemke 2000) and should be resistant to bacterial, fungal, insect, nematode and viral attacks, (Clatfelter 1997; Hughes and Hughes 2003).

8. BREEDING/GENETIC DIRECTIONS FOR FUTURE RESEARCH

Cytological and genetic studies should be conducted to determine the causes of non-setting of seeds in *H. rosa-sinensis* and suggest possible remedies. Cytogenetic studies of the species should be enlarged and intensified with the aim of identifying the parental species of the present varieties. This will lead to more confidence in the planning of breeding programmes.

Modern biotechnology methods should be applied to research in *H. rosa-sinensis.* Much useful information can be obtained from *in vitro* cell culture, embryo rescue, and protoplast fusion studies of the species. Such information could lead to the production of new bursts of recombination and the subsequent selection of new varieties, with the potential of extending the species' range. *Hibiscus* researchers around the world should cooperate, exchange information to avoid duplication of efforts and advance research, especially those that work on *H. rosa-sinensis.*

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Chapter 18

LACHENALIA

Lachenalia spp.

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Abstract: *Lachenalia* species are geophytic endemics of South Africa and Namibia. This chapter includes a short overview of the taxonomic and breeding history of the genus. Diversity present in the genus is discussed in terms of morphology, distribution, propagation and genetics. The large diversity and absence of a key for the identification of species emphasizes the importance of investigating the diversity within species and establishing species boundaries. Crossing mechanisms and available information on the reproductive biology of *Lachenalia* are mentioned. The influence of production methods on the selection criteria and selection procedures is discussed. Information on the extent of reproductive barriers in the genus is included. Present and future breeding strategies and research needed to overcome these barriers are discussed. The chapter concludes with future perspectives for research and breeding in the genus.

Key words: bulbs, geophytes, Hyacinthaceae.

1. INTRODUCTION

The genus *Lachenalia* Jacq. f. ex Murray consists of small bulbous geophytes endemic to South Africa and Namibia. This winter-growing genus belongs to the Hyacinthaceae and consists of more than 100 described species (Dold & Philipson, 1998, Duncan, 1998).

Although *Lachenalia* has a long history, commercial products are relatively new to the international flower market. The Roodeplaat Vegetable and Ornamental Plant Institute of the Agricultural Research Council (ARC-Roodeplaat) developed commercial potted plants from the genus in South Africa (Niederwieser *et al.,* 1998). The breeding history and slow commercialization discussed are thus related

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to specific circumstances and South African conditions. Without state funds, invested during the early years of the development, this program would never have been successful.

The large diversity present in the genus was one of the main reasons for selecting it for development. Various phenotypic characters (Duncan, 1988) as well as an unusual variation in chromosome numbers (Kleynhans & Spies, 1999, Spies et al., 2002) describes this diversity. Preliminary studies on the molecular systematics of the genus also revealed high molecular diversity (Kleynhans & Spies, 2000, Spies et al., 2002).

Since the start of work on the genus in 1965 in South Africa, hundreds of crosses have been made and several reports published (Lubbinge, 1980, Malan et al., 1983, Lubbinge et al., 1983a, Lubbinge et al., 1983b, Lubbinge et al., 1983c, Lubbinge et al., 1983d, Ferreira & Hancke, 1985, Hancke & Coertze, 1988, Coertze et al., 1992, Kleynhans & Hancke, 2002). Several species and intra-species varieties of *L. aloides* (L.f.) Engl. have long been available in small numbers, but the developed cultivars offer a superior product to the consumer. Several cultivars have been registered with Plant Breeders Rights and ten cultivars were internationally available during 2001. This successful commercialization would not have been possible without the development of the required production research.

The multiplication phase takes place in South Africa under license to ARC-Roodeplaat. Dry bulbs are exported to commercial forcers abroad where bulbs are then forced, potted and marketed. Bulbs also make good garden and patio subjects. The bright coloured cultivars will provide a rewarding show of flowers for two to four weeks depending on the temperature and climatic conditions. The colour variation available and the good keeping quality are two of the advantages that this new flower bulb crop present to the consumer.

This chapter includes a short overview of the taxonomic and breeding history of the genus. Diversity present in the genus is discussed in terms of morphology, distribution, propagation and genetics. The large diversity and absence of a key for the identification of species emphasizes the importance of investigating the diversity within species and establishing species boundaries.

Crossing mechanisms and available information on the reproductive biology of *Lachenalia* are mentioned. The influence of production methods on the selection criteria and selection procedures is discussed. Information on the extent of reproductive barriers in the genus is included. Present and future breeding strategies and research needed to overcome these barriers are discussed. The chapter concludes with future perspectives for research and breeding in the genus.

2. HISTORY

2.1 Taxonomy

The taxonomic history of *Lachenalia* (Hyacinthaceae) extends over a period of more than three centuries. The earliest record of the genus is a painting of *L. hirta* (Thunb.) considered to be a painting used by Simon van der Stel of the Dutch East India Company to illustrate his diary of the expedition undertaken to Namaqualand during 1685/86. During the period from 1686 to 1784 several reports were made of plants now classified as *Lachenalia.* The first published report on the genus *Lachenalia* appeared in "Linnaeus Systema Vegetabilium" Ed. 14 in 1784. The correct citation for the genus is *Lachenalia* Jacq. f. ex Murray (Duncan, 1988). Jacquin named the genus after a Swiss Professor, Werner de Lachenal.

In 1896-1897 Baker published his monograph on the genus in "Flora Capensis" Vol. VI (Baker 1897; citing forty-two species. Since the publication of Baker's monograph, Winsome F. Barker undertook most of the taxonomic work on *Lachenalia*. She published forty-seven species and eleven new varieties for the genus (Barker, 1933a & b, Barker, 1966, Barker, 1969, Barker, 1972, Barker, 1978, Barker, 1979, Barker, 1980, Barker, 1983, Barker, 1984, Barker, 1987, Barker, 1989).

In 1988, Graham Duncan of the Kirstenbosch Botanical Gardens published "The Lachenalia handbook" (Duncan, 1988). This handbook contains introductory notes on history, identification and cultivation, with descriptions of 88 species and colour illustrations. Graham Duncan has continued the work of Winsome Barker and is still in the process of describing new species (Duncan, 1996, Duncan, 1997, Duncan, 1998, Duncan, 1999 a, b, c & d, Duncan, 2001a &b).

As yet no formal key to the identification of the more than 100 described species has been published. Graham Duncan is working on a morphological revision of the genus by investigating morphological character diversity in and among species (Pers. Comm., G Duncan, January 2002).

2.2 Breeding and Commercialisation

Rev. John Nelson raised the first authenticated hybrid of *Lachenalia* in or about 1878 (Moore, 1905, Crosby, 1978). This was a cross between *L. aurea* and *L. tricolor luteola.* As these are now both varieties of *L. aloides* this was thus an intra-species cross. Other hybrids reported earlier proofed to be self-pollinations. The first inter-species cross (a cross between *L. aurea* and *L. reflexa* Thunb.) was also made by Rev. Nelson (Moore, 1905). Since then several hybrids have been mentioned in literature (Moore, 1905, Crosby, 1978 for review), but none of these became commercial products probably because the so-called hybrids were selfs of

pure species. Other combinations were published but never referred to again (Crosby, 1978). Crosby (1987) therefore concluded that the majority of early claims of inter-specific hybridization in *Lachenalia* could not be substantiated. The only reliably authenticated inter-specific hybrids (made on more than one occasion) seem to be crosses between *L. aloides* and *L. reflexa.* Crosby (1987) and Lubbinge (1980) were the first to report on a number of different inter-specific crosses in later years.

Despite all these claims of hybrids, *Lachenalia* has commercially remained relatively unknown with only limited numbers of claimed hybrids and species being commercially available until recently (De Hertog & Le Nard, 1993). The first improved hybrids developed in South Africa became commercially available during 1997/98. Progress in the breeding program was slow due to several problems, of which some are unique to the South African environment (Kleynhans & Hancke, 2002, Niederwieser et al., 2002]. One unique problem was the political isolation of the country up until the early 1990's. Isolation prevented researchers from networking with colleagues abroad.

Progress made since 1965 with the breeding programme in South Africa can be divided into five phases (Niederwieser et al., 1998, Kleynhans et al., 2002]. During Phase I which extended over a seven-year period from 1965-1972 the programme consisted of a small gene bank. Basic procedures for breeding and gene bank maintenance were determined and the first inter-species crosses were made. Selections based on phenotype were made. Material was supplied to several South African growers for evaluation. This phase was concluded with the South African flower growers association's recommendation that hybrids had a commercial potential.

Phase II from 1973-1982 is regarded as the actual start of the breeding programme. During this period a large number of crosses were made and superior hybrids were produced. Initial characterisation and evaluation work was done. The first problems arose when growers emphasised the susceptibility of *Lachenalia* to the *Ornithogalum* mosaic virus (OMV). The virus problem was addressed by the initiation of tissue culture propagation (Coertze et al., 1992, Klesser & Nel, 1976, Nel, 1983, Niederwieser & Vcelar, 1990, Niederwieser & Van Staden, 1990a & b, Van Rensburg &Vcelar, 1989) for the supply of disease-free stock material to growers. This phase was concluded by the application for Plant Breeders Rights for 5 cultivars.

The approach during the first three phases of the project was that the bulb growers in S. Africa and The Netherlands had enough expertise to develop suitable cultivation practices. This, however, proved to be a fatal mistake that delayed the commercialisation of *Lachenalia* for several years. The extent of this mistake became apparent during Phase III (1983-1992).

During Phase III local growers experienced problems because propagation material was not available in sufficient quantities and there was no cultivation and virus control information or information available. Furthermore they did not have

the expertise or resources to conduct in-house cultivation trials (Niederwieser et al., 1998, Kleynhans et al., 2002). These problems were emphasised when the first trials were conducted in Holland. The Dutch applied techniques used for the production of well-known winter bulbs such as hyacinth on *Lachenalia* with detrimental results. Conditions in the Northern Hemisphere, which differs greatly from those in SA, also had a large effect on the growth habit of the plants (Kleynhans et al., 2002, Niederwieser et al., 1998). This emphasised the need for a whole new set of production protocols to commercialise the product. Early attempts at commercialisation were thus unsuccessful due to the absence of cultivation information. Despite the lack of cultivation information, the availability of sustainable state funding during these earlier phases was indispensable for the development of superior hybrids (Niederwieser et al., 1998, Kleynhans et al., 2002).

Nineteen ninety-two is seen as the watershed year for the *Lachenalia* programme. The finding that for successful commercialisation of a new crop all the relevant information including production protocols must be available led to a whole product approach. This meant that ARC-Roodeplaat had to take a strong lead in not only the commercialisation of the product but most importantly the development of the required technology for production and the continuous supply of disease free propagation material (Niederwieser et al., 1998, Kleynhans et al., 2002). The decision to implement this approach led to the start of a multidisciplinary research programme with a large committed team.

During Phase IV, which extended from 1993-1996 the breeding programme was revitalised. An average of 250 crosses per year were made and a hybrid evaluation system including all the relevant selection criteria (desired phenotype, multiplication and pot plant characteristics) were established, implemented and improved. Several propagation methods were tested and the basic cultivation requirements were determined. There was a tremendous improvement in pot plant forcing methods. A plant improvement scheme was established which included an *in vivo* and *in vitro* production unit at ARC-Roodeplaat. Regular working group meetings were held with local growers to exchange the acquired information and technology (Niederwieser et al., 1998, Kleynhans et al., 2002).

Several actions were also taken to commercialise *Lachenalia* during this phase: Royalty administration and distribution agents were appointed; plant breeder's rights were obtained to protect ten varieties internationally; a trade name "Cape Hyacinth" was registered for the lachenalias; and a commercial pot plant grower (forcer) was identified in Holland (Niederwieser et al., 1998, Kleynhans et al., 2002).

A smaller research programme and the development of a production system for *Lachenalia* characterised Phase V, 1997-present. A market study conducted by Fides in 1993 estimated the potential market for *Lachenalia* in Europe at 20 million bulbs per annum. Initial trials in the USA (by USDA-ARS Maryland [Roh et al., 1995]) were completed and other markets have yet to be targeted. The potential for successful commercialisation thus existed, but the problem of producing large

numbers of flowering bulbs needed to be overcome. The solution to this bottleneck to commercialisation lay in the mass production of bulbs by commercial growers (Niederwieser et al., 1998, Kleynhans et al., 2002).

Here again ARC-Roodeplaat was prompted to take a strong lead in facilitating the commercial production of *Lachenalia* bulbs. During 1997 and 1998 there was only one commercial grower with exclusive rights to produce *Lachenalia* bulbs. However, when the number of bulbs produced did not grow as predicted, ARC-Roodeplaat had to identify additional commercial growers (Niederwieser et al., 1998, Kleynhans et al., 2002).

In order to convince these commercial growers to become involved in the production of *Lachenalia,* researchers needed detailed information in terms of production schedules, infrastructure and financial requirements. Accordingly ARC-Roodeplaat spent a considerable amount of time during 1998 and 1999 developing production systems that could address the market demand and the commercial grower's requirements (Kleynhans et al., 2002).

This led to the development of a production system consisting of propagators, who receives propagation bulbs and multiplies planting stock, producers, who produces commercial sized bulbs and forcers, who plants potted plants for marketing. Propagators and producers are located in South Africa while the forcers are in the areas (mostly abroad) where the potted plants are marketed. The development of this system is not unique. Production systems like these also exist for other flower bulb crops (De Hertog & Le Nard, 1993).

The development of this production system is, however, seen as one of the most important steps toward successful commercialisation. In 2000, commercial growers produced one million marketable bulbs and in 2001 an estimated 3 million using this system. Due to the small numbers of bulbs sold and the resulting small royalty income, the research during this period was limited to certain aspects of crop production and the evaluation of hybrids (Kleynhans et al., 2002).

In a world where funding is becoming limited, the development and commercialisation of a new crop like *Lachenalia* will require suitable funding and a concerted effort of all parties involved. This includes researchers, commercial producers and marketers. The importance of a whole product approach, where all relevant information is available to producers as early as possible, can not be stressed enough.

3. DIVERSITY

3.1 Morphology

The genus *Lachenalia* is unusually diverse in phenotype. There are a number of "complex" species consisting of many forms, which grade into one another (Duncan, 1988). These are difficult to separate into distinct varieties or species. Along with this the absence of a formal key makes identification difficult.

The morphological diversity among species starts with the tunicate bulbs that can be minute (5-9mm diameter) for *L. patula* Jacq. compared to the large fleshy bulbs of *L. bulbifera* (Cyrillo) Engl. (up to 35mm diameter) (Duncan, 1988). The number of leaves produced may vary from one to numerous, although two leaves are the most common. The leaves themselves may vary from robust and broad (certain forms of *L. bulbifera*) to short and cylindrical (succulent leaves of *L. patula*). The foliage is usually produced in an upright or spreading position, but in certain species, like *L. latifolia* Tratt., they lie flat on the ground (Knight, 1987, Duncan, 1988).

Simple or stellate hairs occur on the leaves of several species. These hairs may be on the upper or lower surface of the leaf or may be restricted to leaf margins. Some species also show undulate leaf margins. Spotting and banding on *Lachenalia* leaves is a conspicuous feature of many species. The colour (green, brown, magenta) and density of spots varies and although usually on the upper surface, sporadic spots may also occur on the undersides of leaves (Knight, 1987, Duncan, 1988).

A wide variety of banding-patterns on the leaf-bases occur in many species while some species (*L. pustulata* Jacq.) bear pustules on their leaves. These pustules range in size from fairly large irregularly scattered ones to small, dense ones. In the majority of species leaves and flowers are present simultaneously, but in certain species (*L. muirii* W.F. Barker) leaves appear after the flowers (Knight, 1987, Duncan, 1988).

Three different types of inflorescence are encountered in the genus. Firstly the spike where the flowers are sessile and attached directly to the rachis; secondly the subspicate inflorescence, where the flowers are attached to the rachis by very short pedicels and thirdly the raceme, where the flowers are attached by long pedicels. Flowers range in shape from long and tubular to small and campanulate, while the position of the flowers on the rachis varies from pendulous to erect (Knight, 1987, Duncan, 1988). The perianth is zygomorphic and the inner segments usually protrude. The stamens vary in position from included to well exerted. Flower colour varies from white, green, blue and purple to red, pink, yellow and brown. Some species are pleasantly scented. Lastly the black usually shiny seeds vary considerably in size from 0.7 mm diameter to 2 mm diameter (Duncan, 1988).

3.2 Distribution and Habitat

The genus is mainly found in southern Africa where it is widely distributed from the south-western region of Namibia, southward throughout the Northern, Western and Eastern Cape provinces of South Africa to as far inland as the south-western Free State Province (Duncan, 1998). With one exception (*L. pearsonii* (Glover) W. F. Barker) the genus is exclusively winter growing, with a pronounced dormant period during summer months. Even species occurring in predominantly summer- or intermediate rainfall areas, nevertheless follow the typical winter rainfall growth cycle (Duncan, 1998).

Due to its wide distribution, the genus is encountered in a very wide range of habitats such as semi-desert conditions in deep sand, rocky outcrops in humus rich soil, mineral rich, barren stony flats, limestone outcrops, seasonally inundated flats and marshes and high rainfall montane conditions (Duncan, 1992, Duncan, 1998). As can be expected those species which are widely distributed are, morphologically, widely variable. However, even within species with relatively small distribution ranges, a remarkable degree of variation exists (Duncan, 1998).

3.3 Propagation

Lachenalia species can be propagated through seeds, offsets, bulbils, stolons, supernumerary bulblets, leaf cuttings or tissue culture propagation (Duncan, 1988, Roodbol & Niederwieser, 1998, Niederwieser & Ndou, 2002). Some species produces an abundance of seed after self-pollination. Others however, produce no seeds or a limited number of seed (Kleynhans & Hancke, 2002).

The best time to sow *Lachenalia* seed is in autumn (March for the Southern Hemisphere) (Knight, 1987, Duncan, 1988). Seeds can be sown onto several mixtures (Knight, 1987, Duncan, 1988) as long as they are thinly covered and kept moist. Seeds will germinate over a period of two to six weeks. Optimum temperature for germination is 10-20^{o}C (Malan, 1969). Hybrid seeds are treated the same as species seed. An added advantage of seed production is the elimination of OMV infections. The virus is not seed transmissible. Other methods of multiplication do not eliminate the virus.

Offsets are side-bulbs which develop out of the mother-bulb, from which they eventually break away (Duncan, 1988). Not all species reproduce readily by this method. Offsets are generally too slow for commercial production. Certain species produce small bulblets at or above ground level on the leaf surface, which are commonly known as bulbils (Duncan, 1988). Some species (*L. namaquensis* Schltr. Ex W.F. Barker) reproduce by means of stolons. Roodbol and Niederwieser (1998) reported supernumerary bulblets (adventitious buds formed from axial meristem). These methods are species-specific. Depending on the inheritance of the method hybrids can be propagated to a greater or lesser extent using propagation methods of

the parent plants. The inheritance of a production method, thus influences the specific production scheduling of different cultivars.

Leaf cuttings has also been described as method of propagation (Cook, 1931, Duncan, 1988, Perrignon, 1992). Most species can be successfully multiplied by means of leaf cuttings (Niederwieser & Ndou, 2002). Leaves are severed above ground level and planted vertically in a well-drained rooting medium and kept moist. Bulblets and roots start to form on the basis of severed leaves after about one month (Ndou et al., 2002). These bulblets can be harvested at the end of the growing season and stored until the next planting season. Commercial production of cultivars is done via leaf cuttings (Kleynhans et al., 2002, Niederwieser & Ndou, 2002).

Propagation through tissue culture is also possible and well established for *Lachenalia* (Klesser & Nel, 1976, Nel, 1983, Niederwieser & Vcelar, 1990, Louw, 1995, Niederwieser & Ndou, 2002). In both leaf cutting and tissue culture propagation the genotype, tissue age, physiological stage of donor plants and medium components influence the success of multiplication (Van Rensburg & Vcelar, 1989, Perrignon, 1992, Niederwieser & Vcelar, 1990, Niederwieser & Van Staden, 1990b, Niederwieser & Van Staden, 1992, Niederwieser et al., 1992, Ndou et al., 2002, Niederwieser & Ndou, 2002).

3.4 Genetics

The genus exhibits a remarkable variability with regard to chromosome number. Numbers ranging from 2n=10 to 2n=56 have been reported in literature (Moffett, 1936, De Wet, 1957, Riley, 1962, Mogford, 1978, Ornduff & Watters, 1978, Nordenstam, 1982, Crosby, 1986, Hancke & Liebenberg, 1990, Johnson & Brandham, 1997, Hancke & Liebenberg, 1998, Kleynhans & Spies, 1999, Spies et al., 2000, Spies et al., 2002, Van Rooyen et al., 2002). The basic chromosome numbers of x=7 or 8 are the most frequent but x=5, 9, 10, 11, 12, 13 and 15 have also been reported (Ornduff & Watters, 1978, Johnson & Brandham, 1997, Hancke et al., 2001).

The origin of and relationship among these different basic chromosome numbers are still unclear. Johnson and Brandham (1997) investigated karyotypes and found that all the basic numbers x=7-13 and 15, produced structural diploids. The authors stated that it was, however possible that diploids with 2n=2x=30 (x=15) could actually be allotetraploids derived from taxa with x=7 and x=8 following hybridisation and doubling of the chromosome number. They also state that plants of *L. mutabilis* Sweet with x=5 (2n=10) are derived from plants with 2n=14 via Robertsonian fusions. Spies et al. (2002) differed from the former authors in their explanation of the chromosome number variation in *L. mutabilis*. They found a basic chromosome number x=6 for this species, but dismissed the explanation of Johnson and Brandham (1997) due to the absence of any long chromosomes (as a result of a

Robertsonian fusion). They suggested the existence of an aneuploid series in this species.

Polyploidy is fairly common in the genus. Although some species have few to no polyploid specimens (Johnson & Brandham, 1997, Spies et al., 2000, Spies et al., 2002], others include many polyploid specimens (Johnson & Brandham, 1997, Kleynhans & Spies, 1999, Spies et al., 2002). A polyploid complex ranging from tetraploid, hexaploid, heptaploid to octoploid was identified in *L. bulbifera* (Kleynhans & Spies, 2002). Another species in which a polyploid complex seems to be present is *L. elegans* (Johnson & Brandham, 1997). Polyploidy seems to be more common in species with x=7 as basic chromosome number (Spies et al., 2002).

Meiotic information on *Lachenalia* was limited (Moffett, 1936) up until the 1990's, when published reports increased (Hancke & Liebenberg, 1990, Hancke & Liebenberg, 1998, Hancke et al., 2001, Du Preez et al., 2002). Hancke and Liebenberg (1990) described the presence of B-chromosomes in both mitotic and meiotic material. Much of the variation in chromosome numbers described by earlier workers could probably be ascribed to the wrong identification of the B-chromosomes. Incorrect species identification could also have contributed to inaccurate reports on chromosome number (Crosby, 1986).

B-chromosomes in *Lachenalia* have no specific staining pattern. They may stain slightly lighter or darker or similar to other chromosomes in the chromosome complement. The B-chromosomes are similar in size to the smallest chromosomes of the complement (Hancke & Liebenberg, 1990). The staining patterns and similar size makes it difficult to identify B-chromosomes.

The meiotic behaviour of hybrids gives an indication of the relationship between the parental species and can indicate the possible origin of the different basic chromosome numbers. Investigations into the meiotic behaviour of hybrids (Hancke & Liebenberg, 1998) showed that *L. aloides, L. orchioides* (L.) Ait*., L. reflexa* and *L. viridiflora* W.F. Barker were closely related. These species all have a x=7 chromosome complement. However, the relatedness of *L. mutabilis* (also x=7) to these species is unclear from the results and needs further investigation (Hancke & Liebenberg, 1998).

A study on chromosome pairing in three dibasic inter-specific hybrids revealed a high incidence of bivalents (Hancke et al., 2001), which indicates that the parent species are closer related than initially expected. The same study also revealed a degree of homoeology between two chromosomes of the x=7 karyotype and three chromosomes of the x=8 karyotype. This indicated that the x=7 plants differed from the x=8 plants by at least two exchanges of chromosome material and the loss of one centromere from the x=8 karyotype. The results thus imply that the change in the basic chromosome number of *Lachenalia* involves a reduction in chromosome number. The process of change was, however, not straight forward since the x=8 karyotype has no acrocentric chromosomes. The change was thus not just the result of simple centric fusion as suggested by Johnson and Brandam (1997).

Initial studies on meiosis in hybrids between species of the x=8 complement (*L. carnosa* Bak.*, L. splendida* Diels.*, L. unicolor* Jacq.*, L. namaquensis* Schltr. Ex W.F. Barker and *L. framesii* W.F. Barker) again revealed a high level of relatedness (Du Preez et al., 2002). Chiasma frequencies that were very similar to those of the parental species were found.

Genetic variation in 21 accessions of the polyploid complex of the species *L. bulbifera* revealed genetic distance values ranging from 0.11 to 1.08 (Kleynhans & Spies, 2000). A dendogram constructed from the RAPD banding profiles clustered certain accessions together. These clusters were supported by the geographical locality and chromosome data of accessions. Accessions with the same chromosome number, but from different geographical localities did not group together (Kleynhans & Spies, 2000). Accessions within the species *L. bulbifera* having the same chromosome number are thus not necessarily closely related. Further investigations are needed to clarify these relationships.

Studies on the species delimitation of *L. hirta* and *L. unifolia* Jacq. revealed that the genetic variation between the two species, as revealed by DNA amplification fingerprinting was only marginally higher than the variation within any of these species. Consequently it was suggested that these species probably represent two subspecies of the same species rather than two separate species (Van Rooyen et al., 2002). These and other genetic studies (Spies et al 2002) stress the fact that the variation in the genus needs further investigation.

3.5 Sub-Generic Delimitation

In the first attempt for sub-generic delimitation Baker (1897) described five subgenera, i.e. *Eulachenalia*, *Coelanthus, Orchiops, Chloriza* and *Brachyschypha.* These subgenera were based on morphological differences. Crosby (1986) reclassified the genus, using five groups, each based on a typical species, i.e. *L. aloides, L. orchioides, L. pusilla, L. unicolor* and *L. unifolia* groups. Crosby added chromosome numbers and crossability within groups to the morphology and used these three criteria for his groupings. Duncan (1988) used the ratio between the lengths of the perianth and stigma, as well as the inflorescence form as criteria and divided the genus into 10 subgroups. None of the methods above were exhaustive. Many new taxonomic aids, of which molecular methods are only one can be used to assist in the delimitation of species.

In a preliminary study, chromosome numbers and sequencing of the *trn*L-F region was used to determine the phylogenetic relationship between 19 *Lachenalia* species (Spies et al., 2002). The aim of the study was to determine which of the above mentioned subgeneric classifications corresponded best to the phylogenetic relationships within this group. The results indicated that none of the sub-generic groupings conform to the natural phylogeny as found in the preliminary study. The system proposed by Crosby is, however, the most natural one (Spies et al., 2002).

From the results of this study as well as studies on meiosis and morphology mentioned above it is clear that a full revision of the genus is urgently needed. Various additional taxonomic aids (anatomy, cytogenetics, molecular systematic, etc.) should be implemented in combination. Such a method may culminate in the best natural classification possible for *Lachenalia.* In view of a considerable amount of within species diversity it might be difficult to obtain a complete survey of all possible variation in the genus. However, a system including multiple taxonomic aids will contribute to a better understanding of the diversity in the genus.

4. BREEDING

The international floriculture market continuously requires new products (De Hertog & Le Nard, 1993). *Lachenalia's* wonderful variety makes it suitable to be used breeding to satisfy this demand. The spectacular diversity gives rise to a multitude of possible combinations for the breeder to create, but also presents several problems and challenges.

The *Lachenalia* breeding program is still relatively young as compared to those on other large bulbous crops. Breeding methodology is basic and has not required advanced techniques. Naturally occurring genetic diversity presented the breeders with enough variation to produce new products. However, this situation is changing fast so that advanced breeding techniques to overcome crossing barriers will become an important part of future breeding strategies.

Despite numerous claims of inter-specific hybrids produced during the late eighteen hundreds and early nineteen hundreds, very few of the named cultivars survived to the late nineteen hundreds (Crosby, 1978). This lack of commercial success is understandable in light of ARC-Roodeplaat's problem to commercialise *Lachenalia* (2.2). It was only after intensive cultivation research and the establishment of a scheme for the supply of disease free material, that large enough numbers of plants could be raised, making it amenable to commercialisation.

4.1 Reproductive Biology

Crosses are made by hand pollinating after emasculation of flowers (Lubbinge, 1980). *Lachenalia* has a three-year breeding cycle from seed to flower (Kleynhans & Hancke, 2002). Flowers may be obtained in the second year from some hybrids, but these flowers are generally of poor quality (few florets and small inflorescences) which makes evaluation difficult. Once a hybrid has been selected propagation is done vegetatively. Marketable bulbs are then obtained within two years.

A study of the ontogeny of the stamen and the organography of the stigma and style of *L. rubida* Jacq. revealed that the stigma of this species was dry with papillae (Coetzer and Van der Walt 1994). Other species will have to be investigated to see

whether this is generally true for the genus. The style of *L. rubida* is of the open type and has three stylar canals (Coetzer and Van der Walt 1994).

Lachenalia flowers are protandrous (Malan 1969; Moore 1905). Initial studies indicated that anthers dehisce one day after the flower has opened but that the pistil is receptive only 4 days later (Malan 1969). This is, however only true of a few species. In some species the anthers might dehisce before the flowers open fully. Anthers of *L. mutabilis* for instance will dehisce before the flower is opened completely, while anthers of certain *L. bulbifera* accessions dehisce after the flower has been open for one to three days (Hancke, et al. 1994). The correct time of emasculation thus has to be determined for each species. Even ecotypes within a species might differ with regard to time of dehiscence (Kleynhans, et al. 1995).

Emasculation takes place one or two days before dehiscence. Anthers are collected in gelatine capsules and left in a desiccator overnight to dehisce. Capsules are then closed and stored in tightly sealed glass bottles. Storage of pollen is important because of the diverse flowering times (April-November) among the species (Duncan, 1988). Although reports on pollen storage at room temperature in a desiccator for two years without loss of viability have been made (Lubbinge, 1980), other results have indicated differently. The best storage is at –4^{o}C or in liquid nitrogen (Kleynhans et al. 1995; Sorour 1988). Providing that pollen was kept dry, it retained 80% of its germination ability when stored for up to two years in a refrigerator (Kleynhans et al. 1995). Similar figures were obtained with storage in liquid nitrogen. Longer periods have not been tested for liquid nitrogen. Pollen stored at room temperature lost viability rapidly after one month. In tests on the pollen of *L. mutabilis* and *L. unicolor,* pollen viability decreased to below 50% after only five days in the desiccator at 25^{o}C (Sorour 1988). Pollen germination tests were done by the hanging drop technique with 10% sucrose and 0.01% boric acid (Hancke & Liebenberg, 1998).

Stigmas are receptive from one to seven days after anthesis (defined as anther dehiscence for this discussion). In trials done on six species *(L. aloides, L. mutabilis, L. bulbifera, L. rubida, L. liliflora* Jacq. and *L. unicolor)* the optimum period of receptiveness varied greatly (Figure 18-1). In all species stigmas were receptive one to two days after anthesis, although the percentage of receptive stigmas (% from number pollinated) in most cases were low. For *L. rubida* and *L. bulbifera* the optimum time for pollination was anytime from three to more than seven days after anthesis. For *L. aloides* times varied from two to six days. For *L. mutabilis* one to three days, for *L. liliflora* two to four days and for *L. unicolor* two to three days were found to be optimal (Figure 18-1). The time of receptiveness of the stigma and the style length appeared to be correlated. *L. bulbifera, L. rubida* and *L. aloides* all have long flowers and styles, whilst the flowers of *L. liliflora, L. unicolor* and *L. mutabilis* are shorter. According to these results the best time for pollination was three to five days after emasculation (Hancke et al. 1994; Kleynhans et al. 1995). Large flowered species can be pollinated even later. Seeds are ready for collection

approximately two months after pollination. Seed capsules turn brown and are harvested before they split open.

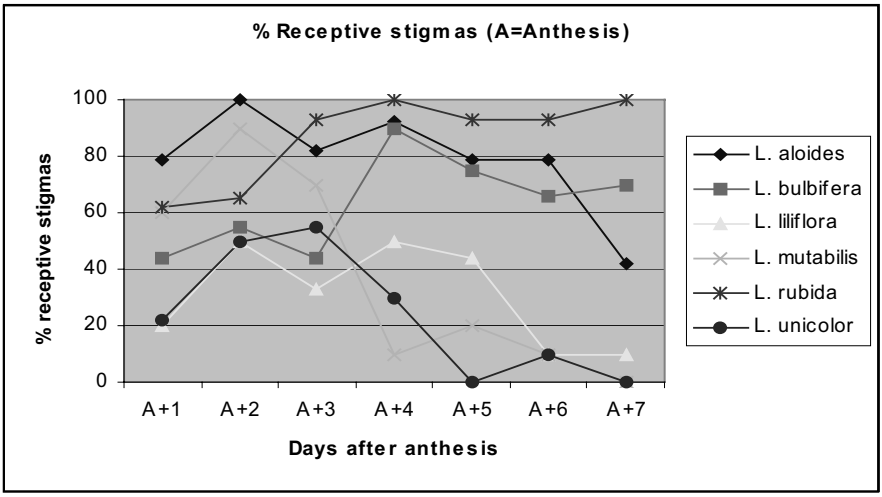

Figure 18-1. Percentage of receptive stigmas after self-pollination 1-7 days (respectively) after anthesis in six *Lachenalia* species.

4.2 Selection Procedures and Commercial Production

The complete selection procedure for *Lachenalia* encompasses a 13-15 year period from making a cross to the commercial availability of a new cultivar. Initial selections take place in the third year (three-year breeding cycle) after making a cross. This first phase in the selection procedure of hybrids is a phenotypic selection based on specific characteristics. These characteristics include the ratio between inflorescence and leaves (60:40 to 50:50), flower colour, density of inflorescence, longevity and sturdiness of the peduncle. Characters like scent and attractive leaf spots are noted. These add value to the product, but are not essential selection criteria.

After initial phenotypic selection (year 3), material has to be multiplied (year 4-5) to have enough bulbs to evaluate the propagation rate of the hybrid in terms of commercial production methods. In the five to seven years of production evaluation the requirements of three commercial growers are evaluated. The first step (year 6-7) involves the propagator who mainly does commercial propagation of cultivars through leaf cuttings. Leaf-section position and physiological stage (best before flowering) of the donor plant influences the leaf cutting performance of cultivars (Perrignon, 1992, Ndou et al., 2002, Niederwieser & Ndou, 2002). Cultivars may differ in their reaction towards these aspects. New hybrids are evaluated taking these

differences into account and information is released to growers. Growers are then able to do commercial scheduling by utilising the production figures obtained during evaluation.

The second step (year 7-8) in the evaluation addresses the requirements of the producer who receives planting stock from the propagator and produces commercial sized bulbs. Only bulbs larger than 6 cm. in circumference are suitable for pot plant production and are thus of commercial size. Bulblets harvested from leaf cuttings may vary from less than 2 to 4 cm. in circumference. The rate at which bulblets grow to a commercial size is dependent on cultivar, the size of bulblets produced by leaf cuttings, the level of fertiliser (Roodbol et al., 2002, Roodbol & Niederwieser, 2002) used and the specific micro-climate under which production is done (Du Toit et al., 2001a, Du Toit et al., 2001b, Du Toit et al., 2002). The evaluation of these aspects in order to supply production figures is thus extremely important to the commercial grower.

The last step (year 8-9) in the evaluation process involves the forcer who receives commercial sized bulbs from the producer. The forcer buys dry bulbs from the producer after harvest and forces these bulbs to produce flowering pot plants at specific times. Forcing includes certain temperature regimes during storage to obtain flower initiation (25^{o}C) and year round flowering (low temperatures followed by initiation temperatures), as well as specific planting temperatures to ensure good quality and short glasshouse periods (Louw, 1991, Louw, 1993, Roh et al., 1995). Cultivars again differ with regard to glasshouse period, keeping quality and growth habit. The microclimate in the pot plant production area (mostly outside South Africa) also has a large influence on the quality of pot plants produced (Kleynhans et al. 1995). Each grower will have to fine-tune his production plan accordingly. The forcer can adapt his specific conditions during production following the indications on the performance of hybrids during the pot plant evaluation.

Cultivar ‘Ronina’ or its close relative ‘Namakwa’ is used as crop ideotype throughout the evaluation to compare new hybrids to existing production protocols and scheduling over years. Specific climatic changes occurring in different production seasons can influence the production statistics. These cultivars were selected because they have often been used in cultivation research (Slabbert & Niederwieser, 1999, Du Toit et al., 2001a, Du Toit et al., 2001b, Ndou et al., 2002, Roodbol et al., 2002, Roodbol & Niederwieser, 2002).

After successfully passing all evaluation stages (9-11 years), Plant Breeders Rights can be registered for new hybrids. Commercial growers require another four years after release for the multiplication of substantial numbers for commercialisation.

4.3 Gene Bank

A representative and well-characterised gene bank is important for any breeding programme. The *Lachenalia* breeding programme was thus initiated with the acquisition of several species and species ecotypes through collection trips to the natural distribution areas (Lubbinge, 1980, Niederwieser et al., 1998). The acquisition of species is still an important aim in the programme. Currently the gene bank at ARC-Roodeplaat comprises 500 ecotypes from 55 species.

Initial gene bank accessions were characterised according to colour, shape, size and number of flowers, scent, markings on the leaves and number of leaves (Lubbinge, 1980). Later characteristics such as the ratios between inflorescence and leaf lengths and inflorescence and peduncle length; discoloration of flowers; keeping quality; time of flowering; multiplication method and production potential; chromosome numbers; pollen fertility; self seed set potential and disease status were found to be important for breeding.

Characterising gene bank accessions in a genus so diverse as *Lachenalia* is essential for proper planning of crossing strategies. Characteristics recorded and evaluated must be updated on a regular basis to keep up with the newest breeding aims and market requirements.

4.4 Reproductive Barriers and Breeding Strategies

Initial results obtained from studies of reproductive biology and inter-species hybrids indicate external and internal isolation barriers to inter-specific crosses (Hancke et al. 1994; Kleynhans et al. 1995; Lubbinge, 1980). The external barriers can easily be overcome by growing the plants in controlled conditions and the successful storage of pollen for a 12-month period (Kleynhans et al. 1995; Sorour 1988). The internal barriers have not been studied in detail.

Internal isolation barriers are encountered either before or after fertilization. Sixty five percent of all inter-species crosses made at ARC-Roodeplaat did not succeed, either because no seeds (pre-fertilization) or non-viable seeds (post-fertilization) were formed (Table 18-1). The death of seedlings accounts for an additional 3%. The reason for the death of these seedlings can not necessarily be ascribed to hybrid breakdown. Seedlings are susceptible to all kinds of rotting diseases and with the absence of specific data on these crosses conclusions can not be drawn. The extent and exact processes causing these failures needs further investigation.

Table 18-1. Success of interspecific crosses between large and small flowered *Lachenalia species*, classified according to crossing result (Kleynhans, unpub. data).

Interspecific cross type (style length)	Tot. no per type	Successful crosses	Unsuccessful –no seed set[a]	Unsuccessful - abnormal seeds [b]	Unsuccessful – seedling death [c]
Short X Short (10-15mm)	150	57	40	49	4
Short X Long (10-15mm X +20mm)	284	75	75	125	9
Long X Short (+20mm X 10-15mm)	121	9	92[d]	15	5
Long X Long (+20mm x +20mm)	169	93	24	50	2
Total no. of crosses	724	234 (32%)	231 (32%)	239 (33%)	20 (3%)

a=Mechanical isolation
b=Pre-fertilisation barriers present
c=Post-fertilisation barriers present
d=Possible hybrid breakdown

Lubbinge (1980) described mechanical isolation as the first pre-fertilization barrier. Large flowered species of *Lachenalia* have flowers of over 25mm long whilst in smaller flowered species the flower length can be even less than 10mm. Pollen from small flowered species is thus not adapted to traverse the long distance from the stigma to the ovary of large flowered species (Stebbins, 1950). Reciprocal combinations, utilizing the small flowered species as maternal plants have been successful in overcoming this barrier (Lubbinge, 1980, Table 18-1) but does not guarantee success (Table 18-1).

Studies on pollen tube growth have indicated that self-incompatibility vary from species to species. It can be either just below the stigma (*L. mutabilis)*, or in or at the bottom of the style (*L. aloides)* as well as in the ovule (*L. unicolor)* with abnormal penetrations (Hancket et al. 1994; Kleynhans et al. 1995). In *L. aloides* pollen germinated on the stigma after self-pollination but, limited growth of pollen tubes were observed in the style. Most pollen tubes stopped just below the stigma or grew less than halfway down the style. Few pollen tubes entered the ovary. Only single penetrations of ovules could be observed. Thickened and branching pollen tube tips (Figure 18-2a) were often observed in the style of this species. Incompatibility in one accession of *L. mutabilis* occurred just underneath the stigma where most pollen tubes stopped their growth. Pollen tubes again had thickened tips. A second accession of *L. mutabilis,* however, displayed little incompatibility in the style and penetrations were observed. Incompatibility in *L. unicolor* on the other hand seemed to be situated in the ovule. Numerous abnormal penetrations occurred (Figure 18-2b)

(Hancke et al. 1994; Kleynhans et al. 1995). These abnormalities imply a gametophytic incompatibility system. The extent to which these barriers are carried over to inter-specific crosses are being investigated.

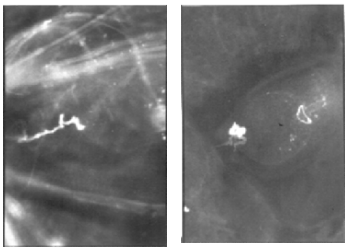

Figure 18 -2. Pollen tube growth in *Lachenalia*: a) Branching and thickened tubes in the style of *L. aloides* after self-pollination b) Abnormal penetrations in the ovule of *L. unicolor* after self-pollination, magnification x400.

Lubbinge (1980) also mentioned polyploidy as one reasons for the failure of inter-species crosses. The author speculated that relatively large, thick pollen tubes produced by some polyploid species (Stebbins, 1950) have difficulty in penetrating the smaller styles of diploids. Recent results did not support these conclusions (Table 18-2). Most inter-specific crosses (68%) where polyploid *L. bulbifera* plants were used as pollen parent were unsuccessful because of the production of abnormal or non-viable seeds. The barrier is thus at the post fertilisation stage and should rather be ascribed to reduce hybrid viability, most probably caused by disharmony either between the parental sets of chromosomes or between the developing embryo and endosperm. There were also no obvious differences between polyploid and diploid accessions of the same species (Kleynhans & Spies, 1999) with regard to flower size, leaf size etc. as is often found in other polyploid plants. In *L. bulbifera* large flowered and small flowered tetraploid accessions were found (Kleynhans & Spies, 1999). Hexaploid accessions also showed large and small flowered ecotypes.

Table 18-2. Results of inter-specific crosses between diploid *Lachenalia species* and polyploid accessions of *L. bulbifera* used as pollen parent.

Result of cross	2*n*=2*x*=28 (tetraploid)	2*n*=2*x*=42 (hexaploid)	2*n*=2*x*=56 (octoploid)	Total %
No. of successful crosses	0 (0%)	4 (8%)	2 (4%)	12
No. of crosses without seed set	3 (6%)	6 (13%)	1 (2%)	21
No. of crosses with abnormal seeds	1 (2%)	23 (48%)	8 (17%)	67

Post fertilisation barriers are important problems that require investigation. Thirty-three percent of all inter-specific crosses attempted did not succeed because of the production of non-viable seed (Table 18-2). When the large diversity in the cytotaxonomy of the genus is taken into account, this is not surprising. Accordingly, the utilisation of embryo or ovule culture to successfully produce hybrids is becoming a priority in the breeding programme.

Embryo clearing was done from 5 to 13 days after pollination to determine the developmental stages of the embryos. Only pro-embryos were visible after 13 days (Kleynhans et al. 1995). The correct time for ovule culture has not yet been established, but the initial results indicated that a period of more than 13 days after pollination was needed before ovules could be collected for successful culture.

Another aspect mentioned by Lubbinge (1980), was the variance of results obtained when plants of the same species from different ecotypes were crossed in varying combinations. The variance was most probably due to hybrid breakdown. These results and the large variation that can occur within one species (Duncan, 1988, Kleynhans & Spies, 1999, Kleynhans & Spies, 2000) stressed the importance of having more than one ecotype of a species. Utilising different ecotypes in the same inter-species cross might give totally different results (Lubbinge, 1980, Kleynhans et al., 2002). Variation in ecotypes can also be exploited by making intra-species crosses first and then combining different intra-species hybrids in stead of the pure species ecotypes (Table 18-3). These bridging crosses can enhance the success of the specific species combination.

Table 18-3. Differences obtained after using intra-species hybrids of *L. rubida* instead of pure ecotypes of *L. rubida* in crosses with *L. bulbifera*.

Female parent (ecotype)	Male parent (ecotype)	Result – no of seeds obtained
L. bulbifera (A)*	*L. rubida* (I)	No seed set and <10 non-viable seeds
L. bulbifera (A)	*L. rubida (J)*	No seed set and <10 non-viable seeds
L. bulbifera (B)	*L. rubida (I)*	<10 viable and <10 non-viable seeds
L. bulbifera (C)	*L. rubida (K)*	<10 viable seeds
L. bulbifera (C)	*L. rubida (L)*	No seed set
L. bulbifera (D)	*L. rubida (I)*	No seed set
L. bulbifera (E)	*L. rubida (K)*	<10 viable seeds
L. bulbifera (F)	*L. rubida (L)*	>50 viable and less than 10 non-viable seeds
L. bulbifera (F)	*L. rubida (K)*	>50 viable and less than 10 non-viable seeds
L. bulbifera (G)	*L. rubida inter-species (IxK)*#	>50 viable and less than 10 non-viable seeds
L. bulbifera (G)	*L. rubida inter-species (IxM)*	>50 viable and less than 10 non-viable seeds
L. bulbifera (H)	*L. rubida inter-species (LxJ)*	>50 viable and less than 10 non-viable seeds

Female parent (ecotype)	Male parent (ecotype)	Result – no of seeds obtained
L. bulbifera (H)	*L. rubida inter-species (IxL)*	>50 viable and less than 10 non-viable seeds
L. bulbifera (H)	*L. rubida inter-species (IxK)*	>50 viable and less than 10 non-viable seeds
L. bulbifera (H)	*L. rubida inter-species (IxM)*	>50 viable and less than 10 non-viable seeds

* Letter indicating different ecotypes of the same species.
# Letters indicating ecotypes used as parents for the hybrid crosses

Reduced hybrid fertility is another post-fertilisation barrier that occurs especially when species with different basic chromosome numbers are combined (Hancke et al., 2001). This barrier can again be overcome with bridging crosses. Crosses between different species within the different basic chromosome groups are first made before the dibasic hybrids are produced. The presence of these barriers and strategies to overcome them stresses the importance of basic studies especially in terms of chromosome numbers, genetic relationships and reproductive studies to develop advanced breeding techniques.

Besides overcoming reproductive barriers certain breeding strategies are followed to obtain specific goals. Current breeding strategies have two main focus areas. First the development of similar but better adapted hybrids and secondly, and most importantly, the development of new hybrids. Characteristics for better-adapted hybrids include higher production rates and increased longevity. New hybrids are required to be different from any current hybrid. This can be achieved by including new colours in the cultivar range or by making new combinations of colour and flower form.

The aim, to replace or to breed new hybrids, directly influences the breeding strategy as well as the selection criteria used. If the aim is to replace an existing cultivar with a better-adapted hybrid, the selection criteria will become more stringent. Replacement hybrids will be evaluated directly against the old cultivar and will have to outperform the existing hybrid with regard to the required aspects in order to replace the existing one. The cultivar 'Romelia' presents an example of problems experienced during commercialisation and marketing that can be improved through breeding. This yellow flowering cultivar tends to produce small bulbs during commercial production. This is probably due to the abundant production of supernumerary bulblets, a highly heritable trait inherited from *L. aloides*. The energy spent in the development of bulblets is not available to the mother bulb to increase in size resulting in the production of low numbers of marketable bulbs.

Producing new cultivars mainly focuses on new colour variation in the current range of cultivars or the production of a new range of cultivars. The current range of

cultivars (Figure 18-3) consists of large flowered hybrids with yellow, red, lilac, apricot, and lemon-green inflorescences. Different colour variations of white, purple, blue, green, orange and pink are still available in the gene bank and can be utilised to expand this range. In addition to different colours there is a large potential in the species for developing a range of smaller flowered cultivars. These hybrids will probably have to be marketed separately because they are smaller and more compact than the existing ones. There is a number of small flowered species (See Figure 18-3 for example) that is very floriferous and thus ideal for the development of such a range of cultivars.

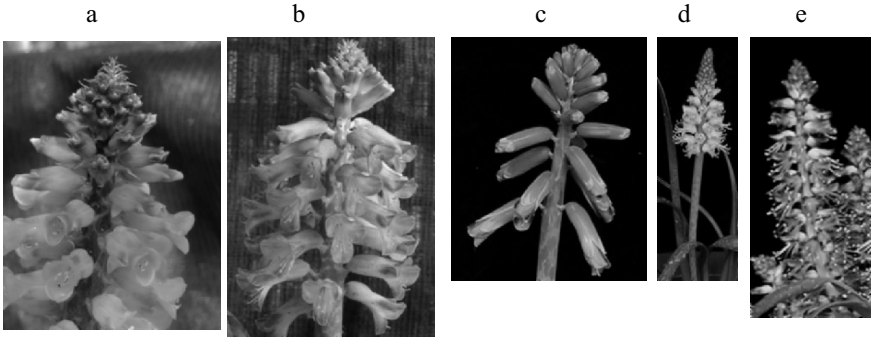

Figure 18-3. Three commercial cultivars and two smaller flowered species of *Lachenalia* a) 'Romaud b) 'Rupert', c) 'Rosabeth', d) *Lachenalia pustulata*, and e) *Lachenalia splendida*.

5. FUTURE PROSPECTIVES

Investigation of the diversity in the genus is currently receiving a lot of attention. Studies on morphological diversity [Graham Duncan, personal communication] and molecular systematics [Spies et al] are being undertaken to enhance the understanding of total diversity. The availability of species boundaries will assist in the correct identification of species. Knowledge on relatedness between and among species will supply the breeders with the basic information to exploit the diversity.

Future perspectives for *Lachenalia* breeding will have to concentrate on advanced breeding techniques for the successful development of new cultivars. However, utilisation of advanced techniques will require some basic research. Developing techniques for successful embryo-rescue after inter-species crosses is a high priority. Determining the extent of pre-fertilisation barriers will assist breeders in utilising the correct techniques to overcome these barriers. Mutation technology presents another strategy of developing new cultivars that can be utilised in *Lachenalia*. This should also receive attention in the near future.

With the large increase in commercial production over the past three years, several disease-related problems have arisen. Virus-related problems have been and will be of great importance in *Lachenalia* production. Successful production will always be directly linked to the presence of a plant improvement scheme. Besides Ornithogalum Mosaic Virus, tobacco necrosis virus has also been identified in *Lachenalia* (Kleynhans et al. 1995). Other virus diseases may occur in the future. Fungi such as *Fusarium, Pythium* and *Penicillium* can cause havoc in the production, if not treated correctly. Results from commercial producers indicate that some cultivars are more susceptible to these problems than others. These problems can thus be overcome by breeding for more resistant cultivars in the future. *Embellisia hyacinthii* was also recently documented to occur in *Lachenalia* (Kleynhans et al. 1995, 2000) Although mostly a secondary fungus it is destructive on *Lachenalia* if not controlled. This fungus can cause losses of up to 50% of the production of commercial sized bulbs in certain *Lachenalia* cultivars. The extent of these and new problems will determine the specific research needed to correct the problems through resistance breeding and specific production practices.

In conclusion the development of cultivars from a new genus can never stand on its own. A successful breeding programme does not end with the production of a superior hybrid but needs a concerted effort from all involved. Supply of required technology for successful production, involvement in actual commercialisation and establishment of a successful marketing channel must all be taken into account.

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Chapter 19

LILY

Lilium hybrids

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Abstract: *Lilium* consists of ~80 species native to the Northern Hemisphere; commercial hybrids are used in a wide range of floricultural products, including flowering potted plants, cut flowers, and garden plants (herbaceous perennial). While lily breeding has been conducted for centuries, most breeding breakthroughs occurred during the past 50 years. These have included interspecific hybridization, intersectional hybridization, and various methods to overcome gametophytic self incompatibility and embryo abortion using pre-fertilization (cut style, grafted style), post-fertilization (ovary-slice culture, ovule culture, embryo culture) methods. The continued use of these and other modern techniques (polyploidization, molecular biology) will ensure creation of new phenotypes for the market.

Key words: cut style, embryo rescue, grafted styles, polyploidization, self incompatibility.

1. INTRODUCTION

1.1 Lilies – General Aspects

The lily has a history dating back at least 36 centuries. It can be traced to the Middle Minoan IIIA-B period (ca. 1750-1675 B.C.), when Cretan vases and frescoes illustrated its beauty, pure white colour and elegant fragrance (Evans, 1921, 1930; Woodcock and Stearn, 1950). The genus *Lilium* is in the family Liliaceae and comprises over 80 species (Comber, 1949; De Jong, 1974). The native *Lilium* species are spread over the Northern Hemisphere (10° to 60°) and centered mainly

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in Asia, North America, and Europe. Currently, the lily occupies a prominent place in horticulture as a cut flower, potted, and garden plant. In 2000 over 1500 million bulbs were produced worldwide. The Netherlands leads in bulb production with 4523 hectares in 2002. There is, however, production in Japan, the United States and more recently in the Southern Hemisphere, e.g. Australia, Chile and South Africa. As a cut flower, the lily is now the fourth most important crop in the Netherlands (Anonymous, 2002b).

1.2 Trends in Lily Hybridization for Commercial Market

Lily breeding goes back about 200 years (Shimizu, 1987). Significant breakthroughs are, however, only 50 years old, starting with the breeding of Asiatic hybrids (McRae, 1998). It has only been since the 1970's that the lily has become, after tulip, the most important flower bulb and cut flower. At present Dutch lily breeding companies are dominant. Over 100 new cultivars have been released annually for the market over the past two decades. Asiatic hybrids were the leading group until 1980's. Since then Oriental hybrids have become the most important group. This has been due to their outstanding flower shape and fragrance even though their forcing time is a few weeks longer than most Asiatic hybrids. This is especially true for old cultivars such as 'Casa Blanca' and 'Star Gazer'. Although 'Joy ('Le Reve')' has an early flowering habit, it has a rather short stem length, which is not desirable for cut flower production in high light and warm climates. For the last 10 years, many Oriental hybrid cultivars have been released with early flowering habits, large flowers and various flower colours.

Many researchers (Skirm, 1942; North and Wills, 1969; Ascher, 1973a; Asano and Myodo, 1977a,b; Asano, 1978, 1980; Van Tuyl et al., 1986, 1991, 2002) have successfully carried out interspecific hybridization studies and this opened the possibility of intersectional hybridization between, e.g. *L. longiflorum* x Asiatic hybrid. The cut style method (**CSM**) was developed to overcome pre-zygotic incongruity, which hinders pollen tube growth in the style between intersectional crosses. This technique in combination with embryo rescue methods such as embryo culture, ovule culture and ovary slice culture, has opened possibilities to develop numerous new hybrids. In the early 1990's a new class of lily cultivars were introduced to the market. They are called **LA** (**L**ongiflorum x **A**siatic) hybrids. In most cases **LA** hybrids are triploid and were derived from back crossing with the F_1 hybrid of *L. longiflorum* x Asiatic hybrid to an Asiatic hybrid (Genome composition of **LA** hybrids is indeed **ALA**). Although they have many desirable characteristics such as rapid bulb growth, healthy leaves, strong stems, large flowers and fragrance, they have negative characteristics such as flower malformation, weak petals and non pure flower colours. In the 1990's Dutch breeding companies focussed on Oriental hybrid breeding. Some outstanding cultivars released were 'Sorbonne' to replace

'Star Gazer' (both pink colour) and 'Siberia' to replace 'Casablanca' (both white). In 2002, the total bulb production area of lily in the Netherlands was 4368 ha (Anonymous 2002a). The **LA**-hybrids occupied 590 ha, which was a 90% increase when compared to 1999. This indicates that the production of these popular interspecific hybrids have increased over *L. longiflorum*, Asiatics, and Orientals in recent years.

2. GENETICS

2.1 Classification

The genus *Lilium* was classified into seven sections by Comber (1949) and later revised by Lighty (1968) and De Jong (1974). The seven sections are: Martagon, Pseudolirium, Lilium (Liriotypus), Archelirion, Sinomartagon, Leucolirion, and Daurolirion. This scientific classification is very different than the one used by Royal Horticultural Society (RHS), where the genera include a large number of species or many cultivars are often subdivided into groups, based on a particular characteristic or combination of characteristics. The RHS classification is reported in *The International Lily Register* (RHS 1982-2002). This classification is presented below:

(I) Early-flowering Asiatic Hybrids derived from *L. amabile*, *L. bulbiferum*, *L. cernuum*, *L. concolor*, *L. davidii*, *L. x hollandicum*, *L. lancifolium*, *L. leichtlinii*, *L. x maculatum* and *L. pumilum*
- (Ia) Upright flowers, borne singly or in an umbel
- (Ib) Outward-facing flowers
- (Ic) Pendant flowers

(II) Hybrids of Martagon type, one parent having been a form of *L. hansonii* or *L. martagon*

(III) Hybrids from *L. candidum*, *L. chalcedonicum* and other related European species (excluding *L. martagon and L. bulbiferum*)

(IV) Hybrids of American species

(V) Hybrids derived from *L. formosanum* and *L. longiflorum*

(VI) Hybrid Trumpet Lilies and Aurelian hybrids from Asiatic species, including *L. henryi* but excluding those from *L. auratum*, *L. japonicum*, *L. rubellum* and *L. speciosum*.
- (VIa) Plants with trumpet-shaped flowers
- (VIb) Plants with bowl-shaped flowers
- (VIc) Plants with flat flowers (or only the tips recurved)
- (VId) Plants with recurved flowers

(VII) Hybrids of Far Eastern species as *L. auratum*, *L. japonicum*, *L. rubellum* and *L. speciosum* (Oriental Hybrids)
(VIIa) Plants with trumpet-shaped flowers
(VIIb) Plants with bowl-shaped flowers
(VIIc) Plants with flat flowers
(VIId) Plants with recurved flowers
(VIII) All hybrids not in another division
(IX) All species and their varieties and forms

All *Lilium* species are diploid (2n=2x=24), except some triploid forms of *L. tigrinum* and *L. bulbiferum* existing in nature (Noda, 1966, 1978; Noda & Schmitzer, 1992). Early chromosome studies (cytogenetics) of lily were performed by Sato (1932) and Stewart (1947). The genome size of *Lilium* belongs to one of the largest in plant kingdom (Bennett & Smith, 1976, 1991). Differences in DNA-content can be measured efficiently using flow cytometry, which is used generally for discrimination between diploids and tetraploids (Van Tuyl et al., 1992). The variation in DNA content within the genus *Lilium* was studied by Van Tuyl & Boon (1997) and ranged from 69 to 96 pg/2C. It appeared that interspecific hybrids in general have intermediate DNA content of the parents. This method was used for the identification of the hybrid character of interspecific hybrids in a very early stage.

Lilium longiflorum has a strong gametophytic self incompatibility (SI) system and has been a model crop for studying the mechanism. Heat treatment and cutting the styles appeared to be methods to overcome this S-allele regulated system (Ascher, 1973a,b, 1977; Lindquist, 1991).

2.2 Interspecific Hybridization

Many important horticultural characters are present in the different *Lilium* species. Commercially important characters include:

1) Resistance to diseases such as bulb rot (*Fusarium*), *Botrytis* and several viruses (TBV, LSV and LVX) (Löffler et al., 1996);

2) Phenotypic characteristics such as flower shapes, sturdy stems, new colours, and fragrance;

3) Physiological characteristics such as tolerance to low-light intensity and heat, leaf scorch, year-round forcing ability, long-term storage ability, and rapid bulb growth.

Van Tuyl et al. (1986) have summarised the goals of interspecific hybridization in *Lilium.* They are as follows:

1) Introduction of desirable characters from various species into cultivars directly or indirectly (*i.e.*, bridge crosses);

2) Creation of new forms and types of lilies;

3) Overcoming F_1-sterility by mitotic and meiotic polyploidization;

4) Generation or expansion of the knowledge regarding on taxonomic relationships, inheritance mechanisms and introgression of specific genes.

Some well-known examples of valuable characters among species are listed in Table 19-1.

Table 19-1. Characteristics of *Lilium species* for commercial breeding.

Species	*Characteristics for breeding*		*Potential for breeding*
	Desirable	Undesirable	
L. longiflorum	Low temperature tolerance, flower shape. White	Susceptible to Fusarium, Virus	High
L. formosanum	Year round forcing, up right, growth vigour, fragrance	Weak stem, virus susceptible	Medium
Aurelion hybrid	Upright, yellow colour, fragrance, flower type	Susceptible to Fusarium, virus, weak stem	High
L. nepalense	Pea-green flower colour	Susceptible to virus, late flowering	Low
L. henryi	Vigour, virus and Fusarium resistance	Flower shape, weak stem	Medium
L. concolor	Upright flower, flower shape and size	Weak stem, leaf and growth vigor,	Low
L. tigrinum	Vigour, resistance to virus, large flower, bulbil formation, resistance to Fusarium	Hair, spots	Medium
L. callosum	Small, many flowers per stem, flower colour	Late flowering, weak growth vigor	Low
L. davidii	Resistance to Fusarium and virus	Short stem,	High
L. dauricum	Resistance to Fusarium	Short plant height	High
L. auratum	Large flower, fragrance, vigour, disease resistance, early flowering	Fusarium susceptible,	High
L. speciosum	Pink colour, fragrance	Spots, late flowering	Medium
L. nobilissimum	Pure white flower, fragrance, sturdy stem, upright	Late flowering	Medium
L. rubellum	Very early flowering, pink flower colour, fragrance	Short stem, susceptible to Fusarium	Medium
L. candidum	Low-temperature and low-light intensity tolerance, pure white, fragrance	Susceptible to Virus, weak growth vigor	Low
L. hansonii	Many flowers, long vase life	Flower fragrance, short stem, weak growth vigor, susceptible to virus	Low
L. martagon	Purple, small flower, long vase life, many flowers	Strong fragrance, susceptible to virus	Low
L. tsingtauense	Resistance to Botrytis	Short stem, weak growth, fragrance	Low

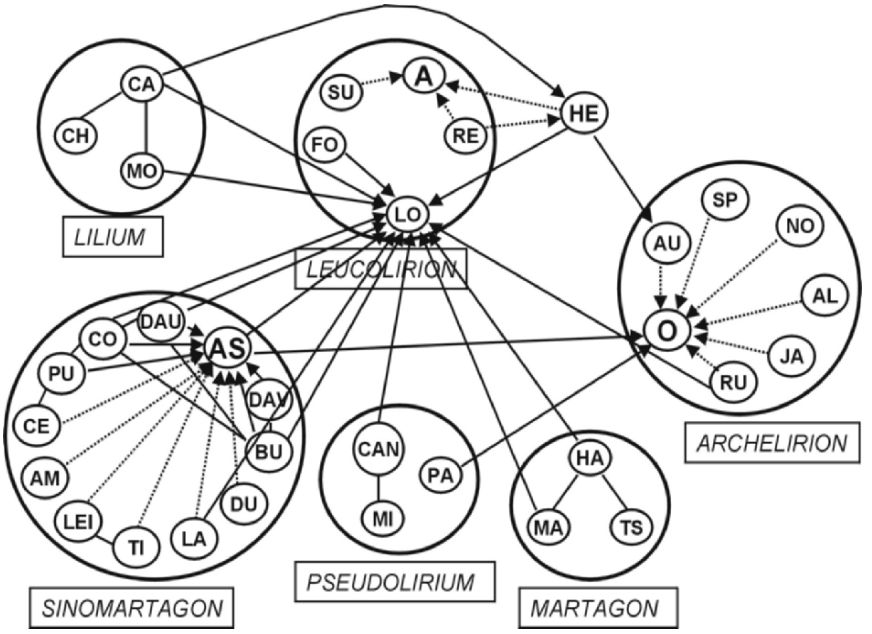

Figure 19-1. A crossing polygon of the genus Lilium including all successful crosses of species between different sections of the genus Lilium developed at Plant Research International, The Netherlands. In this figure, the connection between the Asiatic, Aurelian, and Oriental hybrid groups (large ellipses) are shown by dotted lines. In successful crosses between species (small circles) of different sections (large circles) the arrows point towards the female parent. Abbreviations: A: Aurelian hybrids; AL*: L. alexandrae*; AM: *L. amabile*; AS: Asiatic hybrids; AU: *L. auratum*; BU: *L. bulbiferum*; CA: *L. candidum*; CAN: *L. canadense;* CE: *L. cernuum*; CH: *L. chalcedonicum*; CO: *L. concolor*; DAU: *L. dauricum*; DAV: *L. davidii*; DU: *L. duchartrei*; FO: *L. formosanum*; HA: *L. hansonii*; HE: *L. henryi*; JA: *L. japonicum*; LA: *L. lankongense*; LEI: *L. leichtlinii*; LO: *L. longiflorum*; MA: *L. martagon*; MI: L. michiganense; MO: *L. monadelphum*; NO: *L. nobilissimum*; O: Oriental hybrids; PA: *L. pardalinum*; PU: *L. pumilum*; RE*: L. regale*; RU*: L. rubellum*; SP*: L. speciosum*; SU: *L. sulphurenum*; TI: *L. tigrinum*; TS: *L. tsingtauense*.

Both pre-fertilization and post-fertilization barriers restrict interspecific hybridization between the different sections (Van Tuyl et al., 1991). Several techniques, such as the cut-style method (Asano and Myodo 1977a,b), the grafted-style method, and *in vitro* pollination techniques have been developed to overcome pre-fertilization barriers (Van Tuyl et al., 1991). However, even if fertilization is successful, post-fertilization barriers can inhibit the growth of hybrid embryos (Van Tuyl et al., 1991). *In vitro* pollination and rescue methods such as embryo culture. (Skirm, 1942; North and Wills, 1969; Ascher, 1973a; Asano and Myodo, 1977a,b; Asano, 1978, 1980), ovary-slice culture, and ovule culture have been developed to circumvent these barriers (Van Tuyl et al., 1991). Plants were obtained from cultured embryos of *L. henryi* × *L. regale* by Skirm (1942). Ascher (1973a,b)

succeeded in obtaining plants of *L.* 'Damson' × *L. longiflorum*. Asano and Myodo (1977b) reported the culture of immature hybrid embryos between *L. longiflorum* × *L.* 'Sugehime' and *L.* 'Shikayama' × *L. henryi*. Asano (1980) produced many interspecific hybrids between *L. longiflorum* × *L. dauricum*, *L. longiflorum* × *L. amabile*, *L. longiflorum* × *L. pumilum*, *L. longiflorum* × *L. candidum*, *L. auratum* × *L. henryi*, *L.* 'Sasatame' × *L. henryi*, *L.* 'Royal Gold' × *L. speciosum* and *L. regale* × *L. leichtlinii maximowiczii*.

Later, Van Tuyl and coworkers (1991 and 2002) succeeded in making numerous new intersectional hybrids between many sections of the genus *Lilium* by the use of various pollination and embryo rescue methods. Examples include *L. longiflorum* (Leucolirion section) × *L. monadelphum* (Lilium section), *L. longiflorum* × *L. lankongense* (Sinomartagon section), *L. longiflorum* × *L. martagon* (Martagon section), *L. longiflorum* × *L. candidum* (Lilium section), *L. henryi* (Leucolirion section) × *L. candidum*, *L. longiflorum* × *L. rubellum* (Archelirion section), *L. longiflorum* × Oriental hybrid, Oriental × Asiatic hybrid, *L. longiflorum* × *L. canadense* (Pseudolirium section) and Oriental hybrid × *L. pardalinum* (Pseudolirium section). The crossing polygon (Figure 19-1) shows the crossing compatibility within and between the sections achieved by our research group so far (Van Tuyl et al., 2002).

2.3 Overcoming Pre-Fertilization Barriers

2.3.1 Cut Style Method (CSM)

CSM is the most commonly used technique to overcome pre-fertilization barriers that exist mainly on the stigma and in the style. The inhibiting chemicals are eliminated by cutting off most of style on the day of flowering and subsequently pollinated by placing pollen paste consisting of mixed pollen and stigmatic exudate on top of the cut surface. In this case, the number of germinating pollen can be reduced; however, the numbers of germinating pollen, which penetrate into ovary, is increased. Recent studies have been carried out by comparing of normal and CSM pollination for the production of F_1 and BC_1 interspecific hybrids. The results indicate that CSM is superior for the generating F_1 hybrids and normal pollination method is superior for generating BC populations. This finding can be explained by the crossing barriers between species. Once the genome composition is heterozygous, such as in case of F_1 interspecific hybrids, backcrossing by normal pollination method showed normal pollen germination and pollen tube growth through the style.

2.3.2 Grafted Style Method (GSM)

The GSM was developed because the CSM normally produced only a few embryos per pod. When using the CSM, pollen tubes remain short and most of them do not penetrate the micropyle. GSM should be used in combination with *in vitro* pollination (Van Tuyl et al., 1991). This method is carried out as follows: desirable pollen from the donor plant is pollinated onto compatible stigma in vitro for 1 to 2 days and then the style is cut above 1-2 mm above the ovary. Subsequently, the cut style is joined to the short cut style with ovary of recipient. This graft should be kept for two days, until the pollen bundle enters the ovary of the recipient completely. After five days, the ovary is cultured following the ovary-slice method. This technique is a highly delegated method and labour intensive and only for combinations, which have been unsuccessful with other techniques.

2.4 OverComing Post-Fertilization Barriers

2.4.1 Ovary-Slice Culture

Ovary-slice culture was applied by Kanoh et al. (1988) and Van Tuyl et al. (1991) for the production of interspecific *Lilium* hybrids. Ovaries are harvested 7 to 10 days after pollination, sliced into 2 mm thick disks, and placed on a medium containing 10% sucrose. Within 30 days, ovules or embryos can be separated from the ovary-slice and cultured individually until germination.

2.4.2 Ovule Culture

The ovule culture method must be applied during the course of embryo growth and before the embryo is degenerated. The time for ovule culture is dependent on the cross combination and ranges from 30 to 45 days after pollination. Because the embryo rescue method is labour consuming, ovule culture method can be used when large numbers of hybridized ovules have to be carried out within a short period. A drawback, however, is that the germination efficiency of ovule culture method is lower than embryo culture (Figure 19-2a, b).

2.4.3 Embryo Culture

North and Wills (1969) have reported the successful culture of embryos from seeds without an endosperm and originating from interspecific crosses involving *L. lankongense*. Embryo culture can be applied successfully in crosses in which the degeneration of embryo progresses slowly (Figure 19-2c). This normally occurs with crosses between relatively closely-related species. In most cases, embryos can be

rescued when the globular stage is reached. This technique is very reliable and embryos grow fast without any abnormal development (Figure 19-2d). The best time for embryo rescue method is about 40 to 60 days after pollination.

2.5 General Methodology to Overcome F_1 Sterility

A primary impasse to achieving introgression by backcrossing is sterility of interspecific hybrids. This can be due to several reasons, e.g. as chromosome aberrations, genetic incongruity (genic sterility), or other unknown factors (Asano 1982). Meiotic division of the wide interspecific hybrids is often disturbed due to factors such as unbalanced chromosome assortment, chromosome bridges, chromosome lagging during anaphase I and II, time discrepancy between chromosome movement, and cytokinesis (Asano, 1982). Any pollen generated through these disturbances is lethal. Although the chromosomes of two distantly related genomes have high levels of chromosome association, the pollen will be predominantly sterile or unbalanced due to the random distribution of homoeologous chromosomes during meiotic division (Asano, 1982; Hermsen, 1984; Ramanna, personal communication). Unreduced gamete formation is an exception in this type of material and circumvents these balance disturbances. This phenomenon was demonstrated by 83.6% of restituted pollen in the hybrid of *L. auratum* (Archelirion section) × *L. henryi*, and 52 % in *L. longiflorum* (Leucolirion section) × *L. leichtlinii* (Sinomartagon section) (Asano, 1982), *L.* 'Connecticut Yankee' (Sinomartagon section) × *L. longiflorum* (Leucolirion section) and *L. aurelianense* (Leucolirion section) × *L. longiflorum* (Ascher 1973a,b and 1977). When in natural conditions fertile pollen formation by abnormal meiosis is rare in intersectional hybrids. However, researchers are developing artificial methods using temperature fluctuations and chemical treatments at the optimum stage of flower bud development.

2.6 Importance of Introgression Breeding

Introgression is one of the main goals in interspecific hybridization in order to introduce a restricted number of traits from the donor species to the recipient. For example, in the Oriental hybrid group (Archelirion section) no orange flower colour is present. In addition they are susceptible to *Fusarium*, but resistant to *Botrytis*. The Asiatic hybrids (Sinomartagon section), however, are available in a range of colour, but are generally susceptible to *Botrytis* and resistant to *Fusarium*. Thus, to combine these traits is clear that interspecific hybridization must be used, especially when compared to alternative methods such as genetic transformation or mutation breeding.

2.7 Polyploidization

2.7.1 Mitotic Polyploidization

As indicated most interspecific hybrids between distantly related *Lilium* species are highly sterile. Therefore, even after successful interspecific hybridization between diploid species (2n=2x=24), the sterility of interspecific hybrids imposes a significant restriction to introgression breeding. Polyploidization can solve this problem, which can be distinguished into mitotic and meiotic polyploidization. The former is obtained through artificial chromosome doubling by treatment of vegetative tissue with spindle inhibitors such as colchicine (Blakeslee and Avery 1937; Emsweller and Brierley (1941) or oryzalin (Van Tuyl et al., 1992). In many cases, however, there are serious problems for the homoeologous recombination between parental species in allotetraploid of F_1 interspecific hybrid (Lim et al., 2001b). Due to preferential pairing between homologous chromosomes at metaphase I of the meiosis, homoeologous chromosomes have absolute or almost no pairing and therefore homoeologous recombination is reduced dramatically. In most cases allotriploid progenies are formed between diploid and allotetraploid, and finally triploid plants might not be fertile. This is not relevant for further crossing. Tetraploidization can contribute some advantages to make triploid progeny. They are more vigorous, healthier, and have larger organs than diploids. But in most cases triploid cultivars are not fertile, which commercial breeders prefer to avoid further crossings by competitors. From a theoretical point of view, combining three genomes (Longiflorum, Asiatic, Oriental) have an increased opportunity to bring diverse characters into one plant.

2.7.2 Meiotic Polyploidization

An alternative and preferred method to use interspecific hybrids involves the use of 2n-gametes that occur occasionally in interspecific hybrids of lilies (Van Tuyl et al., 1989). The plants derived from crosses with 2n-gametes are in their parental characteristics genetically more homozygous (SDR effect), or more heterozygous with some degree of heterozygosity depending on recombinations (FDR effect) (Hermsen, 1984). An important feature of 2n-gametes is that, depending on the mode of origin, a certain level of intergenomic recombination can occur during the meiosis I division. Fertile n- and 2n-gametes have been produced in interspecific lily hybrids (*L*. 'Enchantment' × *L. pumilum*) within the Sinomartagon section (Van Tuyl et al., 1989). In fact, fertile n-gametes with homoeologous recombination are ideal for introgression breeding without increasing the ploidy level of the succeeding generation. However, in most cases, unreduced (2n) gametes are predominantly produced.

BC_1 plants derived from 2n-gamete producing F_1 intersectional hybrids showed a range of fertility and are used for BC_2 progeny. By GISH analysis, BC_2 progeny possess relatively large amounts of donor species chromosome segments together with a number of whole chromosomes. This can be used for the introgression breeding of desirable genes into cultivar (Lim et al., 2001a).

2.8 Chromosome Analysis by Genomic *in situ* Hybridization (GISH) Technique

Genomic *in situ* hybridization (GISH) can distinguish the parental genomes of interspecific hybrids. This technique, which utilizes total genomic DNA from one of the parental species as a probe and from the other counterpart species as a block, provides a new technique for effective parental genome analysis in both sexual and somatic hybrids. This technique also detects translocations involving chromosomes from different genomes is useful to monitor chromosome behavior during meiosis. Therefore, the level of introgression in back-crossed progenies between different species can be measured by GISH analysis. Due to its large chromosome size, lily has advantages in the analysis of the number of parental chromosome composition and homoeologous recombination breakpoints. This is beneficial to determine whether or not recombination has taken place between parental chromosomes. Lim et al. (2000, 2001a,b) have utilized this procedure in their studies.

3. BREEDING

3.1 Important Traits for Commercial Breeding

3.1.1 Flower Colour

In lily breeding flower colour is one of the most important characters in combination with flower shape and size. In order to produce a desirable colour in the next generations or understanding of the inheritance of flower colour is needed. The genetics of flower colour has been determined differently in the hybrid groups. In the Asiatic hybrids, orange flowers are the dominant wild type, while yellow is determined by a single-recessive gene. Another gene determines the absence of carotenoid flower colours resulting in white or pink flowers. In Orientals, dark pink is dominant over white. In this group, the carotenoid colours have a minor role (Banba, 1975).

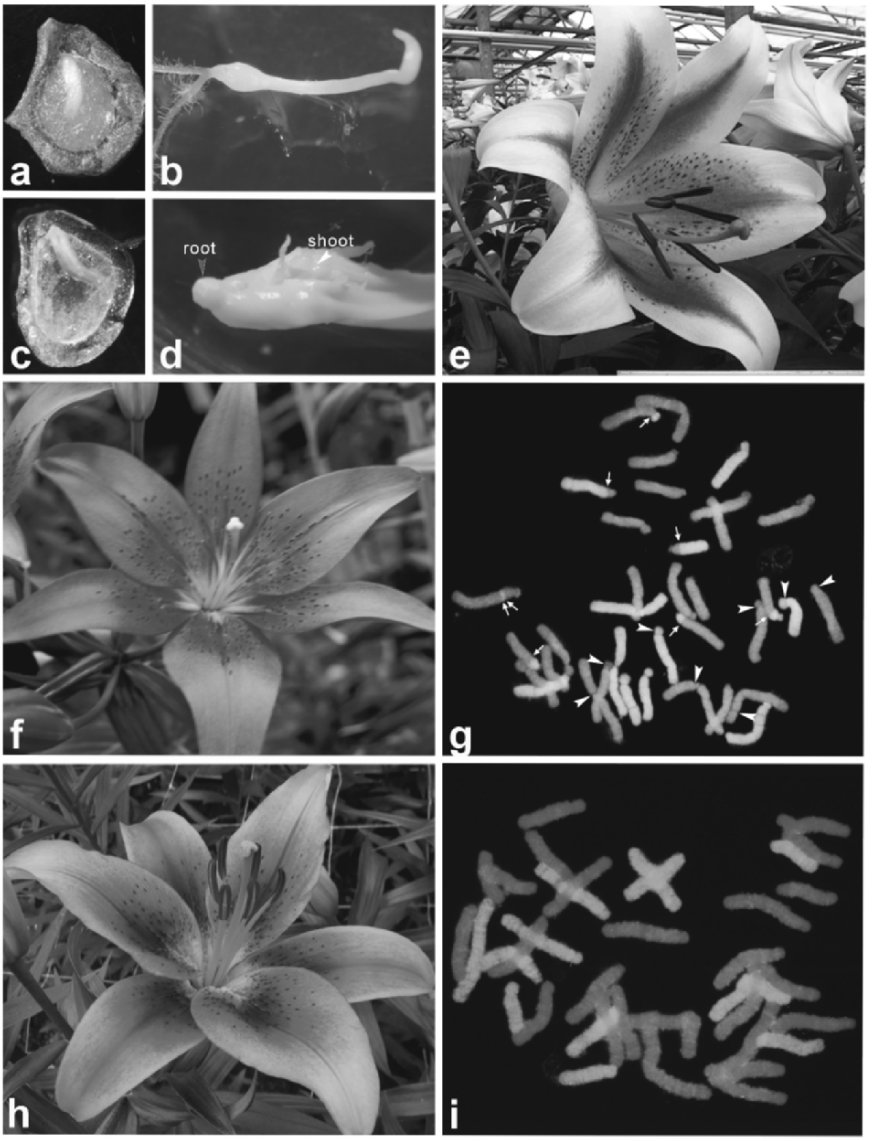

Figure 19-2. a. Normal embryo at 50 to 60 days after pollination, b. a hybrid plant with roots and bulb, c. abnormal embryo growth from an interspecific hybrid, d. a germinated seedling showing ambiguous shoot and root formation, e. super lily (OLO) with a 27cm diameter flowers, f and g. ALA triploid derived from meiotic polyploidization possessing many homoeologous recombinations (arrows). Arrow-heads indicate nucleolar organizer regions (NORs), h. An interspecific triploid hybrid between Asiatic and Oriental (AOA) derived from mitotic polyploidization of an OA hybrid. i. Chromosome painting showing chromosome composition -Asiatic (red) and Orientals (yellow).

3.1.2 Disease Resistance

Fusarium oxysporum var *lilii*, *Botrytis* and viruses are the most severe pathogens for lilies. Research has been performed at Plant Research International for the evaluation of disease resistance for *Fusarium* and *Botrytis* and useful screening techniques has been developed (Straathof and Van Tuyl, 1992, 1994). A high degree of *Fusarium* resistance was found in some Asiatic cultivars and in some species e.g. *L. dauricum.* In contrast, all Oriental hybrids were relatively susceptible. On the other, hand Oriental hybrids were highly resistant to *Botrytis*, while many Asiatic hybrids and some species e.g., *L. pumilum*, *L cernuum* were very susceptible (Van Tuyl and Lim, unpublished results). Resistance to Lily Mottle Virus (Tulip Breaking Virus) was found in some Asiatic cultivars and it segregates as a single gene (Van Heusden et al., 2002).

3.1.3 Flower form, Shape, and Forcing Ability

Due to a wide range of flower shapes, size, colour and morphological characteristics in combination with strong growth habits and relative high levels of resistance to diseases, the sections Leucolirion, Sinomartagon, and Archelirion sections are the most important groups economically. Interspecific hybrids within the sections, especially Asiatic hybrids, have been bred since the early 1800's (Shimizu, 1987). The distinctive characters of three important hybrids groups for cut flowers are:

1) The Longiflorum hybrids (**L**-genome) group in the Leucolirion section have trumpet-shaped, pure white flowers, a distinctive fragrance, year-round forcing capabilities, and mostly outward-facing flowers;

2) The Asiatic hybrids (**A**-genome) group in the Sinomartagon section possess a range of flower colours (orange, yellow, white, pink, red, purple and salmon), mostly upright-facing flowers, and are early (*L. pumilum*, *L. cernuum*) to late (*L. callosum*) flowering types;

3) The Oriental hybrids (**O**-genome) group in the Archelirion section have large, pink or white flowers, a strong fragrance, sturdy stems, wide dark-green leaf shapes, and early (*L. rubellum*) to late flowering (*L. nobilissimum*) habits.

The commercial Asiatic hybrids originated from interspecific crosses between species of the Sinomartagon section, Oriental hybrids were derived from crosses in the Archelirion section, and *Longiflorum* hybrids obtained from *L. longiflorum* Thunb. or crosses of *L. longiflorum* and *L. formosanum* Wallace.

As many as 12 species of the Sinomartagon section are involved in the currently available cultivars of Asiatic hybrids: *L. amabile* Palibin (Korean lily; orange or yellow coloured), *L. bulbiferum* Linn. (Orange, upright-facing), *L. cernuum* (early flowering, white or pink colour), *L. concolor* Salisb. (small flower, early and upright-facing), *L. dauricum* Ker-Gawler (early flowering, upright-facing, hairy, and

Fusarium resistant), *L. davidii* Elwes (orange with black spots), *L. lancifolium* Thunb. (strong stem, vigorous, hairy and bulbil forming), *L. lankongense* Franchet (pink with fire spots), *L. leichtlinii* Hook. (citron-yellow or orange with hairy buds), *L. maculatum* Thunb. (upright-facing) and *L. pumilum* (orange or yellow flower colours and dwarf) (Woodcock and Stearn, 1950).

At least five species of the Archelirion section - *L. alexandrae* Wallace*, L. auratum* Lindley*, L. nobilissimum*, *L. rubellum* and *L. speciosum* Thunb. - have been intercrossed (Beattie and White, 1993). These hybrids are referred to as Oriental hybrids. *L. japonicum* Thunb. was also used as parent for the Oriental hybrids (McRae 1998).

3.2 Commercial Breeding

Commercial breeding of lilies nowadays focus on the one hand on the development of new (interspecific) hybrids like LA, LO, OT, OA etc. for which embryo rescue techniques are needed and can be called “In vitro breeding” (see Section 2.2). On the other hand improvement of the Oriental, Asiatic and longiflorum-hybrid groups are made using classical techniques. This means normal hand pollinations are performed, seeds are sown and in the second (in case of longiflorum) or third (in case of Asiatic) or in the fourth year (in case of Orientals) after crossing seedlings can be selected. The size of these programmes vary from a few thousand to sometimes a half million seedlings per year. Selection for forcing quality is done under greenhouse forcing conditions. In the further processes of selection and propagation (using scaling or in vitro propagation), growth characteristics and disease resistances will be evaluated. Before deciding large-scale propagation, selections will be tested year-round and at different locations (preferably in different countries). The last 5-10 years of the around 100 lily cultivars which are commercialised with breeder’s right (see 3.4) about half originates from interspecific crosses (in vitro breeding) and about half from classical breeding. It is obvious that the technique of *in vitro* breeding is mostly done on a smaller scale, but also that in advanced generations with improved fertility normal sowing procedures is or will be applied. The most important breeders on this moment are Vletter & Den Haan, Marklily, Worldflower, Royal van Zanten, Bischoff Tulleken, De Jong Lilies and Sande (Peterse, 2000). In the VS, Australia, New-Sealand, Germany and some other countries small companies and hobby breeders focus on lilies for the garden market. Depending on the climate these garden lilies originate from a wide range of species and species hybrids. The production is relative small. These cultivars are only registered without application for breeders’rights. A last effort of lily breeding is the seed propagated *L x formolongi* group. In Japan and Korea this type of lilies are grown on a relative small scale. For this purpose upfacing longiflorum hybrids (Raizan, Augusta) are developed by Daichii (Japan).

3.3 Molecular Breeding

Molecular markers linked to resistance traits, especially polygenic complex traits which are difficult to determine, can reduce the breeding and selection processes indirectly, by the so-called, marker assisted selection (MAS). Molecular markers have been used in lily for tracing parentages (Kazuhisa et al., 1998), identifying diversity (Wen and Hsiao, 2001) and for finding linked RAPD-markers to *Fusarium oxysporum* resistance in Asiatic hybrids (Straathof et al., 1996; Jansen, 1996). Straathof et al. (1996) studied the inheritance of *Fusarium* resistance resulting in at least 3 QTL's, which was a difficult trait to repeat during different years. Van Heusden et al. (2002) could repeat these results using AFLP-markers. TBV resistance was clearly a monogenic trait and could reliably to be mapped on linkage group 9. The closest linked marker, however, was still at a distance of about 10 cM. Despite the difficulty screening for *Fusarium* resistance, four significant QTLs were detected. For additional studies of the four QTLs and their individual contributions to the resistance, it will be necessary to convert the linked AFLP-markers to more robust PCR markers (Van Heusden et al., 2002).

Genetic transformation could be a powerful technique for the insertion of genes that do not exist in lily. Both *Agrobacterium* and microprojectile mediated transformation in lily have been accomplished by several researchers (Bino et al., 1992; Van der Leede-Plegt et al., 1992; Watad et al., 1998). The GUS gene was successfully transmitted into next generation. For future applications, a marker-free system is required. Research at PRI is currently focused on developing marker-free technology.

3.4 Lily Registration

Since 1960, at least 7000 cultivars have been cited in the RHS Lily register. There are two organisations that deal with lily cultivar registration either in the RHS lily register or in UPOV (Union for the Protection of New Varieties of Plants). Every year, over 100 new cultivars are registered in UPOV to obtain the breeder's right for commercialisation. In contrast about 200 cultivars are registered in RHS lily register in order to registrar the cultivar name, where the same cultivar name is not allowed. Therefore, RHS lily registration is based on non-profit activity, while breeder's right is primarily for the commercial basis. RHS lily registration does not require a fee, while UPOV registration requires qualification of the commercial value as a new cultivar for one year under standard forcing condition with control cultivars as a comparison. The breeder must provide enough bulbs (50 bulbs, 12-14cm in circumference) for the trial and the judging committee records distinctive characters for the new cultivar, e.g. disease resistance, physiological characteristics, mutation, uniformity and other phenotypic characters. If the first round of evaluation is not satisfactory, the committee can retest the selection. Additional information can

be found at http://www.upov.int/eng/index.htm. Once registered in UPOV, the breeder's right is valid for 25 years, unless cancelled by the applicant.

3.5 History of the Pink Longiflorum 'Elegant Lady'

One of the successful examples of interspecific hybridization is 'Elegant Lady' (triploid, **LLR),** it was derived from crossing *L. longiflorum* and a **LLRR** F_1 interspecific hybrid. *L. longiflorum* possesses long, white, tubular shaped flowers with a pleasing fragrance, and *L. rubellum* has a very early flowering habit (ca. 35 days) and a pink flower with a pleasing fragrance. The **LR** hybrid has pink flower and early forcing habit. Since the **LR** F_1 hybrid is sterile, it was necessary to make a tetraploid by mitotic polyploidization to recover fertile pollen. The amphidiploid (**LLRR**) was fertile and was crossed with *L. longiflorum* to make the hybrid 'Elegant lady' which possess a tubular shaped pink *longiflorum* flower. Genomic *in situ* hybridization (GISH) confirmed that the **LLR** triploid was composed of two sets of *L. longiflorum* chromosomes and one set of *L. rubellum* chromosomes without any homoeologous recombination between parental chromosomes (Lim et al, 2000). The characters were intermediate between *L. longiflorum* and *L. rubellum* (Table 19-2). This plant exhibits elegant tubular-pink flower with very early flowering and a pleasing fragrance. This hybrid was named 'Elegant lady' and was commercially released in 2000.

Table 19-2. Characteristics of *L. longiflorum* and its F_1 and BC_1 hybrids.

Genotype	Plant height (cm)	Flower length (cm)	Forcing time (days)
'Gelria'	94.0	15.9	95.0
'Snow Queen'	116.3	18.2	96.7
LR (F_1)	47.9	11.2	51.7
LLR 'Elegant lady'	79.4	15.9	75.2

3.6 History of ALA Hybrids Derived from Meiotic Polyploidization

LA interspecific hybrids have been commercially available for a decade and currently about 30 cultivars are marketed. Most of them are triploids derived from backcrossing by mitotic or meiotic polyploidization. We analyzed one (**A**)**LA** hybrid 'Fangio' (2n=3x=36, triploid, **A** genome=24, **L** genome=12) and confirmed by GISH that there are many recombinant chromosomes between the **L** and **A** genomes (Figure 19-2f, g). Based on GISH analysis data, it was concluded that this hybrid was derived from spontaneous meiotic polyploidization (Lim & Van Tuyl, unpublished results).

3.7 Breeding Trends

Since 1960 about 7.000 lily cultivars has been registered since 1960 (Leslie, 1982; Mynett, 1996). Active lily breeding work started in Japan between the 1920's and 1940's, in Australia and New Zealand during the 1950's and 1960's and in the United States in the 1960's to 1970's. In the past 25 years lily breeding has been predominantly carried out in the Netherlands. Due to the release of many tetraploid clones from Plant Research International (formerly IVT, CPRO-DLO) to Dutch commercial breeders, the number of polyploid cultivars has steadily increased during the last decade (Van Tuyl et al., 1991; Schmitzer, 1991). With Asiatic hybrids many of the diploid cultivars have been replaced by tri- and tetraploid hybrids, while the **LA**-hybrids are mostly triploids. In contrast, all commercial *L. longiflorum* and Oriental hybrids are still diploid.

Interspecific polyploid cultivars have been produced by the recent employment of new hybridization techniques (Figure 19-2e,f,g,h,i). Examples include, respectively, **LA**-, **LO**-, **OA**- and **OT**-hybrids derived from *L. longiflorum* (**L**) and Asiatic hybrids (**A**), *L. longiflorum* (**L**) and Oriental hybrids (**O**), Oriental hybrids (**O**) and Asiatic hybrids (**A**), and Oriental hybrids (**O**) and Trumpet hybrids (**T**; Leucolirion section).

A major goal of lily breeding is to combine the three distinctive groups, so-called, Longiflorum-, Asiatic- and Oriental-hybrids. For example, **LA**-hybrids have become popular in the market over the past 10 years because of their flower shape and size, upright-facing flowers, sturdy-long stems, early flowering habit and a pleasing fragrance which was not available in Asiatic hybrids. By expanding interspecific hybridization between **LO**-hybrids and **OT**-hybrids, new types of interspecific hybrids will appear in the market together with **OA**-hybrids. **OLA**-hybrids derived from merging the three hybrid groups are also being developed as commercial cultivars.

3.8 Future Prospects

Looking to the future it is clear that after the increase of the Asiatic hybrids in the 1970's and 1980's, which was followed by the exotic and large flowered Oriental lilies, the time for more complex hybrids is here. The LA-hybrids, which have increased from 10 ha in 1995 to more than 590 ha in 2002, demonstrates this trend. The OT and OA hybrids are emerging. It will not take long before one group of hybrid lilies will be developed in which the different species and hybrid groups cannot be distinguished anymore. At the same time, breeding for resistance to virus, *Botrytis, Fusarium* will utilize improved systems like molecular assisted breeding and GISH-techniques.

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Chapter 20

ORCHIDS
Dendrobium

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Abstract: *Dendrobium* orchids are popular flowering potted plants and cut flowers around the world due to their flowering floriferousness, wide range in flower color, size, and shape, year-round availability, and lengthy post-harvest life. Both warm and cool temperature cultivars are grown from *in vitro* germinated seed or clones. Breeders have created polyploid cultivars (amphidiploids, triploids) which have greater flower number/raceme, larger flowers, and other valuable traits. Numerous genes have been identified in the genus with some sequenced genes being used to create transformants. Wide crosses are routinely used to create new genetic variability, as *Dendrobium* exhibit low levels of hybrid breakdown (endosperm-embryo incompatibility) and inbreeding depression. Crop ideotypes prescribe numerous traits for incorporation by plant breeders and geneticists, including flowering >1x/yr, enhanced shelf life, and simultaneous flowering of inflorescences.

Key words: *Ceratobium, Phalaenanthe,* polyploidy, protocorm-like bodies, pseudobulb.

1. INTRODUCTION

Several categories of orchids (Family: *Orchidaceae*) have been successful for use as blooming potted plants. *Dendrobium* orchid is one such category, with increasing popularity in the international floricultural scene. While historically a cut flower commodity, dendrobiums are increasingly being used as potted plants for interiorscaping of hotels and restaurants. Common examples of use are in resort destinations, ranging from lavish use in Las Vegas to the quiet décor of St. Moritz. They are also increasingly visible in offices and homes and make excellent gifts for all occasions. The main attraction of *Dendrobium* relative to other potted orchids is

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their floriferous flower sprays, a wide range of colors, sizes and shapes, year-round availability, and long flowering life of several weeks to months.

2. ECONOMIC HISTORY

The value of sales for *Dendrobium* and other potted orchids has been steadily increasing. Government estimates of sales of potted orchids in the United States, which began in 1996, showed the commodity to be worth over US $100 million in 2000. Hawaii, California and Florida are major potted *Dendrobium* growing regions in the United States. The wholesale value of sales for this commodity in Hawaii has been recorded for several decades. Sales increased dramatically over the last 10 years (Table 20-1). The value of *Dendrobium* attributed to potted plant sales at wholesale in Hawaii more than doubled in this time, from US $2.4 million in 1991 to US $5.6 million in 2000 (Johnson 1999). When the sales include community pots (numerous plantlets per pot) as well as bud/bloom pots (i.e., plants in flower), the value of sales increased by 17% in just one year, from US $5.6 million in 1999 to US $6.6 million in 2000 (Hawaii Agricultural Statistics Service 2001). Potted sales in Hawaii now rival *Dendrobium* cut flower sales of US $6.2 million (total domestic and imported stems, 1998).

Production of potted orchids in the Netherlands is now 40 to 50 million units, with *Dendrobium* increasing in popularity (Hamrick et al. 2002). Taiwan and Thailand are also large producers of potted orchids, with *Dendrobium* occupying a minority but trendy position relative to other exported orchid genera. The USA potted plant market is viewed to have significant opportunities for market growth and thus is a strong target for imports. USA-based potted orchid producers have had some protection against non-USA competition due to quarantine restrictions. Importation of *Dendrobium* orchids in growing media is currently prohibited in the United States. A key to the domestic *Dendrobium* industry holding and expanding its market share in the future will be to continue to provide a superior plant with superior customer service.

Table 20-1. Quantity of consumption and wholesale value of sales of potted *Dendrobium* in Hawaii (Johnson 1999).

Year	Number of pots sold	Value (US$)	Number of growers
1985	0.2 million	1.1 million	88*
1991	0.4 million	2.4 million	47
2000	1.0 million	5.6 million	69

*Number of farms reporting sales.

3. SPECIES ORIGIN AND DOMESTICATION

The genus *Dendrobium* comprises over 1000 species. Some are deciduous and others are evergreen. Many are fragrant. Inflorescences can be erect or arching, pendulous, or even clustered at the pseudobulb nodes. Dendrobiums are sympodial epiphytes that fall into several temperature-watering groups for flower production (Northen 1990). They can be roughly lumped into warm temperature and cool temperature varieties. In the warm group (night temperatures above 60˚ F or 16˚ C) are *D. phalaenopsis, D. gouldii, D. bigibbum, D. antennatum,* and *D. discolor*, for example. Growers may briefly reduce watering of some species in this group as the growth matures and inflorescences are initiated. Regular watering then resumes. Species comprising the cool group (nights of ~50˚ F or 10˚ C) include *D. lindleyi (D. aggregatum), D. parishii, D. pierardii, D. densiflorum, D. chrysotoxum and D. anosmum*. Most of the species in the latter group have rest periods (restricted watering and fertilization) and thus may be less suited for year-round production for the mass-market. The focus of this chapter will thus be on the warm temperature group.

Numerous species originated in Australia, New Guinea, the Philippines, Thailand, India, China and Indonesia, among other countries. Horticulturally important species are clustered into taxonomic sections including *Phalaenanthe, Spatulata* (*Ceratobium*), *Latourea, Formosae* (*Nigrohirsutae) Dendrobium* (*Eugenanthe*) and *Callista* (Schelpe and Stewart 1990). These sections are intercrossable, with variability in percent viable progeny generally determined by the genome or cytogenetic relatededness; more similar genome constitutions have more normal meiotic pairing (Kamemoto and Wilfret 1980). The species constitution of intersectional hybrids can be designated using genome symbols for each *Dendrobium* section represented, with a superscript for the species within that section. The *Phalaenanthe* (P) and *Ceratobium* (C) sections are key contributors to both the cut flower and potted varieties.

Trade in potted *Dendrobium* is largely with hybrids. A registered hybrid is a named cross officially registered with the Royal Horticultural Society in London (http://www.rhs.org.uk/research/registration_orchids.asp). Any hybrid can be registered if a cross between parents with the same names was not previously registered. The pedigree of a registered hybrid can be accessed through Sander's List of Orchid Hybrids or electronically through the Wildcatt Orchids subscription database (http://www.wildcattdata.com/). Thailand's cut flower industry was founded on the French hybrid variety 'Pompadour' (*D*. Louis Bleriot x *D. phalaenopsis*; registered in 1934), with wide-spread production starting in the mid-1970s. *D*. Pompadour is a purple tetraploid comprised of three chromosome sets of *D. phalaenopsis*; and one chromosome set of *D. discolor*, i.e., of a PPPC genome constitution. Other *D. phalaenopsis*-type varieties with round, full, and flat flower faces of 3 inches (8 cm) or greater width continue to be the signature shape of Thai

dendrobiums, including the potted varieties. The overlapping petals and sepals are contributed by the *Phalaenanthe* parent. These hybrids are clonally propagated from meristematic explants in tissue culture and as such are called 'mericlones'. Hybridizers may produce their own mericlones for sale, if they have a tissue culture lab, or they may contract with a specialist propagator. In either case, stock plants are regularly re-started to minimize somaclonal variation.

Hawaii's fledgling cut flower industry was also boosted by polyploid hybrids, in this case doubled diploids (amphidiploids) of *D*. Jaquelyn Thomas (*D. phalaenopsis* x *D. gouldii*) released by the University of Hawaii in the 1970s. Cut flower, and then potted plant, breeding was expanded to include hybrids of several other species producing numerous allopolyploid combinations (Kamemoto et al. 1999a). The flowers of *D*. Jaquelyn Thomas-type hybrids (PPCC) are generally smaller than PPPC-type flowers, measuring about 2-1/4 inches (5.6 cm) long and 2-1/2 inches (6.3 cm) wide with no overlap of petals and sepals similar to the *Ceratobium* parent, but the number of flowers per raceme is greater. The University of Hawaii cultivars are primarily seed-propagated hybrids that are progeny-tested prior to release. The use of one or both of the parents that are amphidiploid permitted good seed set following hybridization and fairly uniform progeny. The first seed-propagated potted plant hybrid, triploid *D*. Lynne Horiuchi (amphidiploid *D*. Macrobig x *D. bigibbum* var. *compactum*) was released by the University of Hawaii in 1985. The majority of University of Hawaii seed-propagated potted plant hybrids utilize species from the *Phalaenanthe* (P)*, Ceratobium*, (C), *Latourea* (L) and *Eugenanthe* (E) sections (Table 20-2).

An additional ten clonally propagated potted plant lines have been released by the University of Hawaii between 1979 and 1999. Worthy of note are *D*. Ethel Kamemoto 'Splendor' and *D*. Ethel Kamemoto 'White Cascade' (Fig. 1). These have as parents *D*. Theodore Takiguchi and *D*. D'Bush Pansy and are compact and very floriferous. 'Splendor' produced 13 racemes on one pseudobulb with an exceptional half-life of about 3 months (Kamemoto et al. 1998b). 'White Cascade' has the unique floral character of pansy-lip (Kamemoto et al. 1999b).

Table 20-2. Some University of Hawaii seed-propagated *Dendrobium* potted plant cultivars.

Cultivar Name	Year released	Genome type*	Color
Lynne Horiuchi (UH613)	1985	PPL	Purple
Caesar (UH921)	1989	PC	Lavender
Samarai (UH988)	1989	CC	White w/ purple lip
Sylvia Yuen (UH1101)	1990	CCE	White w/ lav. lip
Susan Takahashi (UH1999)	1990	PPCC	Purple
Cathy Beck (UH1221)	1991	PCE	Lavender
Pua'ala (UH1182)	1993	PCL	Purple
Betty Nakada (UH1208)	1994	PPE	Purple
Remy Hartmann (UH1307)	1994	PCE	Lavender
Lim Chong Min (UH1382)	1995	PPC	Lavender

Cultivar Name	Year released	Genome type*	Color
Louis Bleriot (UH1392)	1995	PPC	Purple
Miyoko Azuma (UH1121)	1996	PPPC	Purple
Sharon Sewake (UH1419)	1996	PPPE	Purple
Uniwai Sunrise (UH1323)	1997	PPCC	Red-purple
Mari Marutani (UH1420)	1998	PPPC	Purple
Lorrie Mortimer (UH1577)	1998	PCCC	Lavender turning yellow-green
Winifred Ogata (UH1371)	1999	PPPC	Two-tone lavender

*Letters correspond to abbreviations for the taxonomic sections *Phalaenanthe* (P), *Ceratobium* (C), *Latourea* (L), and *Eugenanthe* (E).

4. CROSSING MECHANISMS

Dendrobium can be naturally outcrossed by insect pollinators, as are many other orchids (Arditti 1992). A few species such as *D. antennatum* show cleistogomy and can self-pollinate in closed buds (Kamemoto et al. 1999a). In breeding with most *Dendrobium* species, no emasculation is required for cross-pollination as the plants cannot physically self-pollinate. Pollination is effected by means of a pencil tip that dislodges the adherent waxy pollen mass (pollinium) from one plant for placement into the sunken stigma on the column of the seed-bearing plant. The labellum may then be gently removed to mark that flower as having been pollinated. The floral whorl will wilt and its inferior ovary will expand as a sign that the pollination was effective.

Cross compatiblity among 37 *Dendrobium* species representing ten taxonomic sections has been summarized in Kamemoto et al. (1999a). Some sections of *Dendrobium* showed poor crossability within a species or within a section, such as for the *Callista* group. Other sections showed high crossability at the intraspecific and interspecific (both intrasectional as well as intersectional) levels, such as for *Ceratobium* and *Phalaenanthe* sections.

5. COMMERCIAL PRODUCTION

5.1 Propagation

Commercial hybrids are generally either seed-propagated (almost exclusively Hawaiian in origin) or clonally propagated through tissue culture of apical and lateral buds that proliferate as protocorm-like bodies (see Kim et al. 1970). Protocorm-like bodies resemble true protocorms (germinated orchid seed) in being spherical but are derived from shoot tip or bud explants. *D. nobile* varieties can be

propagated by cuttings in the field and will flower after 1.5 years. The advantages of seed-propagated cultivars over those that are mericloned in tissue culture are that they are easier, faster and less expensive to propagate. Moreover, the resulting seedlings are free of Cymbidium Mosaic Virus, since the virus is not transmitted through seed (Porter et al. 1996). However, chemotherapy can be used to eradicate virus from mericlones during liquid culture using the procedure of Porter and Kuehnle (1997), with recent improvements by S. Wannakrairoj (unpublished data, 2002) by combining two virucides, dithiouracil and ribavirin, into one treatment.

Thailand, and to some extent the Philippines, Singapore and Taiwan, supply many growers throughout the world with flasked material for finishing to blooming stage. The cost of labor for tissue culture in some of these countries is only a fraction of what it is in the United States or the Netherlands, and breeding is very active there to attract customers with a changing selection of hybrids. Micropropagation procedures specific to *Dendrobium* have been summarized (Arditti and Ernst 1993).

For seed propagation, *Dendrobium* seeds are aseptically germinated beginning usually three months after pollination. Green capsules are surface-sterilized, cut open at the blossom end, and seeds tapped out to drop onto most any basal salt medium containing 15% (v/v) coconut water and 2% (w/v) sucrose, pH 4.8 to 5.0. The medium is agar-solidified, although seeds will germinate normally into protocorms and proliferate as protocorm-like bodies in liquid shake culture as well. Alternatively, seed from mature brown, cracked capsules are surface-sterilized and sown in a small volume of water by pipetting onto germination medium. Three months after sowing, seedlings can be transflasked with 75 to 100 plants per 500 ml flask onto a minimal salt medium containing 15% coconut water, 2% (w/v) banana powder (or 7.5% w/v green banana fruit), and 1% sucrose, pH 4.8 to 5.0 (Kamemoto et al. 1999a). Roots can form in either liquid or solidified medium.

5.2 Deflasking Young Plants and Growing On

Nursery practices for growing *Dendrobium* have been recently outlined (Leonhardt and Sewake 1999) in a production guide, available through the website www.ctahr.hawaii.edu, and summarized in Leonhardt (2000). Briefly, rooted plantlets are deflasked into community pots or flats in a disease-free medium that retains moisture, yet is well draining and aerated. Growers may use combinations of Promix, peat, perlite, styrofoam, coconut husk products, sphagnum moss, or chopped tree-fern fiber. Plants are then transplanted into 2 inch (5 cm) or 2-1/2 inch (6 cm) pots, or directly into the finished pot with one or more plants per pot, 4 to 6 months after deflasking. Finished pot sizes range from 2-inch (5 cm) to 6-inch (15 cm) but can be much larger for interiorscaping specimens. Growers or retailers may also market a 2-inch (5 cm) finished plant of about 6 inches (15 cm) stem height inside a 4-inch (10 cm) pot with foam supports and foil, for example, with attractive

and cost-saving results. For growing on, warm-temperature dendrobiums prefer nights above 60˚ to 64˚ F (16˚ to 18˚ C) and days between 75˚ and 86˚ F (24˚ and 30˚ C), with bright light (30% shade in Hawaii for *D.* Jaquelyn Thomas types, 45% for *D.* Pompadour and *D.* Louis Bleriot types). Protected cultivation under plastic or other hardcover greenhouse material is desirable to prevent diseases that lower the grade of the plant.

Preferred media for exporting potted *Dendrobium* include coconut coir and red wood bark chips. Volcanic cinder or cinder mixes are light enough media to be suitable for exportation of plants but they can spill out of the pot. Two pieces of plastic tape across the pot can secure the medium during handling. In Hawaii, plants are 'sleeved' for shipment by first packing shredded paper around the base of the plant and the flowers, if necessary, and then rolling the pot and plant in newspaper, which is taped shut.

Insects, mites and other pests and diseases that are common to cut flower *Dendrobium* also affect potted plants and are well-documented (Hata and Hara 1999; Uchida 1994). One recent pest that causes flower abortion and could impact potted *Dendrobium* is a foliar nematode from the *Aphelenchoides* species for which there is no available chemical control (Uchida and Sipes 1998). Of particular concern for export of potted *Dendrobium* is *Thrips palmi* because of a zero tolerance quarantine action in the continental USA (Hata et al. 1991).

5.3 Time to Flowering and Yield Potential

Potted plants are preferably sold with a minimum of two flowering spikes, with blossoms on the spike being about one-third open. Crop time from deflasking to saleable plant is approximately two to three years. Those plants intended for interiorscaping, however, may have more mature spikes and multiple plants per pot. Application to mature *Dendrobium* pseudobulbs of the cytokinin benzyladenine, by itself or in combination with gibberillic acid, reportedly initiates flowering such that a cut flower crop can be timed (Sakai et al. 2000). Tests still need to be done to examine if this is as useful for juvenile potted plants with developing pseudobulbs. Encouraging data from the private sector indicate that foliar application does indeed appear effective for timing potted plants (K. Sewake, unpublished data 2002). Plants in the first year or two of flowering tend to initiate infloresences throughout the year, with less pronounced seasonal flushes as seen in older plants. Temperature control is a requirement to trigger flowering in *Dendrobium nobile*, requiring nights of 50˚ F (˚ C) (Northen 1990). For post-harvest handling, precautions to avoid ethylene injury are unnecessary, although dislodging of pollinia should be avoided to obviate wilting. Plants are subject to chilling injury below 45˚ F (7˚ C) and thus should be handled accordingly during shipping and distribution.

6. TRAITS AND GENES IDENTIFIED

6.1 Polyploidy

Polyploidy as a trait for selection accounts for improvements in horticultural features of commercial *Dendrobium*. Flowers from polyploids generally are fuller and of greater substance than are flowers from diploids, although they may be borne on slightly shorter racemes with fewer flowers per raceme. Orchid cytogenetics, or the study of orchid chromosomes using cytological techniques, was well underway by the 1940s and 1950s. This permitted documenting the value of polyploidy for show flowers, cut flowers and potted plants, and enabled development of genome breeding as a strategy for commercial cultivar production (Kamemoto 1987). The first documentation of polyploidy in *Dendrobium* was by Ito and Mutsuura (1957). For potted plant breeding, using section *Phalaenanthe* is of interest due to the presence of overlapping, firm sepals and petals in polyploids.

A liquid culture phase is generally used for induction of chromosome doubling. Protocorm-like bodies can be treated with autoclaved 0.1% (w/v) colchicine in orchid seed germination medium for 5, 7, and 10 days at 100 rpm under continuous light before transfer onto solid transflasking medium (Sanguthai et al. 1973). Polyploids may also arise spontaneously during tissue culture or during early development of the embryo. In any case, polyploids among plantlets in flask can be recognized by vigorous growth and enhanced substance of leaves. They can be ascertained by counting chromosomes in root tips or pollen mother cells (Tanaka and Kamemoto 1984) or by determination of nuclear DNA content by flow cytometry (Jones et al. 1998).

6.2 Dendrobium Gene Sequences

Our tools have advanced sufficiently in recent decades such that one may now ask questions on gene expression and gene sequence variation as they relate to orchid production and improvement (early studies on orchid DNA and RNA are reviewed in Kuehnle 1997). Having the sequence lets one look at if, when, and where in the plant the genes are turned on, and then how to manipulate the traits they encode. Beginning in 1993, about 64 genes from seven orchid genera have been published (Mudalige and Kuehnle, 2004). Over two-thirds (67%) of orchid genes were discovered between 1998 and 2001. Some countries active in the research include Singapore, Taiwan, Korea, Germany, and the United States. A summary of published *Dendrobium* orchid genes known to affect plant growth and development is presented in Table 20-3. Only a few of these sequenced genes are directly applicable to orchid production and improvement. These genes concern floral transition (Yu and Goh 2000a, 2000b), flower color (Mudalige and Kuehnle 2002), and senescence (Yang et al. 1996).

Table 20-3. Dendrobium orchid genes affecting plant growth and development.

Gene or cDNA Designation	cDNA library used	Function or presumptive function	Reference
cko1	*Dendrobium* Sonia, Tissue source unknown	Cytokinin oxidase	Yang et al. 2001
DCACS	*Dendrobium crumenatum* flower	ACC synthase	Yang et al. 1996
DcrIc1	*Dendrobium crumenatum*, tissue source unknown	Isocitrate lyase, a key enzyme in glyoxilate cycle	Vellupillai et al. 1999
Dfr	Flower buds of *Dendrobium Jaquelyn* Thomas Uniwai Prince'	Dihydroflavonol 4-reductase, part of anthocyanin biosynthesis	Mudalige and Kuehnle 2002
DOH1	Vegetative shoot apical meristem of *Dendrobium Madame* Thong-In	Knotted1-like homeobox protein, a transcription factor involved in development	Yu and Goh 2000
DOMADS1 DOMADS2 DOMADS3	Transitional shoot apical meristem of *Dendrobium* Madame Thong-In	Transcriptional factors involved in floral transition. MADS-box genes in AP1/AGL9 subfamily	Yu and Goh 2000
Otg 16	Shoot apex of *Dendrobium* Madame Thong-In during floral transition	Putative casein kinase 1	Yu and Goh 2000
Ovg2	Shoot apical meristem of Dendrobium Madame Thong-In	Homeobox protein	Yu and Goh 2000
Ovg11	As above	Function unknown, involved in floral transition	Yu and Goh 2000
Ovg14	As above	Transcriptional regulator of cell cycle regulators, involved in floral transition	Yu and Goh 2000
Ovg15	As above	Function unknown, involved in floral transition	Yu and Goh 2000
Ovg23	As above	Putative copper/zinc	Yu and Goh 2000

Gene or cDNA Designation	cDNA library used	Function or presumptive function	Reference
		superoxide dismutase	
Ovg27	As above	Putative transcriptional repressor	Yu and Goh 2000
Ovg29	As above	Putative 21D7 protein	Yu and Goh 2000
Ovg30	As above	Putative DNA binding protein	Yu and Goh 2000
Ovg37	As above	Putative acyl-carrier protein (fatty acid metabolism)	Yu and Goh 2000
Ovg41	As above	Putative formate dehydrogenase	Yu and Goh 2000
Ovg43	As above	Putative phenyl alanine ammonia lyase (PAL)	Yu and Goh 2000
Ovg50	As above	Unknown function	Yu and Goh 2000
Otg2	Shoot apical meristem of *Dendrobium* Madame Thong-In in floral transition	Putative myosin heavy chain	Yu and Goh 2000
Otg4	As above	Putative cell division control protein	Yu and Goh 2000
Otg6	As above	Putative NADH dehydrogenase, intron region	Yu and Goh 2000
Otg7	As above	Putative MADS box gene, a putative transcription factor	Yu and Goh 2000
Otg9	As above	Putative alternate oxidase in alternate oxidative phosphorylation	Yu and Goh 2000
Otg11	As above	Putative fructose-bisphosphate aldolase	Yu and Goh 2000
Otg14	As above	Function unknown	Yu and Goh 2000
No designation	*Dendrobium crumenatum*	Vacuolar H^+ ATPase proteolipid subunit	Liew et al. 1999

6.3 Flower Color

Anthocyanin pigments comprised of cyanidin glycosides are the predominant pigments extracted from lavender and purple *Dendrobium* (Kuehnle et al. 1997).

These pigments are produced in the phenylpropanoid biosynthesis pathway. Key enzymes that make pigmented products are chalcone synthase (CHS) at the top of the pathway, followed by dihydroflavonol 4-reductase (DFR) and the flavonoid hydroxylases (F-3'H and F-3'5'H) that turn orange to lavender/purple and/or to blue by a simple chemical change. Genetic studies conclude that two complementary gene pairs, *C* and *R*, control expression of purple via duplicative recessive epistasis (Kamemoto and Amore 1990). Genotypes *ccRR* and *CCrr* will comprise white such that selfing produces white progeny and crossing with a member of the other genotype produces color. Thus, breeding for alba by crossing two white parents often results in non-white progeny due to complementary gene action.

A dominant gene *P* is responsible for lip color, or the semi-alba trait of white petals and sepals with a colored labellum, based on crosses between semi-alba *D. dicuphum* and white *D. affine* or white *D. phalaenopsis* var. *compactum* 'Mauna Kea' (Kamemoto and Amore 1990). Semi-alba (*P_*) is dominant to alba (*pp*). A study by Vajrabhaya and Vajrabhaya (1996) suggests that *pp* in the presence of *C* and *R* produces semi-alba, and that the *K* locus accounts for purple keel (*kk* produces white keel).

To effectively produce white *Dendrobium* cultivars, biotechnology-assisted breeding becomes an attractive strategy. Work in several other ornamentals such as chrysanthemum and petunia shows that it is feasible to block color production using the gene sequences for the biosynthetic enzymes (or their transcriptional regulators) of the phenylpropanoid pathway. Chemical analysis shows that DFR and both F-3'H and F-3'5' H are active in lavender *Dendrobium*, due to accumulation of myrcetin and syringetin as well as cyanidin (Kuehnle et al. 1997). Recent cloning of a partial sequence of F-3'5' H confirms this finding (Champagne and Kuehnle, unpublished). DFR is expressed predominantly in buds (Mudalige and Kuehnle 2002) and results in accumulation of the pigment through all layers of the epidermis and into the mesophyll of the petal (Mudalige et al. 1999). This enzyme is a prime target for genetic engineering, and transgenic plants are being grown for flowering (Mudalige, Champagne and Kuehnle, unpublished). Use of a CHS gene in sense and anti-sense fashion to modify anthocyanins in *Dendrobium* is underway (T.F. Chia, personal communication).

Yellows and greens in *Dendrobium* are due to carotenoids and chlorophylls (Thammasiri et al. 1986). Triploidy contributes to improved yellow cultivars. These plants themselves are poor breeders and efforts must be made to produce tetraploid plants. One strategy was described in Kamemoto et al. (1999a) in which a triploid yellow (*D.* Mary Mak) was crossed to a diploid (*D. helix*). Several tetraploids were recovered among a population of mostly aneuploids for use in subsequent breeding.

6.4 Pansy-Lip

Regular peloria, in which the lip reverts to a petal, is a trait in *Dendrobium* referred to as "pansy" type. Crosses of normal-lip *D*. Theodore Takiguchi to pansy type *D*. D'Bush Pansy showed the trait to be due to a single recessive gene (Amore and Kamemoto 1997).

6.5 Flowering

Raceme yield is recorded weekly when about 75% of the flowers are open. Amphidiploids produce on average 17 flowers per raceme, although this number diminishes as the *Phalaenanthe* genome becomes more predominant in the genotype (i.e., flowers are larger and fuller but flower counts per raceme decrease). The flower life on plants is measured as the half-life of racemes, i.e., the number of days from opening of the first flowers until 50% of all flowers wilt or drop. A long half-life of racemes on plants is considered to be 60 days or more, whereas less than two to three weeks is considered unacceptable for potted plants. Flowers for members of the section *Callista*, such as *D. lindleyi* (*D. aggregatum*) last only one to two weeks and are unsuitable for the mass-market, although they make breath-taking potted specimens when in full bloom. Bud drop has a genetic component but is presumably quantitatively inherited; bud drop of less than 5% is the standard in the University of Hawaii breeding program although up to 7% is acceptable for some potted hybrids. Protective covering in some cases reduces bud drop and also alters seasonality of flowering, as do the environmental conditions from year to year.

The *Phalaenanthe* genome has contributed to peak flowering in the fall (August to November) in cut flowers, but some PPPC-type potted hybrids have been produced with flowering into the winter (December through March) as well. *D*. Miyoko Azuma is a dark purple PPPC-type hybrid, between amphidiploid *D*. Jaquelyn Thomas and PPPP-type *D*. Manoa Beauty, with desirable seasonality peaking from October to March. *D*. Mari Marutani (Fig. 20-1) has a PPPC genome combination with peak flowering July to December but moderate flowering in January through March. Another PPPC-type, the two-tone lavender Winifred Ogata (Fig. 20-1), has moderate to strong flowering during fall and early winter but flowers throughout the year (Kamemoto et al. 1999c). Other potted hybrids with good seasonality, with peaks from October to March, are genome combinations of PCE, CCE, and PPE such as *D*. Cathy Beck, and *D*. Sharon Sewake (Table 20-2), with the *Eleutheroglossum* background contributed by *D. carronii* (Kamemoto et al. 1999a).

Peaks in flowering become less of an issue for potted plants when the flowers are long-lasting. For example, *D*. Lorrie Mortimer (Fig. 20-1) has an excellent keeping quality of three to four months. Thus, plants remain attractive during

summer months although the new blooms are produced predominantly from December through May (Kamemoto et al. 1998a).

6.6 Miniaturization

Several species useful for producing compact hybrids with short pseudobulbs are shown in Table 20-4. Some members of the section *Formosae* (*Nigrohirsutae)* are also relatively short (12 to 15 cm) but bear short inflorescences of only two to five flowers.

Table 20-4. Some *Dendrobium species* and hybrids with short pseudobulbs for potted plant breeding.

Species	Section	Pseudobulb height	Color
D. carronii	*Eleutheroglossum*	5 cm	Pink-purplish red
D. canaliculatum	*Eleutheroglossum*	5 – 12 cm	White-yellow-brown
D. phalaenopsis var. compactum	*Phalaenanthe*	5 – 8 cm	Light purple
D. bigibbum var. compactum	*Phalaenanthe*	5 – 8 cm	Mauve
D. 'Mini Gem'*	---	5 – 12 cm	Light lavender

**D. carronii x D. bigibbum var. compactum;* the amphidiploid is used in breeding.

6.7 Fragrance

Many *Dendrobium* species have fairly strong, pleasant scents. Of 140 species evaluated, 40% produced scents ranging from floral to fruity to herbaceous (Kaiser 1993). The mechanism of scent production has also been investigated (Arditti 1992). However, little is known about fragrance genetics. Despite several commercial hybrids being scented, notably *D.* Jaquelyn Thomas hybrids, fragrance has not been marketed per se for *Dendrobium*, either as a cut flower or a potted plant. Nevertheless, fragrance is a desirable attribute that may play a more important role in future breeding. One parent of possible interest for breeding fragrant potted plants is *D. antennatum* (synonym *D. d'albertisii*), with its long-lasting scented and attractive flowers (Kaiser 1993).

7. REPRODUCTIVE BARRIERS

Interspecific crossing barriers are largely absent for many *Dendrobium* hybrid combinations using the horticultural sections named in Section 3. This expands the germplasm pool for hybridization. Also, use of tissue culture for seed germination enables better survival of interspecific hybrids. The *Phalaenanthe*, *Ceratobium*, and *Eleutheroglossum* combinations are the most compatible, as represented by 50 to 100% viable progeny obtained (Kamemoto and Wilfret 1980). The diploid hybrid

progeny resulting from intersectional *Dendrobium* crosses do have poor chromosome pairing in meiosis and reduced fertility. However, some functional unreduced gametes are produced during microsporogenesis such that offspring can be produced when the hybrids are used in further crosses. More normal meiosis and fertility are restored upon doubling of the chromosome number in the hybrids. Such amphidiploids become valuable for breeding programs. Hybrid endosperm-embryo incompatibility appears absent in *Dendrobium* due to insignificant endosperm formation (Kamemoto et al. 1999a).

Inbreeding depression, expressed as loss in vigor and pollen degeneration, is observed with *D. phalaenopsis* and *D. bigibbum*, but not with other species such as *D. antennatum* (reviewed in Kamemoto et al. 1999a). The latter species is a useful parent in potted plant breeding. Moreover, several cycles of inbreeding and selection using an amphidiploid *D*. Jaquelyn Thomas proved useful to increase flower size and lighten color (Bobisud and Kamemoto 1982) and produced valuable stud plants when used subsequently in outcrosses to restore vigor. Inbreeding of hybrids may itself be a viable approach for potted plant development, since the accompanying reduction in plant height and raceme yield may be insignificant, or even desirable, for marketability.

8. COMMERCIAL PRODUCTS

Dendrobium hybrids come in many colors, including lavender, white, pink, yellow, fuschia, and bi-colors. There are dozens of varieties to chose from, depending on the target market and season. In Japan, which is considered to be the world's largest importer of orchids, the nobile-type *Dendrobium* is highly popular for gifts. Color groups, rather than form, tend to define the products being shipped into the continental USA. Popular varieties mericloned overseas and grown in Hawaii include Emma White, Suzanne Neil, Burana Jade, Burana Greenstar, Pathom Red, Sabin, Jiad Gold, Madame Uraiwan and various other yellows and 'blues' from various flask exporters. Examples of several seed-propagated and mericloned hybrids bred and cultivated in Hawaii are shown in Fig. 20-1 and described below.

D. Mari Marutani (Fig. 20-1A) is a stunning large dark purple, seed-propagated potted cultivar with flowering peaks from July to December. Flowers measure 3 inches (8 cm) across, with about 12 flowers per erect raceme. The pseudobulb height at first flowering is about 9.5 inches (24 cm). *D*. Winifred Ogata (Fig. 20-1D) is a two-tone lavender, seed-propagated potted cultivar with peak flowering during fall and early winter. Flowers measure 3-1/2 inches (9 cm) wide and last exceptionally long (85 days) on plants. *D*. Lorrie Mortimer (Fig. 20-1B) is another seed-propagated potted cultivar with flowering from December to May. The light lavender flowers develop attractive yellow-green petals with age and can last up to four months. The petals are twisted and the lip has dark purple veins.

Examples of two clonally propagated hybrids are also shown. Light lavender *D.* Ethel Kamemoto 'Splendor' (Fig. 20-1C) and its white, pansy-lip progeny *D.* Ethel Kamemoto 'White Cascade' (Fig. 20-1E) are floriferous, short statured potted plants bearing flowers during fall and winter. Flowers measure about 1 inch (2.5 cm) across and remain attractive for two months or longer.

9. MARKETING

Plants can be obtained through garden centers, supermarkets, chain discount retailers, home improvement centers and over the internet. Hawaiian-grown plants are graded as Hawaii Fancy (best) or Hawaii Standard, with the former being plants that are free of foliar or floral discoloration, with sturdy upright canes, vigorous root growth, and leaves of normal size, color and shape (Sewake and Uchida 1999).

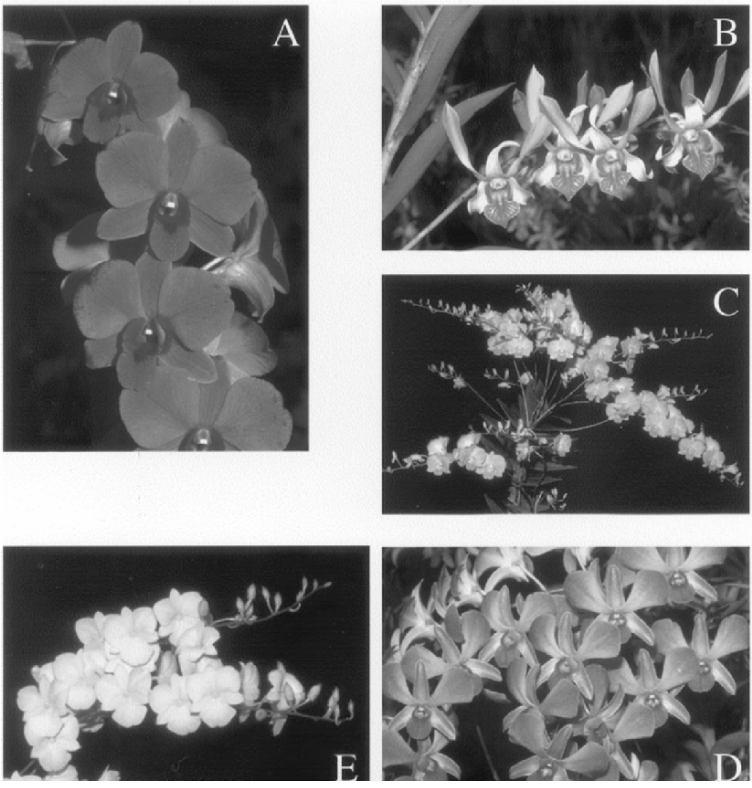

Figure 20-1. Several *Dendrobium* potted plant hybrid cultivars released by the University of Hawaii breeding program. A Dark purple *D.* 'Mari Marutani'; B Lavender lip with yellow-green sepals of *D.* 'Lorrie Mortimer'; C Light lavender D. Ethel Kamenmoto 'Splendor'; D Two-tone lavender *D.* 'Winifred Ogata'; E White pansy-lip *D.* Ethel Kamemoto 'White Cascade'. (Photo credit: H. Kamemoto).

10. CROP IDEOTYPE

Crop improvement for the mass market focuses on compact growth, early flowering, full flowers, an array of color, and suitability for specific pot sizes. It is important to note that many cut flower varieties in containers are being utilized extensively as flowering potted plants for interior decoration in larger spaces. However, they become less suitable as potted plants for use in residences or offices after their first year or two of flowering because of their increasing height and requirement for larger pots (Kamemoto et al. 1999a). Flowering also becomes more seasonal as the plants age, although this can be modified in some cases by treatment with growth regulators (Sakai et al. 2000).

Several characteristics deemed desirable for flowering potted plants were enumerated by Kamemoto et al. (1999a) as follows:

1. Attractive, relatively short, upright to arching sprays.
2. Long lasting flowers.
3. A minimum of two inflorescences (sprays) per plant per flowering period.
4. More than one flowering period per year.
5. Upright pseudobulbs under 24 inches (61.0 cm).
6. Multiple pseudobulbs.
7. Green, pliable, disease-free leaves.

Crop ideotypes such as these must also take into consideration the present and future production practices of growers; breeding and production combined can explore the full potential of this species.

A survey was made in 2001 of *Dendrobium* growers in the State of Hawaii, asking what they thought were desirable attributes for potted plants intended for export (to the continental USA). Results are presented for the island of Hawaii, with 83% of the responding growers exporting near-blooming plants, blooming plants, or plants in bud/bloom as opposed to flasks, community pots, and liners (Table 20-5). The survey and full results are found in Kuehnle et al. (2003).

Table 20-5. Future ideotypes for exported blooming potted *Dendrobium* through breeding and production strategies: Responses from growers on the island of Hawaii in 2001 (Kuehnle et al. 2003).

Trait	Grower preferences (ranking or percentage)
Flower color	Two-tone lavender, yellow, white and other (High) Pink and lavender (Medium)
Flower shape	*D. phalaenopsis*-type (46%), also *D.* Jaq. Thomas (23%), pansy-lip (14%), and *D. antennatum*-type (11%)
Flower size (cm)	12-18 (43%), with 9-12 and 18-24 (each 25%)
Novelty flower shape	High (73%)
Time from flask to market	24 months or less (69%)
Minimum number of sprays on finished plant	2 sprays (59%), with 1 spray also acceptable (32%)
Finished plant size – pseudobulb	72-84 (28%), with between 60-120 also acceptable

Trait	Grower preferences (ranking or percentage)
height (cm)	
Overall plant height, including sprays (cm)	150 – 216 (55%), or no preference (23%)
Early flowering through genetics	Favorable (68%), unfavorable (23%) or uncertain (9%)
Flowering through plant growth regulators	Favorable (55%), unfavorable (14%) or uncertain (23%)
Dwarfing through genetics	Favorable (73%), unfavorable (9%) or uncertain (18%)
Dwarfing through plant growth regulators	Favorable (18%), unfavorable (59%) or uncertain (18%)

11. BREEDING/GENETIC DIRECTIVES FOR FUTURE RESEARCH

Germplasm acquisition and introgression combined with the creation (or selection) and testing of additional polyploid parent plants remains the bread and butter approach to seed-propagated or clonal varietal development in *Dendrobium*. At the University of Hawaii, future breeding directives for potted dendrobiums include improved flower size, growth habit, flower longevity, and greater availability of whites, yellows and art-shades. Breeding programs will be taken to the next step in possibilities through incorporation of molecular biology tools to either supplement or assist hybridization, to facilitate gene screening and selection and, later, to assist plant or gene protection.

Of note in the grower survey results on potted *Dendrobium* attributes (Section 10) is a preference for early flowering and compact growth (dwarfing) through breeding rather than through application of growth regulators. Several parents are available for breeding for compact growth (see Section 6.6). However, little is known about the genetics of the life cycle of orchids to enable more directed selection for a shorter cycle (earlier flowering). Manipulation of the *constans* gene and other genes has induced early flowering under normally non-inducive day-length conditions in a model plant (Nilsson and Weigel 1997) and could be considered in the future as part of an orchid molecular breeding program.

11.1 Molecular Breeding

Molecular breeding promises to be an exciting addition to breeding programs due to the wealth of new orchid varieties that may be created. Recovery of genetically modified *Dendrobium* plants using the microparticle bombardment method is one way this approach can be realized for crop improvement (reviewed in Chia et al. 2001). Other means of DNA transfer include the pollen-tube pathway, *Agrobacterium tumefaciens*, seed imbibition, and protoplasts (Nan and Kuehnle

1995; Nan et al. 1998). *Agrobacterium*-mediated transformation is predicted to become increasingly utilized with *Dendrobium* due to the species surprising affinity for this vector (Nan et al. 1997) and recent successes with this (Yu et al. 2001) and other orchids (i.e., *Phalaenopsis*, Anzai and Tanaka 2001).

Dendrobium genetic engineering projects are exploring traits such as disease resistance (virus, bacteria, fungus), flower wilting, color changes, and fluorescent glow and are largely unpublished work in progress. Pigment manipulation strategies are discussed in Section 6.3 under Traits and Genes. Several comments on disease resistance and flower wilting follow.

11.1.1 Disease Defense

Synthesis of chemical defense compounds enables the orchid to defend itself against invading micro-organisms. Understanding which genes are turned on and how they are turned on will be extremely valuable in the future through development of strategies for controlling plant loss in propagation greenhouses and in production fields. Virus resistance using the viral coat protein approach appeared promising against Cymbidium Mosaic Virus (Chia et al. 1992; Kuehnle 1996), including in a *Nicotiana occidentalis* model (Lim et al. 1999b), but proved ineffective in those transgenic plants analyzed (Chia 1998; Hu, Kuehnle, Obsuwan et al. unpublished). Genes for the lytic peptides, magainins, offer some hope against bacterial and fungal pathogens, with work in progress in Hawaii (Kuehnle, Reyes, Sanford et al. unpublished)

11.1.2 Flower Wilting

Control of flower wilting due to senescence or dislodging of pollinia (during handling) is of great interest to consumers, retailers, wholesalers, and grower/shippers. Ideally scientists want to turn off these genes and knowing the exact orchid gene sequence will let them do that (reviewed in Kuehnle 1997). These genes tend to be just in single copies or in low number, meaning they might be easier to regulate genetically. Although ethylene-induced wilting is of less concern in current commercial *Dendrobium* than in other orchids like *Vanda*, some effort toward breeding in ethylene-insensitivity is proceeding in *Dendrobium* (Chia 1998).

11.2 Viral Vectors and Genomic Markers

A means for fast-track gene screening of traits pertinent to potted dendrobiums, such as novel flower color and dwarfing, is being developed by A. Kuehnle and M. Kumagai (unpublished) using infectious viral vectors for systemic expression. This is expected to be a valuable tool for assessment of phenotypes prior to undertaking

the laborious process of genetic engineering. Another valuable tool may be marker-assisted selection for flower color, as has been used in breeding of other orchids (for example Chen et al. 1994; Lim et al. 1999a). The development of genomic markers for use in early selection of certain traits such as flower color has the potential to accelerate *Dendrobium* hybridization programs. First one must weigh the high costs of doing molecular biology versus less expensive but longer term field observations when deciding if this is a methodology to be used.

Genomic markers also find application in the area of plant patenting and breeder's rights. Orchid hybrids can be registered with the International Registration Authority of the Royal Horticultural Society, but this does not provide any protection of valued hybrids from unauthorized propagation. Filing for and receiving a national or international patent does provide some measure of plant protection. As the breeding of a new cultivar requires many years and effort, patent protection for either a specific trait (utility patent) or the clonal hybrid (plant patent) offers an economic and market advantage for the breeder and licensed propagator. DNA amplification fingerprinting could be developed for use in identification of different varieties of potted *Dendrobium*, as is being done for *Phalaenopsis* (Chen et al. 1994).

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Chapter 21

ORNAMENTAL PEPPER

Capsicum annuum

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Abstract: Ornamental peppers (*Capsicum annuum* L.) are morphologically diverse and admired for their ornamental value. Considerable diversity exists in *Capsicum* germplasm for fruit and leaf shape and size, as well as plant habit. This morphological diversity, together with diverse ripe fruit color and varying hues of green to purple foliar pigmentation, affords a myriad of opportunities to develop unique ornamental cultivars. Easy seed propagation, relatively short cropping time, heat and drought tolerance, and excellent keeping quality contribute to the success of ornamental pepper cultivars. These attributes have enabled the ornamental pepper to be used as pot plants, novelties, cut stems, and bedding and garden plants. The availability of disease resistance in other pod types provides opportunities to introduce resistance into ornamental peppers. Renewed interest in ornamental peppers has stimulated new breeding activities. Knowledge gained from genetic studies and breeding of culinary type peppers affords valuable information for use in developing new ornamental pepper cultivars.

Key words: aji, breeding, chile, genetics, Solanaceae.

1. INTRODUCTION

Ornamental peppers (*Capsicum annuum* L.) belong to the plant family Solanaceae, which has numerous members admired for their ornamental value. These include the *Brugmansia* (Angel's trumpet), *Brunfelsia*, *Browallia* (Bush violet), *Datura*, *Nicotiana*, *Petunia*, and *Salpiglossis*, to name just a few. Two ornamental potted plants in the Solanaceae that are sometimes confused with ornamental peppers are *Solanum pseudocapsicum* (Jerusalem Cherry) and *Solanum capsicastrum* (False Jerusalem Cherry). These plants have white flowers similar to

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Capsicum and yellow, orange, or red egg-shaped fruit. The foliage of *S. pseudocapsicum* and *S. capsicastrum* differs from *Capsicum* and the flowers have bright orange anthers that provide an expeditious way to distinguish them from *Capsicum* with its yellow to blue anthers. It is important to differentiate *S. pseudocapsicum* and *S. capsicastrum* from ornamental peppers because the *Solanum* species fruit is toxic and can make a person very sick if consumed, while the fruit of ornamental peppers is edible, but frequently pungent.

2. HISTORY

Capsicum is native to the Western Hemisphere. Aztec, Mayan, and Inca plant breeders had already developed dozens of pepper pod-types by the time the Spanish arrived (Bosland, 1999a). Peppers were important in religious ceremonies and legends among Indian cultures and were second in importance to the major staple, maize. Pod-types are subspecific categories that distinguish among the specific horticultural varieties within pepper. Pod-types such as ancho, bell, jalapeño, pasilla, New Mexican, and yellow wax, are distinct pod-types that have specific traits for processing and fresh use, flavor, and pungency (DeWitt and Bosland, 1996). Many of these distinctive pod-types had already been developed, including peppers for ornamental use. Ornamental peppers are often grown as "annuals," but in their native habitats, peppers are a frost-tender perennial, living for more than a decade.

Pepper cultivation is fairly ancient in the Americas (Pickersgill, 1969a, b). Archeological evidence of wild *C. annuum* seed that precedes 5000 B.C. has been found at Tehuacan, Mexico. Domesticated forms of *C. annuum* are evident in archeological evidence before the Christina era. Cultivated forms of *C. baccatum,* dated around 2000 B.C., have been found at archeological sites in coastal Peru. *C. frutescens* appears at later levels on the coast of Peru. Peppers were introduced to Europe by Columbus, and then subsequently to Africa and Asia by the extensive trading routes of the Spanish and Portuguese. The first European record of peppers is Peter Martyr's letter of 1493 wherein he noted that Columbus had found peppers more pungent than those of *Piper nigrum* (Heiser, 1976). Numerous peppers were subsequently introduced to Europe. Unlike the tomato and Irish potato, peppers found almost immediate acceptance.

When introduced to Europe in the 15th century, peppers were held in higher esteem as an ornamental plant than as a food source. The herbalists described numerous peppers in the sixteenth and seventeenth century. Ornamental peppers as a potted or bedding plant and a florist crop are still popular today in Europe and are gaining in popularity in the United States (Armitage & Hamilton, 1987, Bosland, 1999b). Seed catalogs list about a dozen different ornamental peppers. A pepper labeled as an ornamental in a seed catalog usually means the pepper plant is

compact, has colorful fruit and does well in a container. Merits of ornamental peppers include easy seed propagation, relatively short cropping time, heat and drought tolerance, and excellent keeping quality.

For many years in the floriculture industry, ornamental peppers have been known as "Christmas peppers" (Harthun, 1991, Hammer, 1980). This is because the lustrous green pepper plants covered with bright red fruits have traditionally been sold during this holiday season. Christmas peppers with their cheery winter color and edible fruits were the most popular Christmas gift plant until about the 1960's, at which time the poinsettia industry began to promote and introduce new, improved cultivars that have made poinsettia the number one Christmas gift plant. Nevertheless, new interest in ornamental peppers has stimulated new breeding activities and the term "Christmas pepper" has all but disappeared from the vernacular with "ornamental pepper" replacing the term.

3. TAXONOMY AND BOTANY

Much is known about the genetics and breeding of *Capsicum*. The cultivated species are self-compatible. The nuclear DNA content of various *Capsicum* species, as determined by flow cytometry, ranges from 7.65 to 9.72 pg per nucleus for *C. annuum* and *C. pubescens,* respectively (Belletti et al., 1995). Most species of *Capsicum* are diploid with 24 chromosomes ($2n = 2x = 24$), and have one or two pairs of acrocentric chromosomes with ten or eleven pairs of metacentric or submetacentric chromosomes. Variability in chromosome karyotype is greater in wild forms of *C. annuum*, *C. baccatum*, *C. chinense*, and *C. frutescens* than in the domesticated cultivars. Ohta (1962) describes karyotypes for several *Capsicum* species.

3.1 *Capsicum* Species

The genus, *Capsicum*, is native to the tropics of Central and South America. The domestication of *Capsicum* was done in pre-Columbian times. By the time, Columbus traveled to the Western Hemisphere, five species of *Capsicum* were domesticated, *C. annuum* L., *C. frutescens* L., *C. chinense* Jacq., *C. baccatum* Willd., and *C. pubescens* Ruis & Pav. and numerous pod types had been developed including ornamental types (Bosland, 1992a). It seems humans have a penchant for selecting novel and unusual forms of *Capsicum*.

Three species complexes based upon species crossability are recognized within *Capsicum* (Tong and Bosland, 1999). The annuum complex consists of the cultivated species *C. annuum, C. frutescens,* and *C. chinense,* and the wild species*, C. chacoense* and *C. galapagoense.* The baccatum complex consists of the cultivated *C. baccatum* and the wild species *C. praetermissum and C. tovarii.* The

pubescens complex is the most isolated of the three groups and consists of the cultivated *C. pubescens* and wild *C. eximium* and *C. cardenasii.* Sexual crosses are possible between species within a complex, albeit sometimes with difficulty. Fertility of interspecific hybrids between accessions of *C. annuum*, *C. frutescens*, and *C. chinense* suggests that hybrid sterility resulting from gene imbalance is the major reproductive isolation mechanism between these species (Greenleaf, 1986; Rao et al., 1988). Although some interspecific *Capsicum* hybrids may exhibit chromosomal sterility, genic sterility appears to be the major cause of gamete abortion (Greenleaf, 1986). Genetic exchange between complexes does not occur in nature.

Morphologically, taxonomists have had difficulty distinguishing between *C. annuum, C. chinense and C. frutescens.* Morphological taxonomic classifications have grouped these together as one species. Nonetheless, the legitimacy of *C. chinense* and *C. frutescens* as separate species is questionable. New molecular evidence has clearly indicated the propriety of three separate species (J. Baral, pers. comm.).

C. annuum is the most widely cultivated and economically important *Capsicum* species today. *C. annuum* is the species most often grown as an ornamental and includes the sweet peppers as well as most of those that are dried for hot peppers, chili powder and paprika. Domestication of *C. annuum* occurred in Middle America, likely in Mexico (Pickersgill, 1971). Cultivated forms of the species are denoted as *C. annuum* var. *annuum* and wild forms as var. *glabriusculum*. Tremendous diversity for pod type, i.e. color, shape, pungency, flavor, size and use, exists in *C. annuum* (Figure 21-1). Bosland (1992a) describes 19 pod types within *C. annuum*. Although pod types do not distinguish peppers at the species level, horticulturists have used pod type as a means of subspecies classification.

Pod types within *C. frutescens* include that of the widely grown cultivar 'Tabasco' (Figure 21-1). In addition to its use in condiments, this cultivar has been considered an ornamental pepper because as the fruits begin to ripen the plants exhibit yellow, orange, and red pods all at the same time. *C. frutescens* is widely distributed as a wild or semi-domesticated species in lowland tropical America and in southeastern Asia. Peppers known indigenously as 'bird pepper' are *C. frutescens*.

C. chinense is widespread in tropical America and is commonly cultivated in the Amazon region. This species includes some of the most pungent of all peppers and is closely related to *C. frutescens*. Wild types of *C. chinense* have small fruit (Figure 21-1), but lack the deciduous fruit habit associated with *C. frutescens* and *C. annuum*. Diverse pod types are present within *C. chinense.* The habanero and Scotch bonnet are the most familiar pod types within *C. chinense.*

Figure 21-1. Diversity *of Capsicum annuum, C. chinense, C. baccatum,* and *C. frutescens* fruit.

Wild forms of *C. baccatum* are centered in Bolivia and surrounding areas. The species is seldom cultivated outside South America. Domesticated types are designated as *C. baccatum* var. *pendulum* and wild forms as var. *baccatum* (Eshbaugh, 1970). Morphologically, *C. baccatum* has characteristic cream-colored flowers with yellow, brown or dark green spots on the corolla. Similar to a number of other *Capsicum* species, pod types are diverse and fruit vary in pungency from mild to very hot, with unique aromatics and flavors (Figure 21-1).

C. pubescens is a highland species that is grown extensively in the Andes and highland areas of Mexico and Central America. Morphologically, *C. pubescens* is the most distinct cultivated pepper species, set apart by conspicuous leaf pubescence and dark, rugose seeds. Instead of white flowers, *C. pubescens* has purple flowers with large nectaries. Other *Capsicum* species produce smooth, light tan-colored seeds. Fruit of *C. pubescens* accessions varies in shape and color, but is not as diverse as that found in *C. annuum.* Wild ancestral *C. pubescens* accessions have not been identified.

The other approximately 20 *Capsicum* species (Table 21-1) lack extensive study on their biology. Many of the known wild species have restricted distribution. These species may contain genes for adaptation to unusual environmental conditions as well as disease resistance, and may harbor genes of interest in ornamental peppers. Unfortunately, the natural habitat of wild pepper species is being lost.

Tropical deforestation is among the most massive and urgent environmental problem facing *Capsicum* germplasm resources (Bosland and Gonzalez, 2000).

Lippert et al. first cataloged a list of known *Capsicum* genes in 1965. The list included 50 genes and guidelines for naming and symbolizing genes. In 1994, Daskalov and Poulos produced an updated gene list. The International Capsicum and Eggplant Newsletter committee for Capsicum Gene Nomenclature has established standardized rules and protocols for new gene names and symbols (CENL, 1994). Seed samples of the named and accepted gene stock are deposited in the Capsicum Genetics Cooperative at New Mexico State University, U.S.A. Duplicate samples are maintained by the originator or at a separate location (CENL, 1994).

Table 21-1. The wild species of *Capsicum.*

Species Complex	Species	
Tubocapsicum:	*C. anomalum*	
Pseudoacnistus:	*C. brevifolium*	
Capsicum:	*C. buforum*	C. *campylopodium*
	C. *cardenasii*	*C. chacoense*
	C. ciliatum	*C. coccineum*
	C. cornutum	*C. dimorphum*
	C. dusenii	*C. eximium*
	C. *galapagoense*	*C. geminifolium*
	C. hookerianum	*C. lanceolatum*
	C. *leptopodum*	*C. minutiflorum*
	C. mirabile	*C. parvifolium*
	C. praetermissum	*C. schottianum*
	C. scolnikianum	*C. tovarii*
	C. villosum	

3.2 Flower Structure and Pollination

Most *Capsicum* species have flowers that are complete and are self-compatible. Self-incompatibility is found in the wild species *C. cardenasii*, *C. buforum*, *C. flexuosum*, and some accessions of *C. pubescens*. Ornamental peppers seem to exhibit no inbreeding depression *per se*, but do show a heterosis effect with hybrids. All species are protogynous and can cross-pollinate. The stigma can be positioned slightly below the level of the anthers or exerted beyond, in which case the chances for cross-pollination are greater.

Even though ornamental peppers are considered self-pollinating, they can be insect pollinated. Depending on the plant material, cross-pollination can range from 2% to 90% (Bosland, 1993). Therefore, ornamental peppers should be considered a facultative cross-pollinating species and seed producers should use caution to prevent uncontrolled cross-pollination. Even though the amount of out-crossing can vary, it is nevertheless sufficient to impede progress in breeding programs. The out-crossing is associated with natural insect pollinators, not rain or wind (Odland and

Porter, 1941; Tanksley, 1984). Honeybees and solitary bees are much more likely to cross-pollinate peppers. The amount of cross-pollination has an effect not only on the precautions needed for seed production, but also on the breeding methodologies used by the plant breeder. In breeding programs, numerous breeding lines and plants must be isolated during seed production. Thus, space for isolation becomes limiting. To ensure self-pollination, a simple and effective plant isolation cage was developed (Figure 21-2; Bosland, 1993).

Figure 21-2. Plant isolation cages for pollination of *Capsicum*.

3.3 Interspecific Hybridization

Interspecific hybridization is a common occurrence in pepper breeding. The ability to hybridize between species is important to introgress disease resistance genes and other unique traits found in other species. Introducing virus resistance from *C. chinense* to *C. annuum* is one of the most productive avenues of pepper breeding. Interspecific hybridizations between species of *Capsicum* can be made with varying degrees of success (Bosland and Votava, 1999). Hybrids of varying fertility have been obtained in most combinations for *C. annuum*, *C. baccatum*, *C. frutescens*, and *C. chinense*. Hybrid sterility is the major reproductive isolation mechanism in *Capsicum*. *C. pubescens* is genetically isolated from the other cultivated species. Numerous hybrids have also been made between the wild and cultivated *Capsicum* species. Embryo culture is useful when "embryo rescue" is required. Embryos from interspecific hybridizations often abort before seed development is complete. Some interspecific hybridization may produce viable

plants if the embryo is rescued at an early stage of development, approximately 28-33 days after pollination (Fari 1995; Hossain et al., 2003).

4. IDEOTYPES

Ornamental peppers are not limited to the potted plant category of ornamentals. Ornamental peppers range in size and shape from short, compact plants with piquin sized fruits, such as 'Holiday Cheer', to plants as tall as three to four feet, with full sized fruits, such as 'NuMex Mirasol'. The great diversity of pod types and plant habit has enabled the ornamental pepper to be used as a pot plant, but other types are used for novelties, cut stems, and bedding and garden plants. Some ornamental peppers, like the 'Peter Pepper', have their ornamental value in their risqué fruit shape. Most all ornamental pepper cultivars bear pungent fruit. Cultivars such as 'Chilly Chili' and 'Medusa' have enjoyed recent popularity due to their non-pungent fruit and concerns for product safety. Miniature sweet colored bell peppers are considered for culinary and ornamental applications (Figure 21-3). This morphological diversity, together with diverse ripe fruit color and varying hues of green to purple foliar pigmentation, affords a myriad of opportunities to develop unique ornamental cultivars. Ornamental peppers include three main ideotypes; potted types, bedding and garden plants, and cut stems.

Figure 21-3. Ornamental sweet miniature bell peppers.

4.1 Potted Plant

Ornamental peppers grown as potted plants are widely marketed for sale in September, October, November and December, with lesser volume at other times of the year. Merits of ornamental peppers as potted plants include easy seed propagation, relatively short cropping time, heat and drought tolerance and excellent keeping quality. Greenhouse growers desire certain traits to enhance profitability of ornamental peppers. The plant should be compact with a polychotomous growth habit, and have colorful fruits that serve as important marketing traits. Potted plants flower continuously, producing peppers of different colors at different times of the year (Figure 21-4). Small, compact plants bearing round or conical Tabasco type fruit perform well in small pots with plant height typically limited to four to six inches. If proper cultivars are selected, no plant growth regulators are required for height control or to promote branching. Selected cultivars must also be ready for shipping to retailers in a relatively short time. Very prostrate and indeterminate/spreading ornamental peppers, normally reserved for bedding plant production, adapt well for hanging basket applications.

Figure 21-4. Ornamental peppers displaying multiple fruit colors.

4.2 Border & Garden Plants

Using ornamental peppers as a bedding and garden plant has attracted renewed interest in recent years. The merits of ornamental pepper as a garden plant include heat and drought tolerance, and vivid colors throughout the summer and fall until the first frost. The use of ornamental peppers in the landscape can create a bed of flashy color in the summer heat. Because ornamental peppers vary in height, they can be used as border or specimen plants, or in mass planting,

The dwarf types with erect fruit showing above the foliage are recommended for border use or massed against a green background. 'NuMex Centennial' and 'NuMex Twilight' are ornamental peppers that work as garden type ornamentals (Bosland et al., 1994). 'NuMex Centennial' has purple flowers and purple foliage. Immature fruit are purple, and then ripen to yellow, then orange, and lastly red. Unlike standard pepper cultivars that have a dichotomous growth pattern, the polychotomous branching of the basal branches make these plants ideal for container production. 'NuMex Twilight' has a white flower, and green leaf color as compared to 'NuMex Centennial's purple pigmentation. Also, the yellow fruit color stage is more pronounced in 'NuMex Twilight'. Both cultivars have erect flower pedicels at anthesis, and fruits are upright and smooth with a cup-shaped calyx. Greenhouse growers have noted that along with the polychotomous growth habit,

Figure 21-5. Ornamental pepper cultivar 'Tangerine Dream'.

the four-colored fruits serve as important marketing traits. 'NuMex Twilight' has also become an important source of cucumber mosaic virus resistance for plant breeders.

Peppers with dual-purpose ornamental and culinary applications are best suited as bedding and garden plants. 'Tangerine Dream' has a spreading prostrate growth habit and produces large sweet, nonpungent erect conical pepper fruit (Stommel and Griesbach, 2004). 'Tangerine Dream's unique plant habit and brightly colored erect fruit provides an attractive ornamental display of edible fruit (Figure 21-5).

4.3 Cut Stems

The cut stem is an important ideotype in Europe, and only recently is it becoming important in the United States. The cultivar, 'NuMex Mirasol' was developed as a cut stem ornamental pepper with branches of fruits to be used in flower arrangements (Bosland and Gonzalez, 1994). This ornamental pepper produces clusters of fruit on a long stem. The fruit is pointed and erect. The multi-stemmed bush produces approximately 16 clusters per plant with about 6 fruit per cluster. 'NuMex Mirasol' with its smooth texture, shiny surface, and brilliant red pods offers a lovely contrast to the foliage and blossoms of standard florist plants. It is also used on decorative wreaths.

A spin-off of this ideotype is the ornamental pepper used for "ristras" or wreaths (Figure 21-6). Ornamental peppers cultivars, such as 'NuMex Sunset', 'NuMex Sunrise', and 'NuMex Eclipse' were developed to provide alternative mature fruit color in the New Mexican pod-type (Bosland et al, 1990). 'NuMex Sunrise', 'NuMex Sunset' and 'NuMex Eclipse' have green immature fruit that mature to yellow, orange, and brown, respectively. They are used primarily as ornamental peppers to make strings of peppers, known as ristras. Only pepper types that dehydrate sufficiently to eliminate rotting can be used to make ristras. Similarly, 'NuMex Sunglo', 'NuMex Sunflare', and 'NuMex Sunburst' are ornamental peppers used to make "mini-ristras" (Bosland, 1992b). Immature fruit color is green, while mature fruit color is yellow, red, and orange for 'NuMex Sunglo', 'NuMex Sunflare', and 'NuMex Sunburst', respectively. The mini-ristra peppers are popular as a tourist item because they are easier to transport than traditional New Mexican-type ristras.

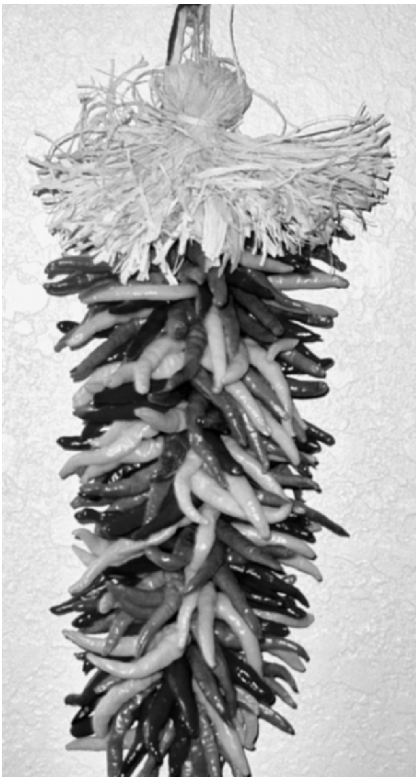

Figure 21-6. Ornamental pepper "ristra".

Table 21-2. Selected commercial ornamental pepper cultivars.

Cultivars A – H	Cultivars H-N	Cultivars N-Z
Aurora	Holiday Flame	NuMex Sunrise
Black Hungarian	Ignite	NuMex Sunset
Black Pearl	Inferno Mixed	Pinocchio
Blast	Jackpot	Prairie Fire
Calypso	Jigsaw	Pretty in Purple
Candlelight	Karneval	Red Missile
Chilly Chili	Little Elf	Riot
Czechoslovakian	Marbles	Salsa
Ember	Masquerade	Super Chili Hybrid
Favorite	Medusa	Sweet Pickle
Festival	Midnight Special	Tangerine Dream
Fiesta	Nosegay	Tequila Sunrise
Fips	NuMex Eclipse	Treasure Red
Fish	NuMex Mirasol	90C40
Fireworks	NuMex Pinata	90C44
Golden Treasure	NuMex Sunburst	90C53
Hearts	NuMex Sunflare	
Holiday Cheer	NuMex Sunglo	

5. ORNAMENTAL TRAITS

Considerable diversity exists in *Capsicum* germplasm for fruit and leaf shape, size and color, as well as plant habit. Relative to tomato, a Solanaceous relative of pepper, we know considerably less about the genetic basis for variation in pepper fruit and plant–related traits. Classical genetic studies have defined the inheritance of numerous genes that influence these characters. The availability of quantitative trait loci from tomato that influence characters such as fruit shape and size (Bernacchi et al., 1998) will facilitate identification of similar loci in pepper. Similarly, ascribing function to phenotypic loci in pepper is moving ahead quickly by virtue of work in related species. Thorup et al. (2000), for example, demonstrated that a candidate gene approach could be used to link specific tomato carotenoid-related metabolic phenotypes and loci that influenced these phenotypes in pepper.

5.1 Color

Color of pepper fruit produced for culinary applications is an important determinant of fresh and processed product quality. For ornamental applications, fruit and foliage pigmentation are very important determinants of cultivar appeal. Ripe pepper fruit color varies in gradation from yellow to orange to red, and includes brown, which is the combination of red and green (Smith, 1950). Color of unripe pepper fruit varies from lilac to dark purple to nearly black, through varying shades of green and yellow to ivory. Whereas ripe fruit color is an important consideration in breeding ornamental cultivars, purple to black pigmentation of unripe fruit provides ornamental interest for the lengthy maturation period that precedes fruit ripening. Purple pigmentation in pepper is attributed to anthocyanin accumulation. Similar gradations in purple pigmentation may be observed in other *Capsicum* plant parts. Foliage and stem color varies from green to varying shades of green/purple to nearly black. Enhancement of cultivars with purple to nearly black foliage color has received renewed interest in ornamental pepper breeding programs. ‘Black Pearl’ is a new USDA cultivar that produces nearly black foliage and dense clusters of round black fruit that mature to red (Figure 21-7). This dark foliage is a striking complement to the red or orange fruit of ornamental peppers. In mixed plantings, the dark foliage is a welcome accompaniment to species bearing red, orange, or white to pale-colored flowers. In pepper, flower color varies from white to greenish white, to purple. Anther pigmentation ranges from blue to purple to yellow. Flower and anther color are often useful taxonomic criteria in distinguishing among different *Capsicum* species.

Figure 21-7. Ornamental pepper cultivar 'Black Pearl'.

5.1.1 Chlorophylls and Carotenoids

The green, yellow, orange and red colors of ripe pepper fruit are due to carotenoid pigments. In addition to the green chlorophylls (a and b), more than 30 different carotenoid pigments have been identified in pepper (Matus et al., 1991). These include the yellow-orange lutein, zeaxanthin, violaxanthin, antheraxanthin, β-cryptoxanthin and β-carotene; and the red pigments capsanthin, capsorubin and cryotocapsin. Capsanthin and capsorubin are the major carotenoids present in ripe red-pigmented peppers, with capsanthin accounting for approximately 60% of the total carotenoids. Violaxanthin and β-carotene are the major carotenoids in yellow to orange-pigmented fruit. The carotenoid biosynthetic pathway in plants is very well characterized. The availability of cDNAs that code for nearly all of the enzymes required for carotenoid biosynthesis in plants (Cunningham and Gantt, 1998) will stimulate continued activity in studying the regulation of carotenoid biosynthesis and manipulation of fruit pigmentation.

Color of mature pepper fruit is influenced by a number of genes. Expression of the dominant *B* locus results in high β-carotene content in mature fruit (Brauer, 1962). Brauer (1962) described a complimentary gene, *t*, which interacts with *B* to produce a range of β-carotene levels. In mature fruit, the chlorophyll retainer gene, *cl*, combines with y^+ (red fruit color) or *y* (yellow fruit color) to produce brown and olive green mature fruit color, respectively (Smith, 1948, 1950). Carotene pigment inhibitor genes, *c1* and *c2,* inhibit carotenoid accumulation in mature fruit (Lippert

et al., 1965). Combinations of *c1*, *c2*, *cl*, *y* and their alleles provide a range of mature fruit colors. Retention of purple anthocyanin pigments in maturing fruit provides additional color gradation.

Numerous genes have been described in peppers that alter normal green foliage pigmentation (Figure 21-8). Foliar variegation is sometimes desirable in ornamental plants. Genes described in pepper that cause variegation include the *m1* to *m4* genes which elicit marbled, distinct green and white zones on foliage and immature fruits (Lippert et al, 1965; Daskalov, 1977). The *pi* gene causes plastid instability, resulting in green and white variegation (Hagiwara and Oomura, 1947; Lippert et al., 1965). Additional variegation genes include the allelic variegated mottled (vg^m) and variegated virescent (vg^v) loci (Lippert et al., 1965). Expression of the *chl* gene, chlorina, results in greenish-yellow variegation or chlorophyll deficiency (Kormos and Kormos, 1955; Lippert et al., 1965). Expression of *A* and *MoA*, described below, introduce purple pigmentation into variegated and non-variegated foliage, resulting in greenish-purple to nearly black pigmentation.

Figure 21-8. Variegated pepper foliage.

Greenish-yellow foliage has been successfully utilized in a number of woody and herbaceous ornamentals to create green color contrasts in mixed plantings. Genes that produce greenish-yellow foliar pigmentation or a general foliar yellowing in pepper (Table 21-3) have not found similar application. Poor plant vigor, lethality, variable expression, and developmental stage-specific expression are associated with many of these genes.

5.1.2 Anthocyanins

Anthocyanin accumulation in pepper fruit is accumulated in variable concentrations and in varying degrees of transiency during fruit maturation. In contrast, purple pigmentation is normally stable through plant development in other plant organs of genotypes that exhibit anthocyanin accumulation.

A number of studies have postulated genetic mechanisms for control of anthocyanin accumulation in pepper. Expression of the dominant gene, *A* (Deshpande, 1933; Peterson, 1959), results in varying intensity of purple pigmentation in foliage, stems, flowers, and fruit. The *A* locus is linked in *C. annum* to *sw* (sulfury white immature fruit color) and *O* (round fruit shape). The action of a modifier gene, *MoA*, intensifies purple color in the presence of *A*. The gene *im* (originally *i)* was proposed for intermediate maturity of purple fruit color in originally nonpurple immature fruit (Hagiwara et al., 1959).

The anthocyaninless gene (*al-1* to *al-5*) prevents purple color, resulting in green nodes and yellow anthers and is epistatic and nonallelic to *A*, *As* (style anthocyanin; purple in absence of *A* or *Asf*), and *Asf* (style and filament anthocyanin; purple in absence of *A*) (Lippert et al., 1965, 1966). Additional anthocyanin-less loci, *al6* and *al7*, from *C. chinense*, and *al8*, from *C. chacoense*, act similarly to anthocyaninless loci described in *C. annuum* (Csillery, 1983).

High performance liquid chromatography (HPLC) analysis of selected *Capsicum* genotypes demonstrated that anthocyanin within the fruit, flower and leaves was identical and identified as delphinidin (Griesbach and Stommel, personal communication). Endo (1953) identified a glycoside of delphinidin in segregating progeny from a cross of two *C. annum* cultivars.

Table 21-3. Capsicum color gene symbols and the character each controls.

Symbol	Character	Reference
A	*Anthocyanin*; Purple pigmentation in foliage, stems, flowers, and immature fruit; also *F*	Deshpande, 1933; Peterson, 1959
al-1 to *al-8*	*Anthocyaninless* gene; prevents purple color, resulting in green nodes and yellow anthers; epistatic and nonallelic to *A*, *As*, and *Asf*	Lippert et al. 1965, 1966; Csillery, 1983
As	*Style anthocyanin*; purple in absence of *A* or *Asf*	Hagiwara and Oomura, 1947
Asf	*Style and filament anthocyanin*; purple in absence of *A*	Odland, 1960
aur	*Aurea*; golden cotyledons and leaves	Zubrzycki and Pahlen, 1974
B	*β-carotene*; high content in mature fruit	Brauer, 1962

Symbol	Character	Reference
c1 and *c2*	*Carotenoid* pigment inhibitor in mature fruit	Lippert et al., 1965
chl	*Chlorina*; greenish-yellow variegation or chlorophyll deficiency	Kormos and Kormos, 1955; Lippert et al., 1965
cl	*Chlorophyll* retainer gene; combines with y^+ or *y* to produce brown and olive green mature fruit color, respectively	Smith, 1948, 1950
flv	*Flavi*; yellow-green leaves, plants shorter and less vigorous	Daskalov and Poulos, 1994
im	*Intermediate maturity* of purple fruit color in originally non-purple immature fruit (originally *i)*	Lippert et al., 1965
lut1 to *lut4*	*Lutescens*; yellow-green mutant, cotyledons and leaves are uniformly yellowish, lighter than normal green, varietal background alters expression	Csillery, 1980
m1 to *m4*	*Marbled*, distinct green and white zones on foliage and immature fruits	Lippert et al, 1965; Daskalov, 1977
MoA	*Modifier* of *A*; intensifies purple color in the presence of *A*	Lippert et al, 1965
mos1 to *mos5*	*Mosaic* variegation on leaves	Csillery, 1980, 1983
pi	*Plastid instability*, resulting in green and white variegation	Hagiwara and Oomura, 1947, Lippert et al., 1965
sw1,sw2,.... swn	*Sulfury white* immature fruit color, dominant alleles influence various green shades	Odland and Porter, 1938; Odland, 1948; Lippert et al. 1965
t	Interacts with *B* to produce a range of β-carotene levels	Brauer, 1962
vg^m and vg^v	*Variegated mottled* and *variegated virescent*, respectively	Lippert et al., 1965
vir1 and *vir2*	Variegation of viridis type (*vir*) wherein young leaves are yellowish, homozygous lethal	Kormos and Kormos 1955; Lippert et al., 1965
xa1 to *xa10*	*Xantha*; seedlings white or yellow, homozygous lethal	Csillery, 1980, 1983
y	*Yellow* mature fruit color	Lippert et al., 1965

Symbol	Character	Reference
yc	*Yellow cotyledon*, yellow green leaves, golden yellow immature fruit, light red mature fruit	Daskalov and Poulos, 1994
Ys	*Yellow spot*; yellow corolla spot of *C. baccatum* var. *pendulum*	Daskalov and Poulos, 1994
yt1 and *yt2*	*Yellow top*; young leaves yellow, mature to green	Csillery, 1980

5.2 Morphology

Diversity exists in *Capsicum* species for plant growth habit, fruit shape, size, and orientation. This variation is of tremendous value in development of new ornamental pepper cultivars for diverse bedding plant, pot culture and cut stem applications.

5.2.1 Plant

Variation in *Capsicum* growth habit affords unique opportunities for development of novel ornamental ideotypes that are well suited for specific applications. Cultivars best suited for pot production are compact, bushy plants, and often determinate. Bedding plants may be determinate or indeterminate, tall or prostrate, and large or dwarf. Cut types are typically tall indeterminate plants with multiple stems bearing fruit at every node, or determinate types with showy terminal fruit clusters. A number of genes have been described that influence plant stature. The dominant gene Dt^+ and recessive gene ct^+ condition indeterminate growth habit. In the homozygous dominant or heterozygous condition, ct^+ is epistatic to *dt*, whereas, dt^+ is epistatic to *ct* (McGamon and Honma, 1984). A dominant suppressor, *Su*, suppresses the epistatic action of ct^+.

Several genes influence compact growth habit, largely due to shortened internodes. Expression of the recessive fasiculate gene, *fa*, results in a compact, bushy plant with short internodes and a more concentrated fruit set (Lippert et al., 1965). The compact gene, *ct*, causes shortened internodes, reducing plant height up to one-half of normal. The recessive *brl* gene causes shortened stem internodes as well as shortened leaf petioles. Genes for dwarf plant stature, *dw1* and *dw2,* reduce plant size substantially (12 to15 cm and 15 to 20 cm mature height, respectively) and cause shortened internodes and thickened dark green leaves. Female fertility is much reduced in *dw* plants. Additional growth habit mutant genes with limited horticultural application are noted in Table 21-4. Spontaneous mutants are common in *Capsicum*, but mutants can also be induced chemically or via ionizing radiation (Daskalov, 1974, 1977; Bhargava and Umalkar, 1989).

Numerous genes have been described that influence leaf morphology (Table 21-4). Potentially useful mutant genes for ornamental applications include the frilly

gene, *fr*, whose expression is characterized by undulating leaf margins. The *anv* gene elicits production of long and narrow leaves, whereas the *rl* locus causes leaf rounding by reducing the length, but not the width of leaves. Presence of the homozygous recessive form of rugose, *ru*, causes rugose or savoyed mature leaves that are darker green than normal, without reduced plant viability. Expression of the *dm* locus results in plants with very small leaves with proportionate decreases in stem and flower size. The propensity of these plants to wilt under moderate water stress limits widespread use of this allele. Most other leaf morphology genes reported have little promise for ornamental application due to gross leaf deformity or deleterious pleiotropic effects such as sterility.

Table 21-4. Capsicum morphology genes and the respective expressed characters.

Symbol	Character	Reference
anv	*Angustifolia variegada*; elliptical cotyledons, long and narrow leaves	Zubrzycki and Pahlen, 1974
bl	*Branchless*; Stems terminate in leaf and flower pedicel at first branching; female sterile	Lippert et al., 1965
brl	*Braquitica latifoliata*; shortened stem internodes, leaf blades wide, large, round, and dark green with short petioles	Zubrzycki and Pahlen, 1974
bv	*Bushy variegated*; small excessively branched plants with white-green mottled leaves	Bergh and Lippert, 1964
ca	*Canoe*; margins of cotyledons and leaves rolled upward	Csillery, 1983
ct	*Compact*; mature plants with more numerous and erect axillary shoots on the main stem; internodes shortened with plants half as tall as normal, fruit maturity slightly delayed	Bergh and Lippert, 1975
dm	*Diminished morphology*; leaves extremely small (2 cm length x 1 cm width), stem and flowers equally tiny with 18-20 internodes on main stem prior to first cyme; wilting under moderate water stress	Csillery, 1983; Daskalov and Poulos, 1994
dt	*Determinate growth*;	McCamon and Honma,

Symbol	Character	Reference
	conditions determinate growth habit	1984
dtr	*Datura leaves*; leaves on the 5-12 nodes irregularly dentate	Csillery, 1983
dvg1	*Deforme variegada*; deformed and undulated green virescent variegated leaves	Zubrzycki and Pahlen.,1974
dw1 and *dw2*	*Dwarf plant*, 12-15 cm and 15-20 cm tall, respectively; short internodes, dark green leaves	Daskalov, 1974
fa	*Fasiculate*; compact, bushy plant, short internodes, flowers and fruit in clusters	Lippert et al., 1965
fb	*Fruit base*; fruit base non-bulging	Deshpande, 1933, Lippert et al., 1965
fi	*Filiform*; threadlike leaves; flower irregularities, female sterility	Lippert et al., 1965
fr	*Frilly*; undulated leaf margins	Csillery, 1980
gd	*Glossy diminutive*, also female sterile	Bergh and Lippert, 1964
H	*Hairy*; pubescent leaf surface	Shuh and Fontenot, 1990
Mf1, Mf2, Mf3	*Multiple flowers* per node; *Mf1* determines expression of multiple flowers when a dominant allele is present at *Mf2* or *Mf3*; recessive homozygosity at *Mf1* modifies expression and reduces multiple flower nodes even with dominant *Mf2* and *Mf3* alleles; recessive homozygosity at any two loci is epistatic to the dominant allele at the third locus	Shuh and Fontenot, 1990; Daskalov and Poulos, 1994
O	*Oblate*, round fruit shape	Khambanonda, 1950; Peterson, 1959
Pt	*Pointed* fruit apex, not fully dominant to blunt	Lippert et al., 1965
pc1, pc2, pc3	*Polycotyledon*; seedlings with three to four cotyledons; fasciated stem; pseudo-dichotomous branching with unequally	Csillery, 1980

Symbol	Character	Reference
	developing shoots	
rl	*Round leaf*; length but not width of leaves is reduced, no remarkable pleiotropic deleterious effects	Greenleaf and Hearn, 1976
ru1, ru2	*Rugose* mature leaves; mature leaves dark green	Csillery, 1983
sd	*Scabrous diminutive*; rough foliar surface	Bergh and Lippert, 1964
Sm	*Smooth* or glabrous leaf surface, interacts with *H*	Shuh and Fontenot, 1990
sp	*Spinach*; ground level whorl of odd, limp leaves; flower buds lacking	Bergh and Lippert, 1964
Su	*Suppressor* of indeterminate growth, suppresses epistatic action of ct^+	McCammon and Homma, 1984
Tl	*Taphrina leaf*; rugose deformed leaves, thin stem	Csillery, 1983
Tu	*Tube*; Cotyledons and leaves rolled up like a tube exposing only abaxial surfaces	Csillery, 1980
Un	*Undulate* leaf surface, small dark green leaves	Pahlen, 1966
up1, up2	*Upright* fruit orientation	Lippert et al., 1965
Wl	*Willow leaf*; leaves narrow, but wider than *fi*; female sterile	Bergh and Lippert, 1964

5.2.2 Fruit

The dominant gene *O* in pepper conditions oblate or round fruit shape. Elongate fruit range from short and blunt to long and pointed. Crosses between round and elongate-fruited plants produce progeny with a continuous range of fruit shapes and sizes, indicative of quantitative inheritance, with the genes for small fruit generally dominant to those for large fruit size. Khambanonda (1950) estimated that approximately 30 genes influence pepper fruit size. Pointed fruit apex (*Pt*) is not fully dominant to blunt (Lippert et al., 1965).

C. annuum characteristically produces a solitary flower at a branch node and hence, sets up to one fruit per node. In contrast, *C. chinense* typically produces two to four flowers per node, providing the potential for multiple fruit set per node (Lippert et al., 1966). Some *C. chinense* accessions develop ten or more flowers at a single node under optimal growing conditions. When combined with the recessive gene for upright fruit orientation, *up*, clusters of immature purple-pigmented fruit or mature yellow, orange or red fruit provide an attractive ornamental display (Figure

21-9). Subramanya (1983) determined that three major genes control multiple flowers and that more genes are required to produce additional flowers per node. Working with a different *C. annuum* x *C. chinense* cross and employing cosegregation of isozymes markers, Tanksley and Iglessias-Olivas (1984) reported that a minimum of five independently segregating chromosomal regions controlled the difference in flowering behavior and that epistatic interactions among independent chromosomal regions played a major role in determination of flower number per node. Greenleaf (1986) noted unpublished results of J.E. Watson and W.H. Greenleaf indicating that seven additive genes determine multiple flowers per node in *C. chinense*. With yet another *C. annuum* x *C. chinense* cross, Shuh and Fontenot (1990) estimated that three genes, plus epistasis, control multiple flowers.

Figure 21-9. Ornamental pepper fruit cluster illustrating the cluster and upright fruit orientation.

Large-fruited peppers bred for culinary use and produced on determinate plants, typically have a long fruit pedicel to allow for expansion of the developing fruit. This is less of a concern for ornamental peppers where fruit are typically small. A longer pedicel length is sometimes desirable, however, with heavily clustered fruit to maximize visual displays. Subramanya and Ozaki (1980) reported that multiple genes controlled pedicel length and that long pedicel was partially dominant to short.

6. BREEDING METHODS

A variety of breeding methods can be used to produce new pepper cultivars. The methods used are determined by the breeder to best fit the goals of the breeding program.

6.1 Mass Selection

This was probably the earliest breeding method to select for ornamentals. If a population had an intriguing trait, the indigenous people of the Americas saved seed and propagated it. Beautiful multi-colored peppers in Peru were selected in this fashion. 'Aji panca' and 'Aji Ayucllo' are examples of populations that can be considered ornamental and are of Inca origin (Bosland, 1997). Land varieties developed using mass selection are the basis of agriculture in many less developed agricultural regions. Successful varietal development depends upon selection of large starting populations where no more than 25% of the inferior lines are discarded and adequate numbers are maintained to minimize unacceptable shift from the features of the original variety (Allard, 1960). Mass selection is a conservative approach to variety development, but requires little effort relative to other programs and can produce a new variety adapted to a specific locale in a relatively short time in comparison to pedigree breeding or F_1 hybrid development. In today's breeding programs that develop ornamental pepper germplasm for commercial use, the method has little value and other more efficient methods are employed.

6.2 Pedigree

The pedigree method involves making single plant selections and self-pollination. The pedigree of subsequent selfed generations is recorded in combination with selection for desired traits. The pedigree method has been widely used in breeding self-pollinated crops and permits the breeder to utilize their skills in selection to a greater degree than other methods often used with these crops. In choosing parents, one parent is selected for its superior performance in a market class or application. The second parent is chosen to complement specific weakness of the first parent. Selection begins in the F_2 generation for superior types. Selection of single plants within families is typically practiced up to the F_5 or F_6 generation when variation within families is minimal and selection among families becomes more efficient. Parent/progeny records utilized in pedigree breeding make it possible to decide which families to advance and which families are best discarded. 'Marbles', 'Riot', 'Tangerine Dream', 90C40, 90C44, and 90C53 are examples of cultivars developed by this method (Baggett and Kean, 1988; Stommel and Griesbach, 1993).

6.3 Mutation

Mutation breeding has not been a major breeding method for the development of ornamental peppers. However, it may be a means of producing novel mutants of ornamental interest. Spontaneous or artificially induced mutations can also have commercial value. 'NuMex Piñata', a sport from 'Early Jalapeno', was released for the home gardener because the mutated *tra* gene causes the fruit to ripen from a lime green to yellow, to orange and finally red (Votava and Bosland, 1998; Votava et al., 2000). Thus, the plant will produce multi-colored fruits for the gardener. Mutations can be induced by ionizing radiation or via chemical mutagens. Bhargava and Umalkar (1989) used both gamma radiation and chemical mutagens to produce an array of pericarp mutations. Alcantara et al. (1996) describe optimal conditions necessary for seed mutagenesis in *C. annuum* using the chemical mutagen, ethyl methanesulfonate (EMS). They produced several novel foliage mutants. Somaclonal variation may also result in novel phenotypes. Ornamental pepper somaclonal variants have not been reported, but investigations in this area are limited.

6.4 F_1 Hybrid

Hybrid ornamental peppers are highly uniform and protect the proprietary rights of the developer. Within *Capsicum*, several systems to produce hybrid seed are possible, including the use of genetic male sterile plants and cytoplasmic male sterile plants. Practical use of male sterility in hybrid pepper production is limited by a number of factors. The production of today's ornamental pepper hybrids is reliant on making hybridizations between the two parents by hand; a very labor intensive and expensive process.

Non-allelic recessive genes, *ms1, ms2* and *ms3* condition genetic male sterility in pepper (Shifriss and Frankel, 1969; Shifriss and Rylsky, 1972; Shifriss, 1973). A homozygous recessive state for either gene is sufficient to produce male-sterile plants. Meiosis proceeds normally, but microspore degeneration occurs soon after the tetrad stage, resulting in non-fertile pollen. Additional *ms* loci have been reported, but allelic tests are lacking among described mutants (Daskalov and Poulos, 1994). Production of the male-sterile parent is an inefficient process because 25% or 50% of the plants, dependent on the presence of one or two forms of the recessive genes, must be identified and rouged from the population of seed parents in a production field. Closely linked markers have not been identified to facilitate elimination of fertile plants in the seedling stage. Nonetheless, sterile plants are easily identified at anthesis. Due to the inefficiency of hybrid production using genic male sterility, its use in hybrid pepper seed production is very limited.

Cytoplasmic-genic male sterility (CMS) has been utilized extensively for hybrid seed production in a number of crops. Unlike genic male sterility, a population of

seed parents can be produced which is 100% male-sterile, thus eliminating the need for rouging and the possibility of non-hybrid seed production. Peterson (1958) first described cytoplasmic-genic male sterility in pepper. Mode of action was similar to classic cytoplasmic-genic sterility systems involving the interaction of a recessive nuclear male sterility inducing gene *ms*, with a sterility inducing S-type cytoplasm. Unfortunately, the cytoplasmic male-sterile system in pepper is unstable, producing fertile pollen under cool weather conditions. The degree of pollen sterility and stability of different CMS lines is also influenced by the genetic background of the S*ms*/*ms* parent (Shifriss and Guri, 1979).

6.5 Haploidy

Haploidy may have application in the development of ornamental peppers. Most *Capsicum* haploids occur naturally from *n-2n* twin seedlings of polyembryonic seeds. Haploidy occurs frequently in *Capsicum*, occurring in 1 per 1,000 to 1 per 10,000 plants (Pochard and de Vaulx, 1979). Different genotypes differed significantly in the frequency of polyembryony. Diploidization can be accomplished by application of colchicine to wounded growing points. These double haploid plants are homozygous at every loci. Many generations of self-pollination would be required to produce this same effect. Pochard and de Vaulx (1979) described inferior fertility and stability in autodiploids, in comparison to standard inbred lines. Autodiploids exhibited heritable sterility, reduced seed yield, and instability in horticultural characters not previously observed in parental F_9 inbred lines. The degree of instability increased with repeated haploid-diploid cycling.

Haploid plants have also been obtained by regeneration of plantlets from microspore culture (Nervo et al., 1995). In contrast with doubled haploids derived from spontaneous mutants, these doubled haploid plants derived from cultured microspores were reportedly homozygous and genetically stable after selfing.

6.6 Biotechnology

Biotechnology is being used in pepper to develop new cultivars, map genomes, and evaluate genetic resources. The most visible and controversial application of biotechnology is the transfer and expression of genes from one species to another. Despite considerable interest in applying gene transfer technologies to pepper, little has been accomplished. Pepper has been very difficult to regenerate with any degree of efficiency from cultured explant tissues. This has limited the use of genetic transformation to introduce foreign genes into pepper. Within other Solanaceous crops, i.e. tomato, tobacco, petunia, etc., excellent progress has been made in plant transformation and regeneration to introduce novel genes into the genome. For unexplained reasons, pepper has been recalcitrant to regeneration.

Many laboratories around the world are addressing this research question. *Agrobacterium tumefaciens* mediated transformation has been reported in both hot (Lim et al., 2001; Manoharan et al., 1998) and sweet (Nianiou et al., 2002; Zhu et al., 1996) pepper. DNA Plant Technology was granted a United States patent in 1993 directed to the transformation and regeneration of pepper plants from cotyledon tissue (Engler et al., 1993). Successful pepper transformation and regeneration has been cultivar specific and of low efficiency. Reports of high efficiency suffer from evaluation of relatively few regenerated transformed plants. Efficient transformation systems that can be successfully applied across a wide range of genotypes/cultivars are needed before this technology will be of significance in development of new pepper germplasm.

Molecular marker technology may aid in the development of improved ornamental pepper cultivars. Molecular markers have proven invaluable for understanding the genetic make-up of agricultural crops. Molecular markers are commonly used to examine genetic diversity, systematics, phylogeny, and in fingerprinting cultivars for intellectual property protection purposes (Lefebvre et al., 2001; Livingstone et al., 1999; McLeod, 1983; Prince et al., 1995). They are used in combination with other markers to construct genetic maps, and are used in linkage studies. Plant breeders can use markers linked to a desired trait in marker-assisted selection (MAS). Selection via molecular markers eliminates the need for costly and sometimes inefficient screenings, and speeds up the process of cultivar development. A number of genetic maps of pepper have been published in the past 15 years. Lefebvre et al. (2002) used restriction fragment length polymorphisms (RFLP), amplified fragment length polymorphism (AFLP), known function genes, and phenotypic markers to develop an intraspecific molecular linkage map of *C. annuum*. A second intraspecific *C. annuum* was developed by Ben Chaim et al. (2001b). Comparative genetic linkage maps constructed from interspecific pepper crosses were developed using AFLP, randomly amplified polymorphic DNA (RAPD), and tomato and pepper-derived genomic and cDNA probes used in RFLP (Kang et al., 2001; Livingstone et al., 1999). An integrated map developed from 6 intra- and interspecific crosses has recently been assembled (Paran et al., 2004). Current mapping studies have reduced the number of linkage groups to 13 and produced a total map length of 1832 cM. Molecular marker research in pepper has focused on identifying markers linked to disease resistance genes (Caranta et al., 1997, 2002; Kim et al., 2002; Moury et al., 2000; Tai et al., 1999) as well as genes that influence fruit quality attributes (Ben Chaim et al., 2001b; Huh et al., 2001; Lefebvre et al., 1998; Popovsky and Paran, 2000).

7. PEST RESISTANCE AND PHYSIOLOGICAL DISORDERS

Similar to peppers produced for culinary use, diseases and pests can affect ornamental peppers. Good cultural methods and pesticides can ensure a healthy and profitable ornamental pepper crop. One of the safest and most efficient means to protect peppers is through the development of disease and pest resistant cultivars. Introducing resistance into an ornamental pepper cultivar also implies that the cultivar meets the horticultural requirements for its market. It is a laborious task to introgress resistance while maintaining horticulturally acceptable characteristics. Therefore, many years may be required before a resistant cultivar can be released, and this task is made even more difficult and time consuming if the genetic nature of the resistance is quantitatively inherited.

7.1 Disease Resistance

Breeding for disease resistance in ornamental peppers has not been a major breeding goal. Few ornamental peppers have been developed with disease resistance as the primary objective. Nevertheless, ornamental peppers can be developed with disease resistance. For example, 'NuMex Twilight' is resistant to cucumber mosaic virus, serendipitously, because it was never screened for this disease. Thus, ornamental peppers can be sources of resistance for plant breeders working on other pod types. Sources of resistance that breeders can utilize to develop disease resistant ornamental pepper varieties include established resistant culinary type cultivars, land races, wild relatives, and closely related species.

Bacterial spot caused by *Xanthomonas campestris* pv. *vesicatoria* has wide geographic distribution and is promoted by overhead irrigation and warm, rainy weather. Clean seed is important in disease management. Bacterial spot is the most serious bacterial disease affecting peppers. Seven bacterial spot races have been identified (Sahin and Miller, 1996). Cultivars with resistance to all seven races have yet to be developed. Bacterial wilt of pepper occurs in the tropics as well as southern parts of the United States, and in warm, humid greenhouses. High levels of resistance to bacterial wilt have been described in *C. chinense*, *C. frutescens* and primitive *C. annuum* germplasm (Greenleaf, 1986). AFLP markers linked to the *Bs2* locus conferring resistance to *X. campestris* have been described (Tai et al., 1999).

Fungi are one of the largest groups of organisms causing disease on pepper. *Rhizoctonia solani, Fusarium* spp.and *Pythium* spp., all soilborne fungal pathogens, can cause damping-off or root rots in the greenhouse. With proper preventative measures, neither is a major threat to greenhouse production (Armitage and Hamilton, 1987). These pathogens may also be problematic in early plantings in beds under cool soil conditions. Rhizoctonia resistant accessions have been

identified (Muhyi and Bosland, 1995). In field environments, Phytopthora blight caused by *Phytopthora capsici* is widespread and can occur at any stage of growth, infecting all plant parts. Partial genetic resistance to *P. capsici* was identified in *Capsicum* (Kimble and Grogan, 1960; Pochard and Chambonnet, 1971) and tolerant commercial sweet bell varieties of *C. annuum* have been developed (S. Czaplewski, pers. com.). QTLs associated with *P. capsici* resistance have been reported (Lefebvre and Palloix, 1996; Thabuis et al., 2003). The causative agent of powdery mildew, *Leveillula taurica*, occurs rarely in cool climates, but is prevalent in warm climates and in greenhouses. Fungicides and available resistant cultivars provide adequate powdery mildew control. Powdery mildew resistance genes have been reported in *C. baccatum* var. *microcarpum*, *C. baccatum* var. *pendulum, C. pubescens*, and *C. annuum* (Ullasa et al., 1981).

The red, orange and yellow pigmentation of pepper fruit is valued in ornamental peppers. This pigmentation is coincident with the ripe stage of fruit development. Ripe fruit are considerably more susceptible to fruit rot pathogens. Bacterial soft rot caused by *Erwinia carotovora* occurs in fruit of outdoor plantings, but is limited primarily to larger-fruited ornamental peppers. Reliable identification of soft rot resistance in pepper germplasm has been difficult to achieve (Bartz and Stall, 1974; Stommel et al., 1996). Occurrence of ripe rot caused by a number of *Colletotrichum* species is enhanced by overhead irrigation and rain fed conditions. Resistance to *Colletotrichum*-induced ripe rot has been reported (Ullasa et al., 1981). Upright fruit orientation of many ornamental peppers prevents moisture retention at the fruit calyx and prolonged free moisture conditions, thus limiting fruit rot problems.

Viruses cause the most serious disease problems of ornamental and culinary peppers in regions where disease pressure is high. A typical virus symptom is leaf mosaic, which is a mottled non-uniform color, leaf curling, distortion, and stunting. Tobacco mosaic virus (TMV) is a Tobamovirus easily transmitted by abrasive contact. Host resistance and good sanitation limit the severity of this disease in greenhouse and field environments. A single dominant gene confers resistance, but two additional factors may be involved (Boukema, 1980; Holmes, 1937). A TMV variant, the Samsun latent strain of TMV (SLTMV), overcomes TMV resistance genes and is comprised of a number of strains that have made breeding for resistance difficult (Boukema, 1980). Use of available resistant varieties and aphid control is critical to limit spread of cucumber mosaic virus (CMV), alfalfa mosaic virus, and pepper mild mottle. QTLs associated with CMV resistance have been identified (Ben Chaim et al., 2001a; Caranta et al., 2002). Similarly, host resistance and insect control limit severity of the leafhopper transmitted beet curly top geminivirus (BCTV) and thrips transmitted tomato spotted wilt virus (TSWV). BCTV is common in the western U.S. Genetic resistance to BCTV has been identified (Ungs et al., 1977; Bosland, 2000). TSWV can be problematic in greenhouse production. Cleaved amplified polymorphic sequence (CAPS) markers for the TSWV resistance allele, *Tsw*, from *C. chinense* have been developed (Moury et al., 2000). Potato

virus Y (PVY) and tobacco etch virus (TEV) are more common in the southern U.S., but sporadic outbreaks may occur in northern states. Planting of PVY-resistant varieties generally helps control TEV because resistance to both viruses is closely linked. There are however, a few strains of TEV that can infect PVY-resistant varieties (Muhyi, et al., 1994). Molecular markers for monogenic and quantitative types of PVY host resistance have been described (Arnedo-Andres et al., 2002; Caranta et al., 1997).

7.2 Insect Resistance

Aphids, thrips and spider mites are the primary insect pests in the greenhouse and under outdoor bedding plant conditions. Whitefly may occur in the greenhouse but can be adequately controlled using predatory insects or pesticides. The green peach aphid may vector over 50 virus diseases, such as cucumber mosaic virus and tobacco etch virus. Four root knot nematode species, *Meloidogyne incognita*, *M. arenia*, *M. javanica*, and *M. hapla*, cause pepper root galling and reduced plant vigor. A dominant gene, *N*, was described by Hare (1956). The *N* gene confers resistance to *M. incognita*, *M. arenaria* races 1 and 2, and *M. javanica* in *C. annuum*, but does not condition resistance to *M. hapla* (Thies and Fery, 2000). Map positions have been determined for the dominant genes Me_3 and Me_4 that confer heat-stable resistance to root knot nematode in pepper (Djian-Caporalino et al., 2001). Declining availability of soil fumigants necessitate use of nematode resistant varieties where soil infestation occurs (Djian-Caporalino et al., 2001; Fery and Thies, 1998).

7.3 Abiotic Disorders

Peppers are sensitive to ethylene. Defoliation of pepper plants for vegetable production during transit can be a serious problem in the transplant industry. Although defoliated peppers recover and result in healthy plants, defoliated plants are slower to establish resulting in delayed maturity. Kays et al. (1976) reported substantial defoliation in transplants treated with ethylene concentrations that were substantially lower than those that may be produced by plants in transit. Removal of ethylene in storage with potassium permanganate greatly reduced abscission. Immature pepper plants were more sensitive to ethylene than mature plants (Hoyer, 1990). Fruit bearing ornamental peppers treated with ethepon sprays to stimulate fruit coloring prior to shipping plants in the dark for 4 days did not result in leaf abscission (Armitage, 1989). A systematic screen of ornamental pepper germplasm for resistance to ethylene-induced defoliation has not been undertaken. Since other Solanaceous relatives have been identified with varying sensitivity to ethylene (e.g. tomato), it is likely that genetic variation for ethylene sensitivity could be incorporated or engineered into ornamental pepper.

Many ornamental crops are sensitive to the effects of air pollution produced as a result of hydrocarbon combustion (Rogers, 1985). Peppers are sensitive to sulfur dioxide injury, resulting in necrotic interveinal tissue. Silvering, bronzing, and sometimes death of lower leaf surfaces may result from exposure to peroxycetyl nitrate (PAN) in susceptible varieties. In contrast, peppers are resistant to ozone, another common air pollutant. Identification of variation for response to air-borne pollutants has not been a high priority in pepper breeding programs.

8. PRODUCTION

Production and sale of ornamentals is a very specialized global industry. Commercial growers have developed production schemes to successfully deliver high quality potted and bedding plants and cut stems to the retail market. The diverse genetic resources available in *Capsicum* offer opportunities to improve production efficiency, reduce product loss, and increase availability of new cultivars.

8.1 Culture

Ornamental pepper is generally propagated from seed. Seed has a prolonged germination period of 10 to 14 days and an optimum germination temperature of about 30^{o}C. The rate of germination and emergence is markedly reduced at temperatures in the range of 15^{o}C to 20^{o}C. Hastening the germination and emergence of pepper seed, especially at suboptimal temperatures, would be of significant value in the production of greenhouse-raised plants. Randle and Honma (1980) described partial dominance for slow emergence at low temperatures in *C. baccatum* var. *pendulum*, with additive and dominance gene action responsible for the expression of this trait.

The seed coat does not cause any mechanical restriction to germination. However, seed size affects the uniformity of pepper plants. A seed size greater than 3.0 mm emerged 2 days earlier, had a significantly better stand, and produced overall better plants than small seeds measuring less that 3.0 mm and weighting less than 5.9 mg. Seed size is dependent on the variety and the growing conditions, usually larger fruits will have larger seeds. Pepper seed can exhibit dormancy. It is more pronounced in wild species than the commercial cultivars and may be overcome by an after ripening period of about 6 weeks at room temperature (Randle and Honma, 1981).

For potted plant production, ornamental peppers are typically grown in 10 cm (4 inch) pots (Ball, 1998). However, 13 cm or 15 cm specimens may be produced with 3 or 4 plants per pot, respectively. Sowings are typically made from April until August 1st for September – Christmas sales. A mid-December planting will yield 10

cm pot specimens for early May sales. Older cultivars benefit from a single or double pinch to promote bushy compact plants. Allow 14 weeks from seeding to sales for plants in 10 cm pots when seeded in the spring or summer and 17 weeks when seeded in winter months (Harthun, 1991; Dole and Wilkins, 1999). Peppers require high light and minimum daytime temperatures between 18°C and 21°C (65°F to 70°F) for maximum fruit set. Lower temperatures are tolerated as plants mature. Yellowing and dropping of leaves are caused by low temperature, low light and insufficient moisture or nutrients.

Production conditions for ornamental pepper bedding plant culture are similar to those for pepper vegetable production. Peppers are a warm weather crop that require night and soil temperatures of 14.5°C (58°F) or higher to promote growth. Plants are produced for sale in cell packs or 10 cm pots (one plant/cell or pot). Allow 6 to 8 weeks for cell packs and 8 to 10 weeks for 10 cm pots. Plants should be ready for sale as early as mid-April in the northern United States for users who plant into beds with night protection. Late-maturing ornamental pepper varieties benefit from a long growing season.

8.2 Growth Regulators

A triazole growth regulator (uniconazole) was tested on potted ornamental pepper for its response on plant height and fruiting (Starman, 1993). Foliar spray concentrations from 5.0 to 15.0 mg/liter gave optimal reduction in plant height. However, 15.0 mg/liter reduced height excessively when applied at eight weeks, but not at ten weeks after sowing. An increase in the percentage of red fruit was seen with an increase in the concentration of uniconazole when applied at ten weeks, but not at eight weeks after sowing.

Ethephon has been tested on ornamentals as a growth regulator to hasten ripening and to control plant height. Ethephon applied as a foliar spray at 300 ppm increased the number of lateral branches, but delayed flowering and reduced fruit production (Khademi and Khosh-Khui, 1977). Concentrations of 150 and 300 µl/liter were effective in accelerating fruit ripening of ornamental peppers (Armitage, 1989). It was also observed that concentrations as low as 75 µl/liter were effective, but a concentration of 600 µl/liter resulted in foliar and fruit damage. Fruit that were less than 3 cm long were less sensitive to ethephon than more mature fruit. When the pH of the ethephon solution was raised from pH 3.3 to pH 6.3, the treatment effect was increased.

Indoleacetic acid and benzyladenine have been evaluated for their effect on lateral branching in ornamental peppers. Concentrations as high as 150 ppm indoleacetic acid and 1200 ppm benzyladenine did not increase the lateral branching of potted ornamental peppers (Khademi & Khosh-Khui, 1977).

The genes for dwarfism and multi-branching are available in pepper germplasm. The use of genetics instead of chemicals to solve these production problems will be a benefit to the grower and the environment.

9. FUTURE PROSPECTS

A small but important group of peppers can be classified as ornamental. Although edible, ornamentals are grown primarily for their unusual pod shapes or for their dense foliage and colorful fruits. Part of the reason for the increased interest in ornamental peppers can be attributed to their wide array of fruit colors and shapes, and the variation in foliage color and plant habit. They rival Chrysanthemum for vivid fall color as a border plant.

Ornamental peppers have the highest per unit value of any pepper product. Ornamental peppers have become an innovative way for small farmers to produce a high-value alternative crop and are a profitable crop for greenhouse production. Breeders will continue to create ornamental peppers in a wide array of dazzling colors and shapes. Tools of the developing biotechnology industry will facilitate introduction of new characters to improve cultivar attributes important for consumer appeal and increase tolerance or resistance to disease and environmental stress. These new and improved cultivars will help fuel the renewed interest in ornamental peppers. These activities will increase the value and importance of ornamental peppers as a potted plant. It is foreseeable that their use as a cut stem and as a garden plant may rival that of roses.

For current information about ornamental peppers, The Chile Pepper Institute maintains a website for those interested in peppers. The Chile Pepper Institute is a research-based resource center dedicated to education about the wonders of pepper. It is a non-profit, international organization housed in the College of Agriculture and Home Economics at New Mexico State University. It maintains a comprehensive bibliography on pepper, containing more than 7,000 references, and is searchable from the Internet (Bosland, 2001). The Chile Pepper Institute frequently sends its members a seed sample of an ornamental pepper. For further information on The Chile Pepper Institute write: The Chile Pepper Institute, Box 30003, Dept. 3Q, NMSU, Las Cruces, NM 88003, or phone: (505) 646-3028, or visit its web site at www.chilepepperinstitute.org.

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Chapter 22

EXACUM

Exacum affine and related species

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Abstract: *Exacum affine* is one of the rare blue-flowering potted plants. In addition to blue flowers, its popularity is attributable to fragrant flowers, shiny green foliage, low-mounding habit, ease of shipping, and post-harvest shelf life. While there are 65 species in the genus, breeders have used primarily *E. affine* for most products. Recent interspecific hybridizations indicate high fertility in progeny, holding promise for future crop transformations. The lack of self incompatibility greatly aids in obtaining intra- and inter-specific seed set. Future breeding goals include the use of male sterility for hybrid seed production, insect vectors for pollination, faster production time, more precise flowering control, disease and insect resistance, increased basal branching, new flower colors, and enhanced post-harvest life.

Key words: Blue flowers, Gentianaceae, Mutation breeding, Persian Violet, Polyploidy.

1. INTRODUCTION

Exacum affine Balf. f. ex Regel (aka Persian violet, Persian gentian, Arabian violet, or German violet) is a beautiful pot plant grown mainly for its many small, sweetly scented, flowers that cover low mounded plants. The leaves are small and form dense covers. Cultivars are available in range of flower colors, all with brilliant yellow anthers making a very attractive combination. The popularity of *Exacum* L. is perhaps due mostly to their ease of shipping and better than average keeping quality for consumers. It is also one of the few potted flowering plants with blue flowers.

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2. BOTANICAL HISTORY AND DOMESTICATION

The generic name *Exacum* was introduced by Linnaeus (1747a, b) from the Latin *ex*, out, and *agrere*, to drive, referring to the purgative properties of members of the genus. However, in 1700, Plukenet had already illustrated the species now known as *E. pedunculatum* L. in his Phytographia (Plukenet 1700). Linnaeus (1753) later described two species of *Exacum*, *E. sessile* L. and *E. pedunculatum* as well as *Chironia trinervia*, now known as *E. trinervium* (L.) Druce, all three from India and Ceylon.

Over the next 100 years, several authors published works describing *Exacum* species. Most notable are Roxburgh (1814, 1820) and Wallich (1831), botanists working for the East India Company. As they explored the Indian sub-continent in search of new flora, they each described several *Exacum* species, some new to science. Within 10 years, Grisebach (1838) published "Genera et species Gentianearum" where he describes 13 species of *Exacum*, including *E. quinquenervium* Griseb., the first species from Africa to be described. Three additional authors, Thwaites (1860), Beddome (1874) and Clarke (1885) were able to identify and describe all but one species that were native to India and Sri Lanka.

At the end of the 19th century, exploration of Socotra and Madagascar had just begun. The first scientific trip to Socotra was accomplished by an English expedition in 1880 and quickly followed by German scientists in 1881 (Klackenberg, 1985). Three of the four known species from Socotra were described on the basis of material brought back by these expeditions, *E. affine*, *E. caeruleum* Balf., and *E. gracilipes* Balf. (Regel, 1883; Balfour, 1884). In modern literature, *Exacum affine* has been attributed to Balfour based on his 1884 publication. However, authority should rightfully be attributed to Regel, as Regel's publication has priority and therefore, the correct binomial name is *Exacum affine* Balf. f. ex Regel (Klackenberg, 1985). Regel based his description of *E. affine* on material sent to him through Mr. Schweinfurth, who collected it during the 1881 expedition to Socotra (Klackenberg, 1985). The germplasm collected by Mr. Schweinfurth is believed to be the first live collection of *E. affine*, as well as the source material that led to the domestication and horticultural production of this species in Germany.

Continuing through the late 19th and early 20th centuries, *E. affine* production increased in Germany and other European countries, as popularity grew for this new and attractive plant. Since these original collections, very little additional germplasm has been collected in Socotra due to its geographical and political isolation.

2.1 Natural History and Centers of Diversity

Exacum species are members of the Gentianaceae or the gentian family. The genus contains approximately 65 species, most of which are annual or perennial

herbs (Klackenberg, 1985). *Exacum* are endemic to four regions; Africa (from Senegal to southern Kenya in the north and to northern Angola to Transvaal in the south); Madagascar; Socotra; and Asia. In Asia, *Exacum* are distributed from Sri Lanka and India to the Philippines and New Guinea with the northern limit in the Himalayas (Klackenberg, 1985).

Although *Exacum* species are found over a very wide geographic area, they are very unevenly distributed. Fifty-nine of the 65 species are found in three, relatively small areas: Madagascar (38 species), Socotra (4 species), and Sri Lanka and the southern tip of India (17 species) (Klackenberg, 1985).

Exacum affine is placed in the section Africana and was considered endemic to Socotra (Klackenberg, 1985). However, it has recently been found in the mountains of the Arabian peninsula. It grows on somewhat moist rocks and alongside streams up to elevations of 1000m. In nature, the species varies in height from 3-50 cm with flower diameter between 10-18 mm. In nature, flowering specimens of *E. affine* have been observed between February and April and again between August to September (Klackenberg, 1985). It is unclear whether these observations are describing individuals that flower twice in one year or whether geography affects the flowering period of different populations.

2.2 Commercial History

Of the approximately 65 species of *Exacum*, only *E. affine* has so far gained economic importance as an ornamental crop. The first report of *E. affine* seed offered commercially is from Ernst Benary Seed Growers Ltd, then located in Erfurt, Germany and now located in Hann. Muenden, Germany. Their 1904/1905 seed catalog listed *E. affine* with the following description (translated from German), "*Exacum affine*, very dwarf, fragrant lilac flowers". The original source of this germplasm appears to be from the 1881 German expedition to Socotra. The commercial production of this seed was accomplished by open pollination, and very little directed breeding was taking place at this time. The next report of a commercial introduction was by Sakata Seed in 1941. They offered "*Exacum affine*, light blue; US$3.20 per 1/16oz, US$5.80 per 1/8oz, US$10.50 per 1/4oz, US$35.00 per oz". Again, this introduction represented an open pollinated cultivar with little directed breeding. However, over the next several decades, interest grew for this crop to the point where several companies undertook active breeding.

In 1968, Sakata Seed introduced the first inbred selection of *E. affine* 'Midget' (Fig. 22-1), by selecting dwarf forms from their in-house germplasm (Suda, 2002). They described this new introduction as "more dwarf and compact than the regular type with a slightly lavender color; suitable for potting". Shortly after the introduction of the cultivar 'Midget', Mr. Claude Hope, of Linda Vista, S.A., in Costa Rica, released a slightly taller cultivar, 'Tiddley Winks', followed by the

subsequent release of cultivars 'Elfin' and 'Improved Elfin'. It is unclear as to where the initial germplasm for this breeding program originated. In 1974, E. J. Small, of Pinellas Park, FL, USA initiated an *E. affine* breeding program with germplasm acquired from a Japanese source; most likely from Sakata germplasm. Their breeding efforts led to the first introduction of a F_1 hybrid cultivar, 'Blue Champion' (Fig. 22-1), specifically bred for pot production.

Figure 22-1. Exacum affine commercial series and cultivars: Top Left- *E. affine* 'Midget White' and 'Midget Blue' (Sakata); Top Right- *E. affine* 'Blue Champion' (E.J. Small); Bottom Left- *E. affine* 'Royal Dane' series (Ex-Plant AsP); Bottm Right- Breeding selection from Sri Lankan interspecific hybrid populations (University of British Columbia).

In the 1980's, a great deal of effort was directed toward the development of improved *E. affine* cultivars. In 1982, Erik Rosendal of Ex-Plants ApS, Denmark, released the cultivars 'Best Blue' and 'Best White', developed from germplasm acquired from both Japan and the USA (Serek and Trolle, 2000). These two cultivars were the first to be bred specifically for the European market, which tends to be more demanding in terms of production performance and consumer appeal. In 1986, Sakata Seed followed their initial 'Midget' introduction with two improved forms, 'Midget Blue' and 'Midget White'. The white form was found as a spontaneous mutation among seedlings of 'Midget' and further inbred to genetically stabilize the white flower color. The 'Midget' series represent the first *E. affine* cultivars specifically bred to create a color series with common production performance. These early introductions were selected for compactness, earliness in flowering, flower size and form, overall vigor, and flower color (Erwin, 1984).

Today, more than 25 cultivars are commercially available with the most popular being 'Royal Dane Blue' ® (Fig. 22-1), produced by Ex-Plant ApS (Leth, 2002). Currently, two companies supply over 98% of the world's supply of *E. affine* cultivars; Ex-Plant ApS in Odense, Denmark, and E. J. Small Growers in Pinellas Park, FL, USA. In recent years, the floriculture industry has again witnessed renewed interest in *E. affine*, with over 24 million units produced worldwide. Denmark leads total production with approximately 65%, with The Netherlands adding an additional 20% (Leth, 2002). However, both North American and Japanese growers are gaining an increasing percentage of overall production (Leth, 2002).

3. BOTANY

3.1 Taxonomy

Recent advances in plant systematics coupled with advances in plant molecular biology have allowed for the realignment and re-classification of flowering plant groups. In the system proposed by the Angiosperm Phylogeny Group (Bremer et al., 1998) *Exacum* is classified among the Asterids. Furthermore, Gentianales (Order) and Gentianaceae (Family) groups are supported and deemed valid. Below this level, the International Association for Plant Taxonomy has accepted the following scheme for *Exacum* (Reveal, 1995): Subfamily – Gentianoideae, Tribe – Exaceae, Subtribe – Exacinae, Genus – *Exacum.* A brief botanical description of *Exacum* follows (Sumanasinghe, 1985):

> *"Exacum*: Annual or perennial, erect, glabrous herbs. *Stems* terete or 4-angular, with or without wings.

Leaves sessile or shortly petiolated, ovate to lanceolate and 3-5 nerved at the base. *Flowers* in leafy, dichotomous cymes, terminal or axillary, 4-5-merous. *Calyces* generally papery, veined and persistent with fruits; lobes winged on back. *Corollae* rotate, often persistent in fruit. *Stamens* inserted in throat of corolla; anthers opening by terminal pores; filaments short. *Styles* long, curved. *Ovaries* 2-celled. Ovules anatropous, unitegmic and tenuinucellar. *Fruits* globose, septicidally 2-valved capsules with numerous small seeds. Endosperm nucleate."

3.2 Cytology and Genetics

The first published chromosome count for *E. affine* reported a gametic chromosome number of n=18 with chromosome lengths of 0.7 and 0.5 µm at the first and second metaphase, respectively (Sugiura, 1936). This report was later confirmed by karyotype analysis of a cultivated plant at the Missouri Botanical Garden (Post, 1967). Darlington and Wylie (1955) reported there were 36 chromosomes in the somatic condition and concluded this to be a tetraploid complement of chromosomes ($2n$=$4x$=36). Sumanasinghe (1986) agreed on the chromosome number for *E. affine* and added new counts for several additional species: *E. macranthum* Arn. ex Griseb. ($2n$=54), *E. pallidum* (Trim.) Klack. ($2n$=52), *E. pedunculatum* ($2n$=56), and *E. trinervium* (L.) Druce ($2n$=60).

Karyotype analyses revealed that chromosome sizes found in *Exacum* are very small as compared to other angiosperms. In cells arrested in metaphase, *E. affine* 'Royal Dane Blue'® had chromosome lengths between 0.6 and 1.2 µm while *E. gracilipes* had chromosomes ranging between 0.3 and 0.9 µm in length (Villemoes, 2000). In *E. trinervium*, chromosome sizes ranged between 0.6 and 2.7 µm in length (Villemoes, 2000). In addition, this species was characterized by the presence of one long and one medium pair of chromosomes as compared to the other Sri Lankan taxa (Sumanasinghe, 1986).

Sumanasinghe (1986) also combined his cytological observations with isozyme analysis in an attempt to refine the phylogenetic relationships among the Sri Lankan taxa. Based on these results, he suggested the following modifications to the phylogeny proposed by Klackenberg (1985): elevation of *E. macranthum* and *E. pallidum* to species rank, and the establishment of two subspecies within *E. trinervium*, subsp. *trinervium* and subsp. *ritigalensis* (Willis) Cramer.

In a recent study, classical cytology and flow cytometry were coupled with molecular analysis to evaluate the phylogenetic associations among *E. affine*, *E. gracilipes*, and a reported interspecific hybrid between the two, 'Mini Lilac', as well as several accessions of *E. trinervium* subsp. *trinervium* and *E. trinervium* subsp.

ritigalensis. Villemoes (2000) reported for the first time a somatic chromosome count for *E. gracilipes* of 20 (2*n*=2*x*=20). She further reported for the first time, total DNA contents for *E. affine* 'Royal Dane Blue'® (3.55 pg), *E. gracilipes* (3.80 pg), 'Mini Lilac' (3.39 pg), *E. trinervium* subsp. *trinervium* (3.02 pg) and *E. trinervium* subsp. *ritigalensis* (3.00 pg). A cladogram based on RFLP data displayed a monophyletic origin for the *Exacum* taxa she evaluated, as expected. Within *Exacum*, two large clades were formed with one clade including all accessions derived from taxa native to Socotra (e.g. 'Royal Dane Blue'®), *E. gracilipes*, and 'Mini Lilac'). The second clade including all taxa native to Sri Lanka (e.g. *E. trinervium* subsp. *trinervium* and *E. t.* subsp. *ritigalensis*) (Villemoes, 2000). These data are in agreement with the previously published phylogeny of Klackenberg (1985) that was based solely on morphological characters.

The genus *Exacum* is hypothesized to comprise a polyploid series of species. Comparing the chromosomal number series found in the genus, *E. affine* is considered to be a tetraploid (Rork, 1949). Darlington and Wylie (1955) suggested the basic chromosome number for *Exacum* to be *x*=9, thereby supporting the assumption that *E. affine* is a tetraploid (2*n*=4*x*=36). However, a basic chromosome number of *n*=10 is also common in this genus. Genetic studies in *E. affine* have demonstrated that simple disomic inheritance is present for many traits and that a high level of fertility is found within the species (Wolf and Craig, 1988). This information is consistent with an evolutionary scheme invoking an allotetraploid origin for *E. affine*. However, Sumanasinghe (1986) speculated that *E. tetragonum* Roxb. (2*n*=2*x*=18) may be the diploid ancestor of the putative autotetraploid *E. affine*, which now functions as an established diploid. In addition, he hypothesized an autoploid origin for *E. macranthum* (2*n*=6*x*?=54) and an autoploid origin with aneuploid reduction for *E. pallidum* (2*n*=6*x*-2?=52) from a common ancestor. He went on to propose an alloploid origin for *E. trinervium* (2*n*=6*x*?=60). However, he made no suggestions on the possible identity of the ancestral species. Table 22-1 lists the chromosome number for all *Exacum* species described to date.

Table 22-1. Chromosome numbers reported for the genus *Exacum*.

Species	*n*	*2n*	Reference
E. affine Balf. f. ex Regel	18		Sugiura, 1936; Post, 1967
E. affine Balf. f. ex Regel		36	Rork, 1949; Darlington and Wylie, 1955
E. affine Balf. f. ex Regel	18	36	Sumanasinghe, 1986
E. atropurpureum Bedd.		34	Mallikarjuna, 1985
E. bicolor Roxb.		62	Mallikarjuna, 1985
E. courtallens Arnott. var. courtallens	34		Mallikarjuna et al, 1987
E. courtallens Arnott. var. laxiflorum Gamble		68	Mallikarjuna, 1985; Mallikarjuna et al 1987
E. foliosum Griseb.		68	Mallikarjuna et al, 1987

Species	*n*	*2n*	Reference
E. gracilipes Balf. f.		20	Villemoes, 2000
E. lawii Clarke		56	Mallikarjuna, 1985; Mallikarjuna et al 1987
E. macranthum Griseb.	28	54	Sumanasinghe, 1986
E. pallidum (Trim) Klack.		52	Sumanasinghe, 1986
E. pedunculatum L.		62	Mallikarjuna, 1985; Mallikarjuna et al, 1987
E. pedunculatum L.	28		Sumanasinghe, 1986
E. perrottetii Griseb.		68	Mallikarjuna, 1985; Mallikarjuna et al, 1987
E. petiolare Griseb.		62	Mallikarjuna, 1985; Mallikarjuna et al, 1987
E. pumilum Griseb.	31		Mallikarjuna, 1985; Mallikarjuna et al, 1987
E. sessile L.	31		Mallikarjuna, 1985; Mallikarjuna et al, 1987
E. tetragonum Roxb.		18	Borgmann, 1964
E. travancoricum Bedd.		68	Mallikarjuna, 1985; Mallikarjuna et al, 1987
E. trinervium (L.) Druce subsp. ritigalensis (Willis) Cramer	30	60	Sumanasinghe, 1986
E. trinervium (L.) Druce subsp. trinervium Cramer	30	60	Sumanasinghe, 1986
E. wightianum Arn.		68	Mallikarjuna, 1985; Mallikarjuna et al, 1987

3.2.1 Interspecific Hybridization

There is only one report of an interspecific hybrid using *E. affine*. The cultivar, 'Mini Lilac' is reported to be the result of an interspecific hybridization between *E. affine* and *E. gracilipes* (Leth, 2002). Recently, in an attempt to verify the parentage of 'Mini Lilac', Villemoes (2000) applied several molecular techniques to evaluate DNA similarity between the hybrid and the reported parental species. Based on RFLP and RAPD techniques, she was unable to definitively assess the interspecific parentage of the cultivar. However, she did find strong support for the close relationship among *E. affine*, *E. gracilipes* and 'Mini Lilac' and stated that the variation observed is consistent with the reported hybridization.

In an attempt to recreate the putative interspecific hybrid 'Mini Lilac', Villemoes (2000) was able to produce primary hybrids between *E. affine* and *E. gracilipes*. All hybrid progeny were female fertile and able to be used as seed parents. However, 50% of the resulting progeny were male sterile, either lacking anthers or lacking

pollen. She speculated that the male-sterile condition could be the result of post-fertilization barriers such as abnormal meiotic pairing during anther development. The cultivar 'Mini Lilac' is fertile so if indeed this cultivar is the result of interspecific hybridization and Villemoes' observations are correct, this cultivar represents a fertile selection out of a cross where fertility can be variable.

In assessing the potential success rate of crosses between *E. affine* and taxa native to Sri Lanka, Villemoes (2000) evaluated several techniques commonly used to facilitate interspecific hybridization. She compared normal pollination to cut-style pollination and the use of mentor pollen on interspecific hybrid production. She observed pollen tube growth through the styles and to the ovary in most crosses. However, no seeds were produced in any parental combination. These data would indicate that species specificity for the production of fertile progeny is related to post-fertilization chromosomal incongruities between phylogenetically divergent taxa, and not any pre-fertilization barrier or incompatibility system.

One other report of interspecific hybridization among *Exacum* species involved several taxa native to Sri Lanka. Sumanasinghe (1986) evaluated crossability among *E. affine*, *E. macranthum*, *E. trinervium* subsp. *trinervium*, *E. trinervium* subsp. *ritigalensis*, and *E. pedunculatum*. He reported no success with any of the *E. affine* crosses. However, he was able to produce intra- and interspecific hybrid seed from the following crosses:

E. trinervium subsp. *trinervium* x *E. trinervium* subsp. *ritigalensis*
E. trinervium subsp. *trinervium* x *E. macranthum*
E. trinervium subsp. *trinervium* x *E. pedunculatum* (limited success)
E. trinervium subsp. *ritigalensis* x *E. macranthum*

Fertility of the resulting primary hybrids from these interspecific crosses was variable depending on the species combination. However, an adequate level of fertility was observed in most crosses to continue breeding. Over the next several generations, fertility of the hybrids was stabilized through selection (Riseman, unpublished data).

3.2.2 Manipulation of Ploidy Levels

There is one published report of a directed effort to double the ploidy level in an *Exacum* species. Semeniuk (1978) applied colchicine to *E. affine* in an attempt to generate functional tetraploids (actual octoploids). He applied a 0.5% colchicine solution to enlarging axillary buds from decapitated seedlings over five consecutive days. He observed several morphological changes associated with the chemical treatment. These included larger flowers and pollen as well as thicker petals, styles, and pedicels,. The pollen was reported to be abundant, uniform and viable. Progeny from 18 different treated meristems all produced uniform plants with the characteristic features of polyploids; heavier stems, stockier plants, darker green leaves, enlarged pollen grains, larger flowers and floral parts, and thicker petals as

compared to untreated controls. Based on these observations, he concluded he had produced plants with higher ploidy level. However, no cytological observations were made to confirm an increase in chromosome number. In evaluating the colchiploid progeny for horticultural merit, Semeniuk (1978) concluded that the visible changes induced in these 'tetraploids' were not horticulturally superior to the original cultivars and the research was discontinued.

Commercial efforts to work with higher ploidy levels in *E. affine* have produced mixed results. The breeding program at E. J. Small Growers, Inc. has periodically discovered 'tetraploid' individuals thought to be the result of spontaneous chromosome doubling through non-reduced gamete production (Cummiskey, 2002). In their evaluations of these individuals, several observations have been made. The 'tetraploids' tended to have reduced capacity for seed production as well as pollen inviability associated with exposure to high temperatures. Despite these problems with reproduction, the putative 'tetraploids' appear to have several desirable traits, including increased resistance to *Botrytis cinerea*, longer flowering periods, more intense flower color, and larger flowers (Cummiskey, 2002). These attributes are of commercial interest and breeding work continues with these individuals.

3.2.3 Mutation Breeding

Due to the relatively narrow germplasm base and low level of variation found in *E. affine*, mutation breeding has been seen as a viable way to generate additional variation. However, the results have been disappointing to date. In addition to the colchicine treatments mentioned before, gamma radiation has been used in an attempt to induce variation and generate valuable mutations. Niedz et al (1979) exposed twenty-nine *E. affine* lines to 30 Gy of gamma radiation. The resultant plants displayed a range of variation including modified plant habit (e.g. vining and compact forms), pollen viability, foliage shape and alterations in flower size, type, and number. However, the variation recorded was deemed inferior to the original forms and breeding was discontinued on these lines.

The two commercial introductions, 'Blue Rosette' and 'Best Rose', were both successfully generated through radiation treatments. These cultivars were the result of exposing meristematic tissue from the cultivars 'Blue Rococo' and 'Best Blue', respectively, to an unspecified radiation treatment (USPTO, 1988). After treatment, 'Blue Rosette' was identified as a mutant displaying a semi-double flower phenotype, while 'Best Rose' was identified as a new color mutant. Production of 'Blue Rosette' is limited to asexual propagation due to its altered phenotype. However, 'Best Blue' bred true to type and was produced by seed.

Currently, the majority of mutations incorporated into commercial breeding lines have been spontaneous and identified within individual breeding programs. These include flower color (e.g. white, mauve, pink, rose, plum), flower form (e.g. semi-double, double), branching habit, and internode length. Beyond these naturally

occurring mutations and few commercial examples, very little genetic advancement has been observed through directed mutagenesis, either by chemical or radiation treatment.

3.2.4 Somatic Variation and Selection

Exploitation of somaclonal variation in *E. affine* has only been realized under specific circumstances. The limited utilization of this technique is likely due to the lack of stability in derived genotypes. However, somatic variation and subsequent somatic embryogenesis have been used to isolate specific genotypes from chimeric tissues created by either natural mutations or chemical treatments (Villemoes, 2002).

3.3 Crossing Mechanisms

To date, no sexual incompatibility, either sporophytic or gametophytic has been identified in *Exacum*. In intra-taxa crosses of *E. affine* lines with common chromosome number, normal pollen germination and pollen tube growth through the style has been observed with normal fertile progeny produced (Villemoes, 2000).

In inter-taxa crosses, variable results have been reported. Pollinations between *E. affine* and *E. gracilipes* produced viable seed with the resulting progeny displaying variation in their levels of fertility. Specifically, the primary interspecific hybrids were 100% female fertile, but only 50% male fertile (Villemoes, 2000). It was postulated that the reduction in fertility was due to chromosomal incongruity between the different germplasm used in the crosses.

In non-*E. affine* crosses, reproductive success has been dependent on the degree of divergence between the two parental taxa used in the cross. The variation in reproductive success has been attributed to post-fertilization barriers, namely, chromosomal incongruities (Riseman, 1990; Villemoes, 2000). At no time has a pre-fertilization incompatibility system been detected. Progeny produced from full diallel crosses among *E. trinervium* subsp. *trinervium*, *E. trinervium* subsp. *ritigalensis*, and *E. macranthum*, displayed variation for several measures of fertility. In these evaluations, Riseman (1990) calculated percent self-pollination fruit set, percent backcross fruit set (to each parental taxa), *in vitro* pollen viability, *in vivo* pollen growth. He observed higher levels of fertility in the interspecific progeny produced from *E. trinervium* subsp. *trinervium* and *E. trinervium* subsp. *ritigalensis* crosses than progeny produced from either of these taxa when crossed with *E. macranthum*. He concluded that these measures of fertility supported the close taxonomic association between the *E. trinervium* subspecies (based in part on common chromosome number), whereas *E. macranthum* has a more distant phylogenetic relationship. In subsequent evaluations of interspecific progenies, near normal fertility has been observed indicating reproductive success can be selected for and stabilized in these populations (Riseman, unpublished data).

4. SPECIES-SPECIFIC TRAITS AFFECTING COMMERCIAL PRODUCTION

4.1 Seed Dormancy

Seed dormancy is not an issue in *Exacum*. New seed that is allowed to dry for a minimum of two weeks, under ambient conditions, germinate readily without any additional stratification or scarification treatments. However, fresh seed that has not been allowed to dry will not germinate (Cummiskey, 2002). This observation would indicate that a germination-inhibiting chemical is present in fresh seed thus preventing precocious seed germination. Upon drying, the chemical inhibitors that were present initially are destroyed or inactivated by the drying process (Deno, 1993). This mode of germination control is a very common mechanism for preventing germination of seed before dispersal among angiosperm species. However, once seed is dried, it is able to be stored at 7˚ C without a reduction in viability for greater than 15 years (Cummiskey, 2002).

4.2 Germination

Requirements for germination of *Exacum* seed are well established. Because there are no identified germination inhibitors present in dried seed, the only requirement for germination, beyond moisture, oxygen, and correct temperature, is light. Sowing and germination instructions for *Exacum* are as follows: 1) Surface sow seed on a light, well drained medium; 2) place sowing container in a pan of water until saturated and allow to drain to field capacity; 3) enclose container in a clear plastic bag or some other type of cover that will maintain high relative humidity; 4) place enclosed container between 7-15 cm from fluorescent lamps; 5) maintain temperature between 20-23˚C. For viable seed, germination rates near 100% should occur between 7-14 days post sowing.

Specific breeding lines have been developed that excel in producing seed that germinate quickly and uniformly. These lines are therefore used extensively in commercial seed production.

4.3 Yield Potential

Yield, in terms of number of seed produced per flower, is of great interest to the commercial breeder because currently, most seed production is accomplished through hand pollination. Seed yields are highly genotype dependent and as such, specific genotypes have been selected and used as the seed parents in most commercial breeding programs. Yields can vary from 50 to 300 seed per flower

depending on genotype, time of year, light levels and temperature (Villemoes, 2002).

4.4 Crop Time

Currently, reduced crop time is one of the most important breeding objectives in commercial breeding programs . Because *Exacum* production from seed can take up to 25 weeks (depending on time of year) to produce a 12 cm pot, this trait has been the focus of intensive selection pressure. Two of the most significant environmental factors that affect crop time are temperature and light levels. As each increases, either together or separately, crop time is reduced. However, there is a significant genotype x environment interaction that allows for the selection of genotypes with much reduced crop times. Currently, cultivars are available that produce a saleable plant in as short as 12 weeks. However, often these cultivars are not superior for many horticultural traits when compared to slower growing cultivars. Nonetheless, their short crop time is seen as an overriding asset to the grower, so sales remain high on these fast crop cultivars (Cummiskey, 2002).

4.5 Additional Breeding Issues

Most *Exacum affine* cultivars are sold as F_1 hybrids with seed production accomplished primarily through hand pollination. The use of male sterility and insect vectored pollination is very rare. Emasculation is normally not required in seed production due to floral morphology and anther structure. In *Exacum*, pollen is released at anthesis through apical pores and requires external manipulation, thereby reducing random pollen dispersion. However, one exception does exist as to whether emasculation should be performed. This exception is based on the level of greenhouse thrips (*Heliothrips haemorrhoidalis* (Bouché)) infestation. When significant thrip populations are present, emasculation should be strongly considered to ensure seed purity. Thrips can act as pollen vectors and will accomplish a significant level of unintended pollinations thereby contaminating seed lots (Cummiskey, 2002). Thrip infestation is particularly difficult to control on semi-double flowered seed parents and requires extra attention with regard to emasculation and insect control measures (Cummiskey, 2002).

The pollen biology in *Exacum* does allow for flexibility in scheduling pollination and seed production. *Exacum* pollen can withstand dry storage up to seven days without a noticeable loss of viability (Cummiskey, 2002) and may actually be able to withstand storage for much longer times under suitable conditions (Riseman, unpublished data). In addition, *Exacum* pollen germinates readily *in vitro* so viability is easily monitored (Riseman, 1990). The ability of pollen to be stored and germinate *in vitro* is indicative of a species that produces binucleate pollen.

5. TRAITS AND GENES IDENTIFIED

To date, very little research has been published on the inheritance of specific traits in any species of *Exacum*. However, the available literature does give some insight into inheritance patterns and dominance relationships of a few horticulturally relevant traits.

5.1 Flower Color and Stem Color

The inheritance of flower color and stem color was investigated using inbred lines derived from several commercial cultivars (Wolf and Craig, 1988). Flower color classes were clearly differentiated as being either blue or white, while stem color was more variable, with the delineation of three classes, green, light red, or red. One locus was determined to condition flower color, with blue completely dominant over white. Inheritance was by nuclear genes and disomic in nature. Stem color was concluded to be conditioned by the same locus as flower color, plus two additional loci. Since plants with pigmented stems produced only blue flowers and those with green stems produced only white flowers, they concluded that red stems and blue flowers were controlled by the single pleiotropic locus, w_1. The additional loci acted as stem color intensifiers. These additional loci, dil_1 and dil_2, were hypostatic to the first locus, w_1. In a second experiment, two true breeding white flowered lines were crossed to produce blue flowered progeny. This suggests that recessive alleles at more than one locus were capable of inhibiting floral pigmentation. Based on these results, they proposed two possible genetic models, one assuming dominant complementary action of these loci, and the other assuming dominant duplicate action with cumulative effects.

In subsequent research, Sumanasinghe (1983) refined the genetic model proposed by Wolf and Craig (1988). He concluded flower color was conditioned by the action of two dominant complementary, non-allelic loci, designated as w_1 and g_1, that are responsible for the threshold limits of flower and stem pigmentation. If both loci are dominant, colored flowers and pigmented stems are produced. If at least one locus is homozygous recessive, pigment production is inhibited. He also proposed the existence of three additional loci (designated as intensifiers d_1, d_2, and d_3) to condition the different shades of blue flower color. The presence of at least one dominant intensifier-locus causes dark blue flowers to be produced. However, all three loci manifest cumulative dosage effects for flower pigmentation. Finally, he confirmed that the w_1 and d_1 loci are pleiotropic for flower color and stem color.

Flower color in several Sri Lankan *Exacum* species has been examined with respect to species-specific contributions to subsequent generations. These studies were not intended to elucidate inheritance patterns, only trends in trait transmission. Riseman (1990) reported that dark blue flower color is exclusively donated from *E. macranthum*. *E. trinervium* subsp. *trinervium*, and *E. trinervium* subsp. *ritigalensis*

both contributed light and medium blue flower color equally to the resulting progeny. Dominance relationships were not identified.

5.2 Leaf Shape

There is one report of an inheritance study investigating leaf shape. Sumanasinghe (1983), working with true breeding *E. affine* lines, determined that a single locus conditioned leaf shape with no dominance detected. In addition, simple disomic inheritance was observed. Alleles in the homozygous dominant state at this locus produce rhombic (diamond-shaped) leaf shape, while the homozygous recessive state conditions deltate (broadly triangular with an obtuse apex) leaf shape. A heterozygous condition produces trullate (shaped like a trowel) leaves, which are intermediate between the rhombic and deltate shapes.

5.3 Petal Shape

Inheritance of petal shape has been investigated once in *E. affine* and once in taxa native to Sri Lanka. In *E. affine*, petal shape is conditioned by a single locus exhibiting no dominance (Sumanasinghe, 1983). When the locus is in the homozygous recessive state, round petals are produced. When the homozygous dominant condition is present, acuminate petals are produced. The heterozygote produces an intermediate form.

Sri Lankan *Exacum* species display species-specific petal shapes, rounded or acuminate. Evaluation of interspecific hybrid progeny revealed no dominance for this trait (Riseman, 1990). When acuminate plants were crossed with rounded plants, a range of intermediate forms was observed. No reciprocal differences were identified. An additional flower form trait, petal spacing, was also investigated in these populations. Riseman (1990) concluded that the imbricate (overlapping) form was dominant to apert (separate).

5.4 Double and Semi-Double Flowers

The double flower form has been discovered independently as a natural mutation in several commercial breeding programs. The phenotypic expression of the mutation is variable and can be 1) a complete conversion of the pistil into petals and a corresponding reduction in stamen size and number, 2) a conversion of stamens into petals with multiple fused pistils, or 3) solely the addition of extra whirls of petals. It is unclear as to whether these phenotypes are all based on a single mutation or multiple mutations (Cummiskey, 2002). However, regardless of the phenotype expressed, a strong environmental interaction is present where exposure to high temperatures further reduces anther production (Cummiskey, 2002). Crossing double flowered plants creates semi-double flower forms; single

flowered plants are characterized by distorted pistil development and near normal stamens. Depending on the propagation method of choice within commercial breeding programs, the mutation has been perpetuated either by vegetative or seed propagation.

Due to the modified floral morphology, development of homozygous lines with double flowers has not been achieved. In attempts to develop commercially acceptable F_1 products with modified flower forms, uniform populations have only been developed for the semi-double type. This has been accomplished by crossing the double form with the single form where progeny are 100% semi-double. In crosses between the semi-double and singles, the resulting progeny segregate approximately 50% double and 50% single (Cummiskey, 2002).

5.5 Other Traits

At this time, no other genes have been identified that are responsible for specific phenotypes in any species of *Exacum*. However, there are reports of significant genotypic effects for several horticulturally relevant traits. Genotypic variation has been reported in *E. affine* for days to flower, days to full bloom, flower diameter, and plant diameter (Rubino, 1993). In addition, a significant genotype x season interaction was identified for all four of these traits where genotype ranking varied depending on season (Rubino, 1993). These data indicate that significant variation for these traits is present and that to fully assess the heritability of these traits, genotypes should be selected only after evaluation under several environmental conditions that include production at different times of the year.

Genotypic variation has been observed in *E. affine* when plants were produced under low irradiance conditions; specific genotypes produced significantly more flower buds and mature flowers than other genotypes (Rubino, 1991; Serek and Trolle, 2000). In addition, variation has been observed for phosphorus efficiency where certain genotypes were able to produce significantly greater dry matter when grown with low phosphorous availability (Riseman, unpublished data). Finally, variation has been observed among *E. affine* lines for tolerance/resistance to both insect infestation (Riseman, unpublished data) and fungal pathogen infection (Cummiskey, 2002). These reports indicate that significant potential exists for breeding new cultivars of *E. affine* that are suitable for low input production that would include reduced energy, fertilizer and chemical inputs.

6. COMMERCIAL PRODUCT EXAMPLES

There are no *Exacum* registries or grower associations that publish cultivar lists. Seed company catalogs appear to be the only source of such information. Commercial cultivars are typically characterized by flower color and form, leaf

shape and color, branching, flowering habits and production times. As of 2002, all currently produced *E. affine* cultivars are listed in Table 22-2 along with the company of origin. The list includes all available color forms within a series as well as double and semi-double cultivars.

In addition to the *E. affine* cultivars listed, there is one confirmed interspecific hybrid currently produced. The cultivar 'Bengal Blue' is the product of a hybridization between *E. trinervium* subsp. *trinervium* and *E. macranthum* developed and produced by Sakata Seed Corporation (Suda, 2002). It is currently produced only by vegetative propagation. However, due to production problems, sales and distribution are limited to the Japanese market.

Table 22-2. Commercially available cultivars of *Exacum affine*.

Cultivar	Commercial Source(s)
Blue Champion	E.J. Small Growers
Mauve Champion	E.J. Small Growers
White Champion	E.J. Small Growers
Royal Blue	E.J. Small Growers
Royal Mauve	E.J. Small Growers
Royal Plum	E.J. Small Growers
Royal White	E.J. Small Growers
Double Royal Blue	E.J. Small Growers
Blue Grandiflora	E.J. Small Growers
Little Champ	E.J. Small Growers
Mini Persian	E.J. Small Growers
Blue Princess ®	Ex-Plant AsP
White Princess ®	Ex-Plant AsP
Blue Star ®	Ex-Plant AsP
Rose Star ®	Ex-Plant AsP
White Star ®	Ex-Plant AsP
Fuji White ®	Ex-Plant AsP
Jupiter Blue ®	Ex-Plant AsP
Royal Dane Blue ®	Ex-Plant AsP
Royal Dane Deep Blue ®	Ex-Plant AsP
Royal Dane Rose ®	Ex-Plant AsP
Royal Dane White ®	Ex-Plant AsP
Midget Blue	Sakata Seed
Midget White	Sakata Seed

7. MARKETING

Exacum affine is primarily marketed to consumers as a pot plant with sales generally through florists and greenhouses. For the grower, there is a relatively

wide range of cultivars to select from with specific cultivars best suited for production in either small (e.g. 7-10 cm) or large (e.g. 12-15 cm) pot sizes or best suited for production in certain geographic regions. In addition, there are several color series available for added diversity but with uniform production requirements.

Most commercial breeders/producers of *Exacum affine* either sell seed directly to growers, sell seed to specialist propagators who then produce plugs for sales through brokers to growers or produce and sell plugs directly to growers. These marketing options are a reflection of the individual company's strategic plan for growth as well as decisions related to resource allocation.

8. CROP IDEOTYPES

An idealized crop of *Exacum affine* would include the following attributes: 1) fast production time (approximately 8-12 weeks from seed) with minimum energy inputs, 2) precision control of flowering, 3) host-plant resistance or immunity to both insect and fungal pests, 4) good basal branching, 5) a high level fertility for parental lines, 6) a wide range of flower colors and forms, all with appealing fragrance, and 7) enhanced consumer performance for both in-house and in-garden use. Many of these attributes are already incorporated in the range of cultivars commercially available. However, if *Exacum* is to witness an expansion in production, the remaining traits need to be identified from suitable sources and incorporated into superior commercial breeding lines.

Currently, most commercially available cultivars possess an adequate level of fertility, good range of flower colors and forms, and acceptable consumer performance. The remaining traits outlined above will vary in their ease of identification and incorporation. For example, a trait that should prove relatively easy to incorporate would be enhanced home and garden performance. Variation for these traits have already been observed in advanced breeding lines and are currently being selected (Cummiskey, 2002). An example of a more difficult trait to be incorporated into commercial lines would be host-plant resistance for white-fly infestation. Variation for this trait has already been witnessed among *Exacum* populations (Riseman, unpublished data). However, whether this observation was based on antixenosis (non-preference) or antibiosis (direct deterrence) has not been established. If this trait is to be incorporated into commercial cultivars, it will need to be more precisely described and the basis clearly understood. Only after this additional research will breeders be able to create an efficient screen for the evaluation of potential donor germplasm. In addition, traits such as pest resistance can often be the result of many genes acting together to create the resistant phenotype. Polygenic traits are inherently more difficult to manipulate and control and thus, require more time and resources.

Potential germplasm sources for these desired traits would include cultivars discontinued in production, accessions maintained in botanical gardens and conservatories, wild populations of *E. affine* and related species. Because such a narrow germplasm base was used in *Exacum* domestication and development, wild populations offer the greatest chance of obtaining the desired variation. However, for traits unknown in *Exacum* (e.g. precision control of flowering), breeding strategies beyond classical techniques may need to be developed. These would include embryo rescue, for use in recovering progeny produced from wide crosses, and genetic transformation and regeneration technologies for use in directed gene transfer from unrelated organisms.

9. FUTURE BREEDING & GENETIC DIRECTIVES

Future breeding directions are highly dependent on an individual company's long term goals. European breeders/producers are typically more interested in developing new cultivars that will be suitable for rational (e.g. low input) production in terms of both energy and chemical inputs. Therefore, breeding objectives include accelerated production under 1) lower temperature and light conditions, 2) reduced fertility, and 3) reduced insecticide, fungicide and growth regulator inputs. These translate into breeding cultivars with high growth rates with low temperatures, cultivars with host plant resistance to fungal and insect pests, and dwarf forms. These are of course in addition to breeding objectives related to general horticultural traits such as improved color range, plant habit, and floriferousness. At this time, the objectives listed are intended only for incorporation into cultivars for pot plant production; outdoor performance is not a primary selection criteria for these producers.

North American and Japanese breeders/producers have traditionally been more concerned with consumer and grower preferences in relation to new cultivars. Therefore, they have concentrated on developing a wider selection of color and flower forms, fragrance and post-production performance. In the future, these companies will continue along these lines and as such, will be developing cultivars with increased flower diameters, additional flower forms represented in a full color series and enhanced consumer performance. In addition, there is a great deal of interest in developing cultivars with superior garden and landscape performance.

The domestication and development of additional species of *Exacum* is of great interest to all current *Exacum affine* breeders. The Sri Lankan species mentioned in this chapter are the taxa that are typically considered for the creation of new and distinct *Exacum* products. The two outstanding traits that these taxa offer are large flowers (flower diameters greater than 4 cm) and upright plant habits. Within this germplasm, the potential exists for the development of not only a new pot plant, but also new bedding plant forms and flower types (Riseman, unpublished data). These

Sri Lankan taxa have formed the foundation of active breeding programs both in the public and private sectors. Within each of these programs, these taxa and their resulting progeny are currently being bred and evaluated. However, development of this germplasm has been slow due to the high level of heterogeneity and heterozygosity found in these populations, significant inbreeding depression, as well as general production issues (e.g. control of the flowering response for accurate scheduling). It is anticipated that these issues will be resolved in the future and that an entirely new floriculture crop will be available for growers and consumers to enjoy.

10. CONCLUSION

The present-day *Exacum* breeder can choose among several options for germplasm enhancement in addition to traditional breeding and selection. The development of biotechnology, in relation to crop improvement, offers many options that were not available a few years ago. However, many of these techniques have not been fully developed for *Exacum* and still require additional research before full application can be realized. To date, the commercial use of *in vitro* technologies is limited to micropropagation of sterile or heterozygous individuals. However, the successful use of this technology is a critical step toward any subsequent biotechnological or genetic engineering research including gene transfer, haploid production, somatic embryogenesis, or *in vitro* selection techniques.

In any breeding program, a clear vision of the breeding objectives is of critical importance in deciding which breeding strategy should be employed. For example, the development of *Botrytis* resistant germplasm will be complex and difficult. If genetic variation for this trait is not identified in the currently available breeding stock, breeders will be forced to evaluate additional breeding options including evaluation and introgression of wild germplasm or related species into their breeding lines or the development and use biotechnological procedures.

A tremendous amount of variation is still available in *Exacum*. Although approximated 65 species of *Exacum* have been described in scientific literature, only one to three have been used to create the range of variation visible in cultivars available today. The large genetic pool still available to breeders will allow for the creation of entirely new and unique floricultural products previously unimagined. However, before this potential can be fully exploited, increased knowledge on the genetics of species within the genus is still required. In addition, more research is required on the use of modern techniques such as embryo rescue, protoplast fusion, and genetic transformation before integration and exploitation of this untapped germplasm can be fully achieved.

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Chapter 23

TULIP

Tulipa gesneriana and T. hybrids

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Abstract: Tulips are commonly associated with The Netherlands, even though they are native to Central Asia. This association began in 1594 and caused the famous 'tulipomania' in the 1600s. This vegetatively propagated crop is currently the most important bulbous geophyte in the world. Modern cultivars (predominantly *Tulipa gesneriana*) are grown for bulb production, cut flowers, flowering potted plants, and landscaping. The Netherlands and France are the primary tulip bulb producers. Continued breeding and improvement of *T. gesneriana* focus on disease resistance, improved floral longevity, and new flower shapes/colors. Interspecific hybridization is hampered by reproductive (pre- and post-pollination) and germination barriers (due to incongruity), and long generation times. Crossing barriers have been overcome with the use of techniques such as bud pollination, cut styles, grafted styles, placental pollination, and pollination of isolated ovules. Haploidization and molecular techniques are being used to create homozygous plants and conduct marker-assisted breeding, respectively.

Key words: Bulb, Geophyte, Interspecific hybridization, Liliaceae, Monocot, Mutation breeding

1. INTRODUCTION

1.1 Tulips – General Aspects

Generally, tulips are associated with The Netherlands. However, the primary gene centre of the genus *Tulipa* L. is located in the Pamir Alai and Tien Shan mountain ranges in Central Asia (Hoog, 1973). Diversification occurred from this

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region, resulting in a distribution from Morocco to Western Europe and eastward to western China. A secondary gene centre has been found in the Caucasus.

Tulips were introduced from Turkey into Europe. They flowered for the first time in The Netherlands in 1594. Around 1630, tulips were extremely popular. The highest price recorded in 1637 for one tulip bulb was fl 5200. - (€ 2360). This was about four times the yearly salary of a middle sized businessman (Dash, 1999). The introduced tulips have been grown and bred for a long time. This has resulted in a wide diversity of flowering, growth, vigour and flower shape. These tulips, whose original species have not been determined, are grouped together and are called *T. gesneriana* L. The current commercial assortment still consists mainly of cultivars from *T. gesneriana* (Fig. 23-1). The second group of cultivars, the Darwin hybrids, has been obtained from interspecific crosses between cultivars of *T. gesneriana* and genotypes of *T. fosteriana* Hoog ex W. Irving.

Tulips are grown either for (1) bulb production, (2) forcing as cut flower and potted plant and (3) landscaping. About 85% of the world bulb production is grown in The Netherlands (Le Nard and De Hertogh, 1993). Another important tulip production area is France. In 1995, the annual turnover for bulb production was about 600 million Dutch guilders and for cut flower production about 274 million Dutch guilders (Anonymous, 1996). More than 70% of the flower bulbs and cut flowers produced in The Netherlands are exported.

The tulip is the most important ornamental bulb crop in the world. The planted acreage in The Netherlands for the season 2001/2002 was about 10.700 hectares (Anonymous, 2002). The *T. gesneriana* and Darwin hybrids consist of more than 1100 cultivars (Van Scheepen, 1996). The 10 most popular cultivars, however, occupy more than 35% of the planted acreage. Only 7% (643 ha) of the total tulip area consist of species, of which *T. fosteriana*, *T. greigii* Regel and *T. kaufmanniana* Regel are the primary species.

1.2 Classification

The tulip is a monocotyledonous plant in the *Liliaceae* family. The number of species ranges from about 45 (Stork, 1984) to more than 100 (Hall, 1940, Botschantzeva, 1962). According to the taxonomic classification by Van Raamsdonk and De Vries (1992, 1995), the genus is divided into two subgenera: *Tulipa* and *Eriostemones* (Boissier). These subgenera are classified into eight sections (Table 23-1).

Figure 23-1. Some examples of the broad tulip assortment, all are *T. gesneriana* types except c and f. a. Barcelona b. Alliance c. Ad Rem (Darwin hybrid) d. China Pink e. Christmas Marvel f. Purissima (*fosteriana* hybrid); g. Mutation breeding: searching for sports a red sport of a new promising cultivar; h. Selecting a good new cultivar in a forcing experiment in the greenhouse; j. The tulip breeding fields of Plant Research International (Institute on background).

Table 23-1. The taxonomic classification of the species of the genus *Tulipa* in the sections (bold names) of the two subgenera, *Tulipa* and *Eriostemones*, according to Van Raamsdonk and De Vries (1992, 1995).

Subgenus *Tulipa*		
Tulipa	***Eichleres*** (Hall) Van Raamsdonk	***Tulipanum*** de Reboul
T. gesneriana L.	*T. ingens* Hoog	*T. agenensis* DC.
T. armena Boiss.	*T. lanata* Regel	*T. systola* Stapf
T. hungarica Borbas	*T. tubergeniana* Hoog	*T. kuschkensis* B. Fedtschenko
T. suaveolens Roth	*T. eichleri* Regel	*T. julia* C. Koch
T. didieri Jord.	*T. fosteriana* Hoog ex W. Irving	*T. aleppensis* Boiss. ex Regel
	T. greigii Regel	*T. praecox* Tenore
	T. albertii Regel	
	T. sosnovskyi Akhverdov et Mirzojeva	
	T. praestans Hoog	
	T. kaufmanniana Regel	
	T. tschimganica Bochantzeva	
	T. dubia Vvedensky	
	T. subpraestans Vvedensky	
Kolpakowskianae (Hall) Van Raamsdonk	***Clusianae*** Baker	
T. altaica Pall. Ex Sprengel	*T. clusiana* DC.	
T. lehmanniana Mercklin	*T. montana* Lindley	
T. tetraphylla Regel	*T. linifolia* Regel	
Subgenus *Eriostemones* (Boissier) Van Raamsdonk		
Australes sensu Hall	***Saxatiles*** sensu Hall	***Biflores*** sensu Hall
T. australis Link	*T. humilis* Herb.	*T. turkestanica* Regel
T. primulina Baker	*T. pulchella* Fenzl.	*T. polychroma* Stapf
T. biebersteiniana Schultes	*T. saxatilis* Sieb. ex Sprengel	*T. biflora* Pallas
T. sylvestris L.	*T. bakeri* A.D. Hall	*T. sogdiana* Bunge
T. whittalii (Dykes) A.D. Hall	*T. aucheriana* Baker	*T. neustrueva* Pob.
T. ophanidea Boiss. Ex Heldr.		*T. tarda* Stapf
T. hageri Heldr.		*T. dasystemon* Regel

2. GROWING TULIPS

Normally tulips are vegetatively propagated. It is only for breeding purposes that tulips are crossed and seeds are harvested. After sowing, seeds require a period of low temperature to induce germination and to initiate a bulb primordium (Niimi, 1978). The embryo produces one cotyledonary leaf, a primary root, and a hollow diverticulum called a 'dropper'. The bulb primordium is positioned at the tip of the dropper. As the dropper grows further into the soil a bulblet is produced at the end of the dropper (Taillandier and Riviere, 1981). This small tulip bulb requires 4 to 5 years of growth before it reaches the critical minimal size for flowering. The minimal size depends on the genotype, but typically *T. gesneriana* bulbs must reach a circumference between 6 and 8 cm. Tulips grown for bulb production, commercial forcing and landscaping are vegetatively propagated. Daughter bulbs develop from the buds which are located in the axil of the bulb scales are used for vegetative propagation. The average propagation rate of most tulip cultivars is between two and three bulbs per year (Le Nard and De Hertogh, 1993).

The tulip bulb has an annual replacement cycle, which can be divided into three phases (Le Nard and De Hertogh, 1993):

(1) Mother bulbs are planted in autumn, when soil temperature decreases. Subsequently, the roots of the mother-bulb grow rapidly until November-December. Concurrently the fully differentiated shoot elongates slowly and the daughter-bulbs exhibit a slight growth. The scales of the mother bulbs begin to senescence slowly.

(2) During early spring, when temperatures increase and after an extend period of low temperature, plant growth becomes very active. Rapid shoot and floral bud elongation occurs prior to flowering. Flowering tulips form two or more leaves after flowering. The growth rate of the daughter bulbs increases to the maximum. Concurrently the mother bulb scales shrivel and progressively disappear.

(3) At the end of the spring, the aerial organs [stem, leaves and flower(s)] of the mother bulbs senescence and growth of the daughter bulbs ceases. During this period the daughter bulbs undergo initiation and differentiation of floral and vegetative buds and root primordia. All these organs are present in the daughter bulbs by the end of summer.

A very important factor affecting the growth and development and flowering of tulip is temperature. For flower initiation (phase 3), a relatively high temperature (17-23 °C or higher) is needed. Thereafter (phase 1), a period of low temperature is required (2-9 °C). Physiological changes occur at low temperatures that are required for optimal floral stalk elongation and flower development at the subsequent higher temperatures (14-20 °C) (phase 2). For commercial flower and potted plant production, flowering is controlled by simulating the temperature conditions required in nature (this called forcing). The cold period can be given partially by storing the bulbs in temperature controlled and highly ventilated rooms, prior to planting the bulbs. This called "precooling". The optimal length of the total cold

treatment varies with the genotype. Le Nard and De Hertogh (1993) have published a review of the physiology of tulips.

3. SEXUAL REPRODUCTION

Normally each tulip flower has one pistil and six anthers. There are variations in semi- and full-double cultivars. The pistil consists of a stigma, a short style and an ovary. The stigma and short style comprise about 20% of the total pistil length. The ovary has three carpels, each containing two rows of ovules. One ovary contains between 150 ovules *(T. turkestanica*) to 210-270 ovules (most species) to 300-450 ovules (*T. gesneriana*).

A central bundle of pollen tubes develops in the ovarian cavity after compatible pollination. Pollen tubes bend sideways and grow towards and into the ovules. At 15˚C, they reach the first ovules at 1-3 days after pollination. This temperature is optimal for most tulip crosses (Kho and Baër, 1971). The lowest ovules are reached about 7-11 days after pollination. The number of ovules penetrated by a pollen tube increases from 3 to 9 days after pollination. On average 68%-83% of the ovules is ultimately penetrated by a pollen tube (Van Creij et al., 1997a). In general, fertilization takes place after penetration of the ovule by a pollen tube. However, Van Creij et al. (1997b) observed no fertilization in several percent of the ovules with pollen tube penetration. Pecenicyn (1972) has described the fertilization process of several *Tulipa* species.

In tulips, the development from zygote to mature embryo follows a different pathway than found in most monocot angiosperms. The zygote of most species divides transversely resulting in an apical cell, which gives rise directly to the embryo, and a basal cell, which divides to form the suspensor. In tulips, the basal cell develops into a proembryonal cell complex and the apical cell gives rise to a part of this cell complex, the suspensor, and the embryo (Ernst, 1901, Haccius and Hausner, 1972, Wafai and Koul, 1982).

The first division of the zygote results in the formation of the basal cell, which is taller and contains more plasma than the apical cell. Haccius and Hausner (1972) observed in *T. tarda* that cell multiplication proceeded from the base to the apex. The developmental sequence of cell division is precisely ordered in *T. tarda*, while it is more variable in *T. altaica* (former name *T. kolpakowskiana* (Van Raamsdonk and De Vries, 1995). Irregularities in division order are frequently found in *T. gesneriana*. These first divisions result in the formation of an irregularly segmented cell complex, the so-called proembryonal cell mass (Van Creij et al. 1997b). Van Creij et al. (1997b) observed ovules containing a proembryonal cell mass starting three weeks following pollination at 15°C. The suspensor is formed at the chalazal side of the proembryonal cell mass starting about 3 to 6 weeks after pollination. The globular embryo develops on top of the suspensor from mostly six weeks after

pollination. In this stage, the suspensor and embryo are surrounded by endosperm. The suspensor degenerates at the advanced globular embryo stage. The globular embryo will elongate in length and develop into a spindle-shaped embryo. Most spindle-shaped embryos are found beginning nine weeks after pollination. Subsequently, the embryo sac is almost completely filled with endosperm at the spindle-shaped embryo stage. The endosperm around the embryo is digested and, ultimately, the embryo is positioned in a cavity filled with fluid. Mature seeds can be harvested about 12 weeks after pollination (Van Creij et al., 1997b).

Aberrations in embryo development were found in compatible crosses within *T. gesneriana* and within *T. fosteriana* (Sayama et al., 1982, Van Creij et al., 1997b). Sayama et al. (1982) observed seeds with endosperm but without embryo in both compatible crosses. Van Creij et al. (1997b) found the percentage of ovules with developing embryos in compatible *T. gesneriana* crosses varying between 16% and 50%. Aberrations in embryo and/or endosperm development were found in about 5% of the ovules with embryos. Most of these ovules exhibited abnormal endosperm (4%) and the majority also had a deformed embryo (3%). The development from zygote to spindle-shaped embryo was retarded in several ovules. This resulted in the appearance of ovules containing a proembryonal cell mass or a globular embryo at the stage seed pods are harvested.

4. BREEDING

4.1 Improvement of Tulips

The production of flowers and bulbs of an optimal quality can be seriously affected by many pathogens. Therefore, disease resistances are important breeding objectives. Introduction of genes for resistance should improve the commercial assortment of tulips. In addition the use of pesticides required should be significantly decreased. Beside the introduction of disease resistance, a shorter cold requirement and forcing period, an improved flower longevity, and new flower shapes and flower colours are important goals for tulip breeding.

Most tulip breeders focus on breeding within *T. gesneriana.* Parents are chosen on basis of forcing qualities, flower colour, flower form and disease resistances. Each year several thousands flowers are hand pollinated resulting in several ten thousands seedlings. After growing the seeds till adult plants, most breeders select around 0.1% - 1% of the best plants (plant habit, colour etc.) during forcing as cutflower in the greenhouses. After vegetative propagation further selection takes place for other characters as disease resistance, bulb production etc.

A very important method for tulip improvement is the exploitation of the genetic variation of other tulip species through interspecific hybridization (see 4.4).

Crossing barriers, however, have prevented the formation of hybrids in many interspecific tulip crosses. *T. gesneriana* has been crossed successfully with only 12 out of the approximately 55 tulip species by using conventional breeding methods (Van Eijk et al., 1991, Van Raamsdonk et al., 1995). Crossing barriers impeding sexual reproduction in interspecific tulip crosses are due to incongruity between the crossed species (Hogenboom, 1973).

The rapid introduction of new cultivars enriched with desirable traits for the tulip production is also hampered by several other factors. The main hindrance is the long period required for the development of a new cultivar. After crossing, 5 to 6 years are needed to obtain a flowering bulb. Subsequently, it takes another 10 to 20 years to screen the tulips for desirable characters and to propagate the bulbs for commercial release. The production of large numbers of bulbs needed for the introduction of a new cultivar could be enhanced if the multiplication rate could be increased. Despite many efforts to develop a rapid multiplication system *in vitro* (Baker et al., 1990, Hulscher and Krijgsheld, 1995, Chanteloube et al., 1995, Kuijpers and Langens-Gerrits, 1997), the production of tulip bulbs still occurs primarily by propagation in fields.

4.2 Disease Resistance

Tulips can be affected by several diseases e.g. bulb-rot, fire, and viral diseases. Host resistance is the best approach to prevent such diseases. The most important pathogens are *Fusarium oxysporum* (bulb-rot), *Botrytis tulipae* and Tulip Breaking Virus (TBV). Also other fungi (*Pythium* spp., *Rhizoctonia tuliparum*/*solani*), viruses (Tobacco Necrosis Virus (TNV) and Tobacco Rattle Virus (TRV)), mites and nematodes (*Trichodoridae*, *Pratylenchus penetrans* and *Ditylenchus dipsaci*) can cause economic losses. The use of resistant cultivars reduces the use of chemical control, increases bulb production, and requires less labor for sorting and selecting harvested bulbs. Resistant cultivars are also important for bulb exports. Bulbs infected with *Fusarium* cause extra efforts to clean the stock and give rise to complaints from consumers.

Breeding research for *Fusarium* resistance was carried out by Van Eijk and co-workers (Van Eijk et al., 1983, Romanov et al., 1991). They produced reliable screening tests not only for clones but also for juvenile seedlings at the pre-selection stage. In these tests, bulbs are planted in *Fusarium*-infested soil and grown under standardized conditions for the entire season. After harvest, bulbs are examined for *Fusarium* infection. Almost absolute resistance was found within the *T. gesneriana* assortment. Since seedling selection produces some susceptible plants (escapes), selected plants have to be re-tested at the clonal level. The inheritance of *Fusarium* resistance was investigated and it was found that the use of one resistant parent can result in resistant descendants.

Research on tulip breaking virus (TBV) (Romanov et al., 1991, Straathof et al., 1997, Eikelboom et al., 1992) resulted in reliable screening tests at the clonal and seedling level. Viruliferous aphids inoculate leaves of flowering plants. Flower breaking was observed one year after inoculation. Absolute TBV resistance was found in several *T. fosteriana* cultivars, e.g. 'Cantata' and 'Princeps'. In crosses between *T. gesneriana* x *T. fosteriana* highly resistant genotypes were found. These crosses, also called Darwin hybrids, are mostly triploid and suffer from F1-sterility. Artificial doubling of the chromosome could solve this problem in the future (Van Tuyl, 1996, Van Tuyl and De Jeu, 1997).

An assay for screening tulips for resistance to *Botrytis tulipae* (Straathof et al., 2002) has been developed. Absolute resistance was found in *T. tarda.* However, this species can not be crossed with the assortment of *T. gesneriana*. In some cultivars of *T. gesneriana* and *T. kaufmanniana* partial resistance was found. At Plant Research International (PRI) breeding for *Botrytis* resistance is in progress.

For the future, resistance to the three main diseases of tulip must be combined in order to have multi-resistant tulips. PRI has been conducting research to accomplish that task in co-operation with a group of tulip growers.

4.3 Flower Longevity

Flower longevity, the life of a cut flower or potted plant is one of the most important characteristics for the consumer. Research has shown that large genetic variation is available in the cultivar assortment (Van Eijk and Eikelboom, 1976, Van der Meulen and Van Oeveren, 1993, Van der Meulen et al., 1997). They developed screening tests in which the vase life of a genotype could be estimated. A good correlation was found between the longevity of the flower still attached to the bulb and the vase life of the flower (Van Eijk and Eikelboom, 1976). The variation in flower longevity among cultivars varied from 8 to 16 days, when evaluated at 14°C. Studying segregating populations derived from these cultivars, the flower longevity varied from 6 to 22 days (Van der Meulen et al., 1997). This means that selection for longer flower longevity, based on additive effects of several genes, is a promising method.

4.4 Methods for Overcoming Crossing Barriers After Interspecific Hybridization

A wide range of techniques has been developed to bypass crossing barriers in many crops. Manipulation of the fertilization process is rather difficult. Most techniques focus, therefore, on bypassing crossing barriers prior to fertilization or post-fertilization.

Pre-fertilization barriers have been bypassed in several different interspecific crosses after bud-pollination (Sink et al., 1978), the use of the cut-style method or the grafted-style method (Van Tuyl et al., 1991, Wietsma et al., 1994), placental

pollination (Zenkteler, 1990, Sink et al., 1978) and pollination of isolated ovules (Stewart, 1981). In tulip, the cut-style method and placental pollination have been studied (Van Creij et al., 2000a). Following the cut-style method, the style is cut above the ovary and subsequent pollinated at the cut surface of the remaining portion of the style. The percentage of ovules with pollen tube penetration did not increase in crosses between *T. gesneriana* and five other *Tulipa* species after the application of the cut-style method. For placental pollination, ovaries were cut longitudinally into six sectors and placed *in vitro*. Each sector contained a placenta with a row of ovules and the ovary wall. Pollen was applied on the placenta. Pollen tube penetration percentages were not increased after placental pollination compared to stigmatic pollination. However, after placental pollination, most of the ovules with pollen tube penetration showed subsequent embryo germination.

Methods for bypassing post-fertilization barriers focus on the survival of hybrid embryos and on restoring the fertility of F_1-hybrids. Embryo culture, ovule culture, ovary-slice culture and ovary culture have been developed to enable hybrid embryos to survive *in vitro* (for reviews see Williams et al., 1987, Sharma et al., 1996). The application of embryo rescue techniques in tulip breeding has been reported by Van Tuyl et al. (1990), Custers et al. (1992, 1995) and Van Creij et al. (1999, 2000b). More embryos could be rescued from an earlier developmental stage (4 wk. post-pollination) with ovule culture, as compared to embryo culture. Also, more embryos could be rescued at each culture date with ovule culture in comparison with embryo culture (Custers et al., 1995). The efficiency of direct ovule culture and ovary-slice culture followed by ovule culture has studied by Van Creij et al. (1999). For ovary-slice culture, ovaries were cut transversely in eight sections and placed on medium. The percentage of germinating embryos increased, in most cases, significantly with a more advanced developmental stage of the embryos at the start of the culture. The results of ovary-slice culture, started at various dates after pollination, were comparable to or better than the results of direct ovule culture. By using ovary-slice culture and/or ovule culture, unique hybrids have been obtained from the crosses *T. gesneriana* x *T. agenensis* and *T. gesneriana* x *T. praestans* (Van Creij et al., 1999*)*.

Post-fertilization barriers may cause sterility of F_1-hybrids. Sterility of F_1-hybrids can be caused by the lack of chromosome pairing during meiosis. In many crops, chromosome doubling has restored fertility. Recently, tetraploid tulip cultivars have been produced after treating tulip stems from bulbs *in vitro* with oryzalin or colchicine (Eikelboom et al., 2001, Van Tuyl et al., 2002).

Various techniques have in most cases to be applied for the production of viable hybrid plants of a specific cross. When prefertilization barriers hinder interspecific hybridization, they must be bypassed. However, once prefertilization barriers are bypassed, embryo rescue techniques must often be used to save the hybrid embryos from a premature death. Finally, sterility of the F_1-hybrids must often be overcome.

In vitro pollination offers the prospects to perform an integrated system of pollination, fertilization and embryo-rescue techniques under optimal environmental

conditions. Van Creij (1997) describes a procedure for *in vitro* pollination of tulip, using compatible intraspecific *Tulipa gesneriana* crosses as model system. Application of *in vitro* pollination offers good prospects for tulip breeding. Pollination methods, such as intra-ovarian pollination, which might bypass prefertilization barriers can be developed. Interspecific crosses showing post-fertilization barriers can be made *in vitro*, or ovary culture can be started at early culture dates. The number of embryos that germinated was doubled after *in vitro* pollination compared to the application of ovary-slice culture followed by ovule culture started 3 weeks after pollination. The bulblets obtained *in vitro* can be used for polyploidization treatments *in vitro*.

4.5 Interspecific Hybridization

Many crosses between *T. gesneriana* and other tulip species have been carried out to enrich the commercial assortment with desirable traits from these species. However, incongruity barriers impede sexual reproduction in many interspecific tulip crosses either in whole or in part. These barriers can prevent or diminish the formation of viable seeds prior to fertilization (pre-fertilization barriers), during fertilization, or post-fertilization. Liedl and Anderson (1993) published a review concerning reproductive barriers.

Crosses between cultivars from *T. gesneriana* and species from all eight sections of the genus *Tulipa* have been carried out by Van Eijk et al. (1991) and Van Raamsdonk et al. (1995). Pre-fertilization barriers and post-fertilization barriers have been studied in interspecific tulip crosses, with *T. gesneriana* as one of the parents (Van Creij et al., 1997a, 1997b). Beside cultivars of *T. gesneriana*, the commercial assortment consists of the Darwin hybrids (crosses between *T. gesneriana* and *T. fosteriana*), which are mostly triploid. The use of triploid cultivars for further breeding is impossible due to F_1 –sterility. In contrast, *T. gesneriana* was found to be compatible with other species of the same section (*Tulipa*). Analysis of pollen tube growth in the pistil and pollen tube penetration in the ovules in reciprocal crosses between *T. gesneriana* and *T. didieri* exhibited pollen tube growth percentages comparable to intraspecific *T. gesneriana* crosses (Van Creij et al. 1997a). Van Raamsdonk et al. (1995), however, found that fewer hybrid F_1 bulbs were produced in crosses with *T. didieri* as pistillate parent when compared to crosses with *T. didieri* as pollen donor. Apparently, post-fertilization barriers diminish the number of viable F_1 bulbs obtained from the cross *T. didieri* x *T. gesneriana*.

Hybrids were produced in several crosses between *T. gesneriana* and representatives of the section *Eichleres* (Van Eijk et al., 1991, Van Raamsdonk et al., 1995). Crosses between *T. gesneriana* (pistillate parent) and *T. kaufmanniana* and *T. fosteriana* exhibited high pollen tube penetration percentages (Van Creij et al., 1997a). Small numbers of seeds were obtained from these crosses (Van

Raamsdonk et al., 1995). Seed production has never been reported for the cross *T. gesneriana* x *T. praestans*, despite relatively high percentages of ovules with pollen tube penetration (Van Creij et al., 1997b). Apparently, post-fertilization barriers hinder or prevent the formation of viable seeds in these crosses. Custers et al. (1995) studied the ovule content of swollen ovules of the cross *T. gesneriana* x *T. kaufmanniana*, six weeks after pollination. About half of the swollen ovules contained embryos that ceased to grow. Others showed remnants of embryo tissue or were empty. Embryos continued to grow in only 10-25% of the swollen ovules. However, the sizes of these embryos were highly variable and most of them remained smaller than control embryos. Most of these ovules did not produce germinating seeds. This confirms the presence of post-fertilization barriers. Either no or a low number of ovules were penetrated by a pollen tube after pollination of pistils of *T. fosteriana*, *T. praestans* or *T. kaufmanniana* with pollen from *T. gesneriana* (Van Creij et al., 1997a). This indicates the occurrence of prefertilization barriers.

Crosses between *T. gesneriana* and species from the section *Tulipanum* did not produce verified hybrids, except for *T. systola* Stapf (=*T. stapfii* Turrill; Van Raamsdonk and De Vries, 1995). Relatively high percentages of pollen tube penetration were found in the cross *T. gesneriana* x *T. agenensis*. Embryogenesis has been studied in this cross and compared with embryogenesis in a compatible *T. gesneriana* cross. Fewer embryos were formed from the cross *T. gesneriana* x *T. agenensis* when compared to the compatible cross. Embryogenesis was also retarded. The first globular embryos and spindle shaped embryos were found at later dates and the relatively lower number of spindle shaped embryos in mature seeds had a shorter average length than in the compatible cross. This retarded development, in combination with the higher percentages of ovules with aberrations in development from 4.5 weeks after pollination, mainly in endosperm formation, reveal that post-fertilization barriers occur at this level. Pre-fertilization barriers prevented pollen tube penetration in the ovules in the reciprocal cross. This resulted in pollen tube penetration percentages lower than 1% (Van Creij et al., 1997b).

Crosses between *T. gesneriana* and species from the sections *Kolpakowskianae* and *Clusianae* and from the subgenus *Eriostemones* have never been successful (Van Eijk et al., 1991, Van Raamsdonk et al., 1995). Pre-fertilization barriers prevented normal pollen tube growth in crosses between *T. gesneriana* as the pistillate parent and *T. altaica*, *T. clusiana, T. sylvestris*, *T. pulchella* and *T. turkestanica*. Pollen tubes had reached no more than 7% of the ovules in some flowers of these crosses. However, pollen tube penetration percentages up to 31% (*T. pulchella*) or 87% (*T. clusiana*) were found in a low number of flowers of the reciprocal crosses (Van Creij et al., 1997a).

Crosses between several other tulip species were also carried out to investigate the possibility of using these species hybrids in bridge crosses. Species of the same section can often be crossed (Van Raamsdonk et al., 1995). The crosses exploited

between species of the section *Eichleres* and the section *Tulipanum* did not succeed. F_1 bulblets were produced from crosses between species of the section *Eichleres* and *Clusianae*. They were, however, never verified on hybrid origin. Crosses made between *T. systola* (section *Tulipanum*) and some species of the section *Eichleres* were also not successful (Van Raamsdonk et al., 1995). Nevertheless, *T. systola* might be used in bridge crosses between species of the section *Tulipanum* and *T. gesneriana*, The species of the section *Eichleres,* which can be crossed with *T. gesneriana,* can only be used in bridge crosses with species of the same section.

Several factors proved to influence the rate of pollen tube growth and pollen tube penetration and the number of seeds obtained in tulip crosses. Reciprocal differences were found in the numbers of ovules with pollen tube penetration. Van Raamsdonk et al. (1995) found reciprocal differences in seed set on the plant. The maternal genotype affected the percentages with pollen tube penetration (Van Creij et al., 1997a). They also found the percentages of ovules with pollen tube penetration differed largely between the different flowers of the specific crosses and between both years. Cultivar-effects, effects of accessions, and year-effects were found to influence seed set in interspecific tulip crosses (Van Eijk et al., 1991, Custers et al., 1995).

4.6 Mutation Breeding

Tulip is a vegetatively propagated crop and during cultivation hundreds of natural mutants (sports) were selected (Van Scheepen, 1996). Mutations can exhibit difference in flower (edge) colour or flower shape (parrots, fringed and double). Differences exist in the mutation sensitivity of cultivars. Many mutants are known from specific cultivars, e.g. ‘Bartigon’, ‘William Copland’, ‘Murillo’ and ‘Apeldoorn’.

In order to produce mutations artificially, the possibilities of Röntgen (X-rays) for mutation breeding in tulips have been investigated (Broertjes and Alkema, 1970, Van Harten and Broertjes, 1989). Mother bulbs, as well as daughter bulbs, can be used for mutation induction. Also the dosage required varies from 350 to 550 rad. The radiation can be applied early in the planting season (August) or late (November). Besides mutations in flower colour and flower shape, mutations also occur in the colour of the leaf edge, in plant height and in bulb production. In the 1970’s PRI released several radiation mutants of ‘Preludium’ and ‘Lustige Witwe’. Private breeders are still using mutation breeding techniques (Straathof and Eikelboom, 1997).

5. PLOIDIZATION TECHNIQUES

5.1 Polyploidization

The number of chromosomes of many cultivars and *Tulipa* species have been investigated (Zeilinga and Schouten, 1968a). The assortment consists mainly of diploids (two sets of 12 chromosomes; $2n = 2x = 24$), some triploids (mainly Darwin hybrids) and a rare tetraploid ($2n = 4x = 48$). The production of tetraploids using laughing gas was described by Zeilinga and Schouten (1968b). By placing plants, which were pollinated a week previously, for one day in a cylinder with laughing gas (N_2O) and 5 to 6 atmospheric pressure, tetraploid seedlings were obtained. To improve the fertility of these tetraploids, they were crossed mutually. Highly fertile tetraploid cultivars of *T. gesneriana* and tetraploids of *T. fosteriana* and *T. kaufmanniana* were released to Dutch tulip companies in 1989 and 1991 (Straathof and Eikelboom, 1997).

Recently, using *in vitro* polyploidization techniques, similar as in *Lilium*, tetraploid tulip cultivars have been produced (Eikelboom et al., 2001, Van Tuyl et al., 2002). For future tulip breeding, this technique will undoubtedly have an important role in overcoming interspecific crossing barriers.

5.2 Haploidization Using Microspore Culture

The production of *in vitro* haploid plants using micropore culture is a technique used in many plant species in order to produce homozygous plants. Research showed that tulip was more promising to develop via this technique these plants than lily (Van den Bulk et al., 1992, Van den Bulk and Van Tuyl, 1997). Embryo-like structures from young pollen (microspores) were obtained in tissue culture. However, due to the difficulties with the *in vitro* propagation of tulips, this research was completed without obtaining homozygous doubled haploids.

6. MOLECULAR BREEDING METHODS

6.1 Transformation

Wilmink and co-worker (Wilmink et al., 1992, 1995), have investigated genetic modification of tulips. Using a Particle Delivery System, transient expression of the reporter gene for beta-glucuronidase was demonstrated. It was shown that the CAMV 35S as well as the TR2' promoter were active in flower stem explants. Regeneration and bulb formation of tulip *in vitro* is, however, extremely difficult and it takes many years for a flowering tulip will be obtained. Seven years after selecting

Gus-positive and PPT-resistant explants only one complete transgenic tulip plant flowered. It proved, however, that stable integration was established.

6.2 Marker-Assisted Breeding

Tulip and the long juvenile period is highly suitable for using molecular marker techniques and this makes pre-selection possible. Marker-assisted breeding has been investigated using AFLP-markers in lily and tulip (Van Heusden at al., 2002). AFLP-markers were found over the entire tulip genome (12 linkage groups). Several AFLP-markers were linked with TBV-resistance (Van Heusden, pers. comm.). In the future, the application of PCR-derived markers for various characters will speed up the breeding process of tulips.

7. FUTURE PROSPECTS

Tulip is a crop with a long juvenile period. It takes at least 25 years to develop a new cultivar after carrying out a cross. When disease resistance tests are performed, this will take even longer. To reduce the use of pesticides, restricted by many governments, disease resistance will be required in the future. Currently, *in vitro* propagation is not an economical method to speed up this process. Therefore, for the future, the application of molecular assisted breeding techniques can be an important tool for reaching the goal of developing a tulip assortment, which can be grown without large disease problems.

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CUT FLOWERS

Chapter 24

LISIANTHUS

Eustoma grandiflorum

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Abstract: Lisianthus, a relatively new floral crop to the international market, quickly ranked in the top ten cut flowers worldwide due to its rose-like flowers, excellent post-harvest life, and blue flowers. It is also widely used as a flowering potted and bedding plant. In addition to blue, a wide range of flower colors are available, as well as floral patterns (picotee, etc.). Lisianthus breeding efforts have focused on F_1 hybrid seed production, uniform flowering throughout the year, lack of rosetting, heat tolerance, flower color, flower size and form, double flowers, disease resistance, and interspecific hybridization. Research on flowering demonstrated that cultivar, temperature, and day length during the seedling stage all affect rosetting. Several traits are bred to differ for product classes, such as inflorescence architecture (flat-topped for cut flowers, tiered or spray for potted/bedding plants). While the crop is self-compatible, it is subject to inbreeding depression. Molecular biology is being used to engineer lisianthus with altered flower colors, modified flowering times, and fragrance. Future classical and molecular breeding will focus on disease resistance (particularly *Fusarium*), day neutrality, heat-tolerance, flowering earliness, and post-transplant survival.

Key words: *Eustoma exaltatum,* Interspecific hybridization, Molecular biology, Rosetting, Soil pH.

1. INTRODUCTION

The plant was commonly known as *Lisianthus russellianus* when first listed in seed catalogues in the early 1980's in the United States. It was only a short time before its scientific name was recognized to be *Eustoma grandiflorum* (Raf.) Shinn. (synonyms *Eustoma andrewsii*; *E. russellianum*; *Lisianthus russellianus*) (Bailey and Baily, 1976; Everett, 1981). Since growers had grown accustomed to the using

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the genus name *"Lisianthus*", the common name for *Eustoma grandiflorum* among growers and the general public today is still lisianthus.

Lisianthus is a relatively new cut flower crop when compared with established floricultural crops such as cut roses, carnations, or chrysanthemums that have been grown as commercial cut flowers in the United States since the mid-1800's. Yet, lisianthus has made a tremendous impact on cut flower markets. It was first available in Japan in 1933 and is now the number one cut flower with over 129 million stems sold in 2001. In Europe, it is ranked as one of the top 10 cut flowers with over 122 million stems sold in 2001. In the United States, their popularity continues to grow not only as a cut flower (over 14 million stems sold in 2002) but also as a bedding plant and a pot plant. Their success story has not been matched for decades by any flower crop, and may not be matched for years to come. That is, it is very rare for a new floriculture crop to climb from a virtually unknown flower to being ranked as one of the top ten cut flowers in a 20-30 year period. It is truly remarkable that a new cut flower would replace an industry standard to become the number one flower marketed. One might compare the development of lisianthus, as a cut flower, to the rapid development of impatiens as the number one bedding plant.

1.1 Origin

Sakata Seed Company offered lisianthus as a new cut flower in the United States in the early 1980's (Sakata, 1982). For some, especially those of us who originate in the mid-west region of the United States, lisianthus was not a "new" flower at all. Growers in the United States soon discovered that this was not a Japanese flower but rather a native to the U.S. (Rickett, 1966). Its natural habitat is the prairies of the plain states. It has been observed in the northern part of Mexico, Texas, Oklahoma, Kansas, Nebraska, Colorado, Wyoming, and South Dakota (Shinner, 1957; Wood and Weaver, 1982). It had common names of Texas Blue Bell, Prairie Rose, and Prairie Gentian. Park Seed Company carried lisianthus in its catalogues as early as 1887 (Parke, 1986). However, all would agree when the Sakata Seed Company re-introduced lisianthus as F_1 hybrids in its catalogues in the early 1980's that lisianthus had a new and improved look (Roh and Lawson, 1984). The selection and breeding improvements were tremendous. Japanese breeders should take great pride in the advancements they accomplished with 50 years of breeding before re-introducing lisianthus. Further improvements that have been accomplished since their introduction have been equally impressive.

1.2 Acceptance in the United States

In the United States, the appearance of lisianthus was met with excitement and confusion. Growers and consumers alike were anxious to try lisianthus because plants had eloquent and beautiful flowers, the flowers had a long vase life, and there

were few "blue" flowers to be found in the cut flower market. However, the first hybrids were not uniform in flowering or vegetative characteristics, and some speculated that these were not true F_1 hybrids (Roh et al., 1989). Production of high quality cut flower stems was difficult and sporadic. This resulted in differing opinions for acceptance of this crop, with some growers working hard to learn how to profitably produce this crop while others rejected it.

A lot of the initial research on lisianthus was done in Japan and articles were written in Japanese, which limited their use for growers in the U.S. For example, an excellent book on the breeding and culture of lisianthus, edited by Ohkawa (1992), contained invaluable information that would have benefited U.S. growers, but its impact was diminished due to language barriers.

In addition to use as a cut flower, some saw the potential of lisianthus as a bedding or potted plant (Grueber et al., 1985; Halevy and Kofranek, 1984; Roh and Lawson, 1987; Tjia and Sheehan, 1984). Disney World displayed lisianthus in its gardens in 1983 and they received much attention (Parke, 1986). Lisianthus are still widely used in their landscapes today. Thus, it soon became evident that lisianthus could become one of the few floricultural crops that could be used as a cut flower, potted plant, and bedding plant.

1.3 Breeding Efforts

Lisianthus seeds were available commercially in Japan as early as 1933 (Azrak, 1984; Ohkawa, 1994). Commercial cut flower growers in Japan did most of the early breeding, and it was not until approximately 1977 that Sakata Seed Company began breeding F_1 hybrids. In addition to private and commercial breeders from Japan, breeders from around the world have joined the effort to improve lisianthus. Charles Weddle of Weddle Native Plants, Palisade, Colorado, was perhaps the first breeder in the United States to formally release F_1 hybrids for cut flowers, and in cooperation with Colorado State University, the hybrid 'Colorado Blue Bell' was released in 1984 (Azrak, 1984).

Initially, most of the breeding efforts in the United States were for pot or bedding plant use. In the early 1990's, three dwarf lisianthus cultivars were released from three different private- and public-sector breeding programs: 'Blue Lisa', PanAmerican Seed Co., West Chicago, Illinois; 'Little Belle Blue', U.S. Dept. of Agr., Agr. Res. Serv., Beltsville, Maryland; and 'Mermaid Blue', Sakata Seed America, Morgon Hill California, (Rubino, 1992). Claude Hope, a world-renowned breeder working in Costa Rica, did the original breeding for the dwarf cultivar 'Blue Lisa' (Aylsworth, 1977). After his death, breeding efforts were continued at PanAmerican Seed Company in its California and Chicago facilities for both pot and cut flower lisianthus. A breeding program was initiated in 1985 at the University of Florida's Gulf Coast Research and Education Center, Bradenton, Florida. The emphasis was on heat-tolerance and basal branching pot and bedding plant types.

'Maurine Blue' (Harbaugh and Scott, 1996) and 'Florida Blue' (Harbaugh et al, 1996) were the first heat-tolerant lisianthus whose seedlings could be grown at 28-31C (82-88F) without rosetting (formation of a basal cluster of leaves with no flowering stems). Recently Goldsmith Seeds, Inc., Gilroy, California, also has released potted and cut-lisianthus.

The author is aware of active breeding efforts in Taiwan, India, and Europe as well as Japan and the United States for both pot and cut flower lisianthus. Indeed, it would be safe to say that breeding efforts are on a worldwide basis at this time.

1.4 Improvements in Culture and Management Practices

Even with all the accolades, the initial enthusiasm for lisianthus as a new flower crop soon turned to frustration for growers. As is common with most new crops, production information lagged behind the desire to economically cultivate the crop. Cultivars lacked uniformity in plant height, flower number, and time of production. Growers who had initial success in flowering lisianthus in the spring (May to June flowering) soon found that many of the plants would rosette when seeds were sown at other times of the year. They also discovered that the length of time from sowing to flowering increased from as short as only four months to 8 months or longer. Many growers became so frustrated with production problems and crop losses that they decided not to grow lisianthus.

At the same time that breeders were working to improve uniformity and other horticultural characteristics, the demand and interest in lisianthus stimulated researchers around the world to investigate production problems. As a result, improved cultural practices have been developed that have aided growers to produce more uniform crops of lisianthus. While still not considered an "easy" crop to produce, the new cultivars and production knowledge have allowed growers to produce lisianthus profitably (Katz, 1997; Newman, 2000). A summary is presented below of some of the important cultural stumbling blocks, their causes, and some solutions.

1.4.1 Factors Influencing Rosetting and Flowering

Many researchers have studied the physiology of flowering of lisianthus in order to determine the cause of rosetting. The results of these research projects have shown that cultivar, temperature, and day length during the seedling stage all affect rosetting, and these factors have interactive effects (Table 24-1). Most commercial lisianthus cultivars have a tendency to rosette when seedlings are exposed to temperatures $\geq$ 25C (77F) (Harbaugh et al., 1992; Ohkawa et al., 1991). However, cultivars differ significantly in their sensitivity to high temperatures (Fukuda et al., 1994; Harbaugh et al., 1992; Li et al., 2002).

Table 24-1. The effect of lisianthus cultivar, temperature, and day length during the seedling stage (14 - 43 days after sowing) on the percentage (%) rosetted plants.

Daylength	Temp.	'Yodel White'	'Lisa Pink'	'Maurine Blue'
SD (12 hr)	28C	100	42	0
LD (18 hr)	28C	42	0	0
SD (12 hr)	12C	0	0	0
LD (18 hr)	12C	0	0	0

Seedlings typically bolt after they develop three to four true leaf pairs if temperatures are ideal. However, lisianthus is sensitive to high temperatures from the time seeds absorb water during germination through the development of three to four leaf pairs. The general rule is that the earlier and longer seedlings are exposed to heat stress, and the higher the temperature to which seedlings are exposed, the greater the percentage of plants that will rosette.

Reports on the effect of photoperiod on flowering have not always been in agreement. Halevy and Kofranek (1984) reported that pink, blue, and white color variants flowered even under an 8 hr photoperiod, and they considered lisianthus a day-neutral plant. Similarly, Azrak (1984) described lisianthus as natural day plants that flowered after they reached a certain age or size. Other researchers considered lisianthus a facultative long-day plant (Grueber et al., 1984; Roh and Lawson, 1984; Roh et al., 1989). Additional research has helped explain the discrepancies. More plants rosetted under high temperature (28C, 82F) when seedlings were grown under short days compared to long days (Harbaugh et al., 1992). In addition, once seedlings bolted, flowering was influenced by photoperiod x temperature interactions, and the response was cultivar dependant (Harbaugh, 1995). That is, some cultivars bolted after receiving a cold temperature treatment, but when grown under short days, flower buds aborted or some plants reverted to a rosette growth habit (i.e., the main stem elongated with several internodes forming and then internode elongation ceased as additional leaves developed). In this test, 'Yodel White' would be classified as a facultative long day plant. A breeding line, 'GCREC-Blue', flowered as a day-neutral plant when seedlings were grown at 28C (82F), but when seedling were grown at 12C (54F), more plants flowered normally under long-days than short-days. These studies led breeding programs to select for day neutral and heat-tolerant plants.

Lisianthus has been described as both an annual and biennial (Farina, 1989; Halevy and Kofranek, 1984; Wilkins and Grueber, 1983), and an understanding of the effect of temperature on seedlings helps explain this. In its native environment, if seeds germinated during summer, seedlings exposed to high temperatures would rosette and survive the winter in the rosette stage. Plants would flower the following year, and thus behave as biennials. If seeds germinated in the spring and seedlings developed under cool spring conditions, plants would flower in the same year. Under these conditions, they would be classified as an annual.

In order to have cut flowers year round, researchers are developing systems to take advantage of the knowledge gained on the effect of low temperatures in the seed development stage, and to reverse heat induced rosetting. Bolting increased when 'Fukukaen' seedlings were grown from seeds ripened on parent plants at 23 C/18C day/night (73F/64F) compared to seeds ripened at 33C/28C (91F/82F) (Ohkawa et al., 1993). Pergola et al. (1992) reported that cold treatment of imbibed seeds at 3C (37F) for 4 weeks increased bolting of an unspecified cultivar. Similarly, cold treatment of imbibed seeds at 10C (50F) for 5 weeks resulted in 100% bolting of 'Fukushihai' and 53% bolting with 'Miyakomomo' seedlings subsequently grown at 33C/18C (91F/64F) (Ohkawa et al., 1993). 'Lilac' seedlings grown for ≥3 weeks below 18C (64F) bolted rapidly when subsequently grown at >22C (72F) (Pergola, 1992). Heat-induced rosetting of 'Fukushihai' seedlings was reversed by low-temperature treatments of 15C (59F) for 4 weeks (Ohkawa et al., 1994). Thus, some plug producers are growing plugs in their colder production areas and shipping older plugs to southern locations. Others are considering vernalization of seedlings with cold temperatures as a technique to ensure uniform flowering. And some growers, choosing suitable cultivars, are actually inducing rosetted seedlings with high temperatures during germination and/or 35-45 days following germination, and then placing these seedlings under cold storage conditions of 12C (54F) illuminated with 5-10 $\mu mol \cdot m^2 \cdot s^{-1}$ at the four-leaf stage for 4 weeks (Ohkawa, 1994). Of course as mentioned, some are also breeding for heat-tolerance and day-neutral characteristics to minimize or prevent rosetting and day length effects.

1.4.2 Soil pH

Soil pH was found to be another major cultural factor that could be a stumbling block to quality production of lisianthus. A relatively high soil pH (optimal 6.7) is required to ensure a good root system (Harbaugh and Woltz, 1991). When the soil pH is <6.2, micro-element toxicities, especially zinc, limit growth and cause undesirable leaf chlorosis and necrosis.

1.4.3 Fertilization Requirements

Lisianthus would be grouped with those floricultural crops that have a high fertilization requirement in the plug stage (Harbaugh et al., 1997), and also when grown in ground beds for cut flowers or as flowering pot plants (Harbaugh et al., 1998). The author has found that a 1 nitrogen : 1.5 potassium ratio (Harbaugh, unpublished data) resulted in high quality plants with excellent postharvest longevity of cut flowers. Lisianthus also grow better with high calcium. Frett et al. (1988) reported that 150 ppm calcium in the irrigation water or fertilizer solution resulted in plants with strong stems and a high bud count. Many cut flower growers use a

calcium/boron spray to prevent stem breakage since the stems can become very brittle if calcium and boron are limited.

1.4.4 Insect Management

While fungus gnats (*Basidysia* spp.) can be more of a nuisance than a pest responsible for crop damage in many crops, they were found to be a major pest of lisianthus (Price et al., 1998). They eat roots, leaves contacting the soil, and even small seedlings. Fungus gnats appear to be attracted to lisianthus. In a greenhouse with many crops, they prefer lisianthus. Thus, constant scouting as part of a pest management program must be in place when growing lisianthus. Other major pests that need to be controlled (Osaki, 1992) include whiteflies (*Trialeurodes vaporariorum*), aphids (*Myzus persicae* and *Aphis gossypii*), beet armyworms (*Spodoptera exigua*), and thrips (several species, including *Thrips palmi* and *Frankliniella occidentalis*).

1.4.5 Disease Management

Unfortunately, lisianthus are susceptible to many plant pathogens. McGovern et al. (1998) discussed the need for effective disease management for the most common diseases in lisianthus. Common disease problems include: Gray mold (*Botrytis* blight), *Curvilaria* leaf blotch (Jones and Harbaugh, 1995), *Fusarium* crown and stem rot (Koike et al., 1996; McGovern and Harbaugh, 1997; McGovern et al., 1998), *Pythium* root rot, and *Rhizoctonia* crown and stem rot. *Impatiens necrotic spot virus* or INSV (McGovern et al., 1997), as well as at least six other viruses, have been reported to cause infection in lisianthus.

1.4.6 Growth Regulators

Gibberellic acid sprays (15-25 ppm) were reported to be useful for rapid stem development after rosetted plants began to bolt (Wilkins and Grueber, 1983), but failed to hasten flower initiation in rosetted lisianthus (Hisamatsu et al., 1999). Roh et al. (1989) also reported GA_3 sprays (250 ppm) promoted stem elongation of cut flowers. Along with genetic dwarfing, several plant growth regulators are useful for height control for pot plants and bedding plants: Ancymidol drenches at 0.25 to 0.5 mg per 15 cm pot (Adriansen 1989); Daminozide foliar sprays applied once at 5,000 ppm or twice at 2,500 ppm (Tjia and Sheehan, 1986); Paclobutrazol drench with 2-4 ppm (Adriansen, 1989); Uniconazol foliar sprays at 5 or 10 ppm (Starman, 1991; Whipker et al., 1994). Many factors influence the need for plant growth regulators, such as temperature, day length, and cultivar, so growers have learned to strategically apply plant growth regulators depending on their conditions.

2. FLOWER AND FLOWERING CHARACTERISTICS

Lisianthus flowers have an exceptionally wide diversity of flower colors, shapes, and sizes, and this diversity was multiplied by the development of double flowers. Double flowering lisianthus were natural selections made by Japanese growers (Ohkawa, 2002). Examples of some of the diversity of flower colors, sizes, shapes, and forms with single and double flowers are shown in Figure 24-1.

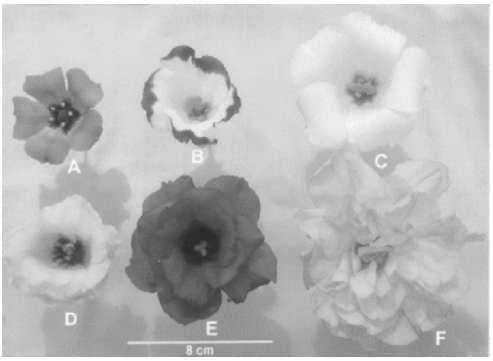

Figure 24-1. Examples of single and double lisianthus flower colors, sizes, and shapes: a. Single, light blue, small, flat/open petals; b. Single, bicolor rim, medium, tubular shape; c. Single, white, large, bell shape; d. Double, pink, small, bell shape; e. Double, purple, medium size, flat outer petal with tubular center petals; f. Double, light pink, large, ruffled petals.

2.1 Single Flowers

Native lisianthus and the first developed cultivars had single flowers. The corolla consists of five petals united at the base with 5 stamens attached on the corolla throat. Occasionally, the author has observed the first open flower on a stem to have 6 petals, but as the other flowers opened, they would have five petals. The ovary is 1-chambered and the stigma 2-lobed.

The calyx is made up of five inconspicuous green sepals that are threadlike at the tip (less than 1-mm wide; 0.04-inch). In the bud stage, the sepals extend beyond the petals and may contain a slight coloration of blue or pink giving one an idea of the petal color long before the petals have developed color.

While a bell shaped flower is the typical flower shape (Fig. 24-1c), there are many variations in flower shape. The flowers were often described as appearing like roses in the bud stage, tulips as they began to open, and as poppies when fully opened. In our breeding program, we have selections with more tubular flowers that have a similar flower form to tulips even when fully opened (Fig. 24-1b). Some of the University of Florida breeding lines, especially when exposed to high light, can appear poppy-like in shape, and others open to a point where the petals are almost

completely flat. The latter have gaps between the petals resulting in flowers that have the appearance of a daisy (Fig. 24-1a). Interestingly, no matter how the open flowers appear, the flower buds resemble rose buds and this no doubt is a primary reason for consumer acceptance.

2.2 Double Flowers

Double lisianthus typically have two to five rows of petals (Fig. 24-1d). However, the author has observed breeding lines with petals so numerous that they are difficult to count. The latter generally do not have normal stigmas. They also may have numerous deformed stigmas and/or they are devoid of stamens. Double flowering lisianthus resemble roses, not only in the bud stage, but also as they open. While Japanese and European markets prefer the single flowers, double lisianthus represent about 80% of the cut flower lisianthus sold in the U.S.

2.3 Petals

Lisianthus generally have very smooth petals. However, some may have ruffled or wavy edges which give quite a different appearance to the flower. Some of the doubles breeding lines from the University of Florida breeding program with many rows of ruffled petals resemble carnations (Fig. 24-1f).

2.4 Flower Size

Flowers are about 7.6-cm (3-inches) across on the native lisianthus and this is probably the most common size today (Fig. 24-1b, e). However, the University of Florida has breeding lines that are as large as 12.7-cm (5-inches) for both single and double flowers (Fig. 24-1c, f). There are now several commercial series which have small flowers about 5.1-cm (2-inches) in diameter when fully opened (Fig. 24-1a, d). Single and double flowers may soon be classified into small (mini), normal, or large as the flower sizes are quite distinct.

2.5 Flower Color

The flower colors for lisianthus growing in its natural prairie habitat range from the predominantly blue-purple hues to white, pink, and a bicolor blue or pink rim. With all the breeding efforts that have taken place, there are as many as 15 colors in a series with vivid blues, light blue, light to very dark purples, light to dark pink, rose, lilac, cream to pure white, yellow, wine red, peach and green. There are also bicolored flowers with the most popular being a white petal with a blue margin (usually termed a ‘picotee’ or ‘rim’). The petals may have just a small speck of color at the tip, a large blotch, or the petal margin completely covered with a 1mm to

5mm (0.2 in.) wide strip of color. Other bicolor flowers include white with just a blush of blue or pink, or white with blue or pink speckles over the entire petal surface.

The eye spot is frequently a dark shade of blue/purple appearing almost black in blue flowers, and burgundy to a shade of brown in pink flowers. The eye spot is usually inconspicuous and relatively small, not adding or detracting from the petal color as it generally blends into the base color of the petals. However, with white flowers, the eyespot can be a pure blue, pink, or green/yellow and can be so large that the flower appears to be bicolored. Some refer to them as a reverse picotee, since the petal margins are white but the base of the petals have color.

2.6 Inflorescence Habit

The first cut lisianthus cultivars could best be described as having monopodial stems with flowers displayed in a racemose fashion. There were few if any side branches. If they had side branches, they were high up the stem. The order of blooming is from the base of the stem upward (acropetally) resulting in open flowers displayed in layers or in a tiered fashion. Since the pedicels were of similar length and the internode differences were relatively large, these original cut flowers had a very open inflorescence.

Breeders later developed cut flower cultivars that had more of a flat- topped appearance (such as with a corymb inflorescence). These new cut flower cultivars are often referred to as spray types with many flowers opening at the same height. For cut flowers, this may result in a desirable characteristic in some flower arrangements. On the other hand, the tiered display of flowers may be desirable for other designs, especially in tall arrangements.

With pot or bedding plants, these two types of inflorescence habits, tiered or spray, are also important. A pot plant with many flowers open at the same level at one time may be very desirable. The plants are purchased for the floral display at the time of purchase, enjoyed for a few weeks, and then discarded. In other words, these potted lisianthus are sold in the same manner as a florist pot mum or poinsettia. With a bedding plant, flowers developing on the same level may not be desirable. As the older flowers mature, the petals often turn brown. If the new flowers open at the same level, they do not hide these brown petals. When the flowers are tightly bunched, botrytis can start on the dead tissue and spread to new petals. With the tiered flower arrangement, new flowers open above the old flowers. The new flowers hide the undesirable dead flowers and they are not as prone to botrytis.

The floral display is not only affected by the type of inflorescence (arrangement of flowers on the stem), but also the pedicel length and internode length on the inflorescence stems. There are considerable differences in pedicel lengths. In dwarf cultivars, the pedicel may only be a few mm long, while we have breeding lines of

cut flowers with pedicels greater than 12 cm (4.7 in.). Similarly, the internode lengths vary from less than 1 cm (0.4 in.) to greater than 10 cm (3.9 in.). In the dwarf cultivars, the short pedicels and internodes can result in the plant having a "stubby" appearance with flowers all opening within a few cm of each other. As stated above, if plants with short pedicels or internodes are produced for bedding plants that are expected to continue to grow and flower in the garden, problems may develop.

2.7 Post-Harvest Floral Characteristics

Although lisianthus cut flowers were reported to have a vase life of two weeks, and this was one of the primary characteristics leading to their acceptance, cultivars differ significantly in postharvest longevity. Vase life ranged from 10 to 31 days (Harbaugh et al., 2000) among the 47 cultivars evaluated. The need for selection of cut lisianthus with longer vase life was evident from these studies.

When flower buds of blue cultivars open under low light conditions in the home, the flowers are very pale blue or gray. Griesbach (1992) reported that low light and alkaline pH within the growing cell lead to reduced color intensity. Similarly, buds of pink flowers open to a very pale pink or even white in the home. However, addition of a preservative, such as 8-hydroxyquinaline citrate, to a 3-4% sucrose solution will prevent flowers from fading and increase vase life (Grueber et. al., 1984; Marousky et al., 1985). Pulsing cut flowers for 24 hr with a solution of 5-10% sucrose (Halevy and Kofranek, 1984) or with BA (N^6-benzyladenine) followed by a 4% sucrose solution has also been reported to increase vase life of cut lisianthus (Huang and Chen, 2002).

3. VEGETATIVE CHARACTERISTICS

3.1 Plant Height

The first lisianthus cultivars introduced into the U.S. market in the early 1980's were cut flowers. They were generally single stems with few or no basal branches. Basal branches can be defined as lateral stems originating from axils of the basal cluster of leaves. Cut flower height ranged from 61 to 91 cm (2-3 ft) depending on the time of year that plants were grown and the cultivar. The author has seen breeding lines reach 121 to 152 cm (4-5 ft) in height. When one considers that dwarf cultivars are only 15 to 30 cm (6-12 in.) tall, the gene pool that is available to breeders concerning plant height is staggering compared to many flower crops.

3.2 Leaf Characteristics

Lisianthus have opposite, sessile, and glaucous leaves. Leaves are largest on the basal portion of the plants and often are >12.7 cm (5 in.) long and 7.6 cm (3 in.) wide. They become much smaller up the stem with upper leaves <5 cm (2 in.) long and 1.3 cm (0.5 in.) wide.

Leaf characteristics vary significantly among cultivars. Leaf shapes typically are from ovate to oblong, but some of our selections have lanceolate (almost grassy in appearance) or oblanceolate leaves. Inflorescence leaves may appear linear on some cultivars.

Leaf color ranges from gray-green (similar to carnation), light green, dark green, to almost a blue-green. Leaves can be very attractive to undesirable. Thus, leaf shape and color must be evaluated in the selection process as well as flowering characteristics. Leaf characteristics are probably more important in potted types, as lower leaves are removed in cut flowers when the stems are harvested.

4. BREEDING TECHNIQUES

Lisianthus is easy to breed compared to many floricultural crops. It is an out-crossing species that is self-compatible, but subject to inbreeding depression. The stigma is positioned well above the anthers in most cases. Pollen starts to dehisce as flowers open, and by one or two days after flowers have opened, pollen is usually plentiful. The stigmas generally do not become receptive until 3-5 days after flowers begin to open so that emasculation is easy to accomplish without accidental selfing. We usually emasculate critical crosses by opening (unraveling) petals one or two days before flowers would naturally open, and thus pollen is not ripe or dehiscing.

Stigmas appear to be slightly sticky as the pollen readily adheres to the stigma. However, brushes used for pollination do not become sticky and are easily cleaned with water or alcohol. This is a welcome attribute compared to other flowering crops.

Pollen may be collected and stored at room temperature (22-26C; 72-79F). The author has successfully used pollen stored three years but have not evaluated storage times and temperature in great detail (Harbaugh, unpublished data). Pollen is stored in packets that are in sealed jars with a desiccant.

Seeds mature in 8 to 10 weeks during the summer, but may require longer during the winter. Each seed pod commonly contains at least 1,000 seeds or more (several thousand) in larger pods (Harbaugh, unpublished data). Seed pods begin to turn yellow at the apex (base of the stigma) and then split longitudinally when fully ripe. If not harvested before they begin to split, seeds may be lost. In addition, under humid conditions, moisture enters the opening seed pod and fungal pathogens find

these conditions ideal. It is common to see mycelium growing inside an open seed pod. The result is that seeds stick together and rescuing seed is very hard if not impossible.

We have stored seeds for many years at 18-20C (64-68F) in envelopes in sealed plastic bags. If seed pods are not immediately dried and seeds removed, germination is greatly reduced. Thus, ensuring seeds are dry before and during storage is critical.

4.1 Inheritance

As with many floricultural crops, much of the breeding has been done by private sector/commercial breeders that do not publish inheritance information. The Public-sector University of Florida breeding program (Harbaugh, unpublished data) has conducted a few detailed inheritance studies. Blue petal color is dominant over white or pink, and pink dominant over white. However, white flowers have at least two mechanisms involved. White flowers may result as a simple recessive gene or as a result of epistasis. In the former, the white is generally a cream color, while in the latter, a pure white results. When a pure homozygous, epistatic white is crossed with a blue/purple flower, the resulting flowers are light blue.

The inheritance of bicolored flowers is more complicated and perhaps involves more than one gene pair. In addition, the rim color seems to be very unstable and expression of the rim color varies with environmental conditions. For example, we have observed flowers on the same stem that range from pure white with only a very small blue tip to completely blue. Plants exposed to cold temperatures may have blue flowers, and then rim flowers develop under warmer temperatures. Eight to nine generations of inbreeding have been required to develop homozygous bicolor lines that have stable color patterns over a range of environmental conditions. This is in comparison to five generations for simple flower color or vegetative traits.

Inbreeding depression is a serious concern with lisianthus. Much vigor is lost with just a few generations of selfing (Harbaugh, unpublished data). The use of backcrossing techniques and/or sibling crosses are necessary considerations for developing viable/strong homozygous parents to be used in making F_1 hybrids.

4.2 Interspecific Hybridization

While the first cut flowers appeared to be improvements from *Eustoma grandiflorum*, *E. exaltatum* may have been used to develop lisianthus with smaller flowers. Dr. Ohkawa (personal communication) introduced seed of *E. exaltatum* in 1986. *E. exaltatum* is found along the sandy coastal areas of the Gulf of Mexico, such as Florida, Louisiana, and Texas, but has also been reported in the Florida Keys and islands of the West Indies. It has common names of coastal dunes lisianthus, seaside Gentian, and catchfly Gentian. It has very small flowers with petals about

2.5 to 3.8 cm (1-1.5 in.) across. When *E. grandiflorum* is crossed with *E. exaltatum*, the first generation has intermediate sized flowers that are about half the size of *E. grandiflorum* (Harbaugh, unpublished data). The author suspected, and Dr. Ohkawa confirmed, that many of the smaller flower types marketed today probably resulted from interspecific crosses.

Some of the progeny resulting from our interspecific crosses had flowers that were quite unusual. However, we have had difficulties with sterile flowers developing with continued inbreeding of interspecific lines. Many of the flowers had poor anther/pollen development.

4.3 Biotechnology

To date, development of new plant forms through various biotechnology techniques or genetic engineering does not appear to have impacted lisianthus to any great extent as far as release of new transformed cultivars. However, since much of this work is kept secret by private sector breeding programs until it is commercialized, one can not know all that is being considered, investigated, or in progress. Recently, several investigators have reported transformation of lisianthus by transgenic means (Deroles et al, 1993; Handa and Deroles, 2000). There has been considerable work on understanding and altering flower color (Deroles et al., 1995; Ledger et al., 1997; Nielsen et al, 2002; Schwinn et al., 1997). In addition, researchers are investigating modifying flowering time and introducing fragrance (Zaccai et al., 2001)

Griesbach and Bhat (1990) produced tetraploid forms of lisianthus using colchicine and found tetraploid plants had stronger stems and reduced plant stature. somaclonal variants were discovered in a tissue-cultured population of lisianthus (Semeniuk and Griesbach, 1987). These variants (dwarf, miniature, or branched compared to the parent that was a tall single stem cultivar) had not been seen in commercial seed sources at that time (Griesbach et al., 1987a, b; Griesbach et al., 1988). The traits of the new variants were stable and progeny produced from these plants indicated genetic transmission of the traits. The authors felt that tissue culture may be important in obtaining new genes that could be used to further improve lisianthus.

5. FUTURE DIRECTIONS

Very few new flower introductions have impacted the floriculture industry in such a rapid and dramatic way as has occurred with lisianthus, and few flowers have had such widespread acceptance in the cut flower, pot and bedding plant arenas. Great strides have been made in development of new flower colors, double flowers, and flower sizes. Similarly, many improvements have been made in vegetative

characteristics such as strong stems for cut flowers and dwarf plants for pots. Cultivars are now available with much better heat-tolerance and day length requirements. Even with all the improvements, however, lisianthus is still a difficult plant to produce. We believe the next improvements in lisianthus will be in the area of disease resistance. Lisianthus is susceptible to at least three *Fusarium* pathogens causing root, stem, and crown rot. For cut flower growers, *Fusarium avenaceum* and *Fusarium oxysporum* have caused major losses. Bedding plants seem to be very sensitive to *Fusarium solani* when plants are transplanted into the landscape. Evaluation and breeding for *Fusarium* resistance would greatly decrease production woes and improve landscape performance.

Continued development of day neutral and heat-tolerant lines that would aid programmed flowering of lisianthus would increase acceptance of this crop. This would also increase the use of lisianthus in parts of the world with high temperatures, or increase the window of production outside the normal April to June flowering time.

Although earliness to flower is linked with heat-tolerance and photoperiod, continued improvements in earliness to flower would significantly impact growers since lisianthus have a relatively long production time. Of course, earliness can not be at the expense of plant quality, which appears to be a breeding problem to overcome. The author believes that lisianthus will be developed similar to snapdragons, with cultivars available in day length response groups for flowering at different times of the year. Already, commercial lines are being sold for early and late flowering (Ohkawa, 1994).

Lisianthus does not transplant easily when in flower and does not perform in the landscape as well as some other bedding plants that are sold in flower. For lisianthus to move into the top 10 bedding plants, breeding has to be directed at improved survival after transplanting into the garden. In addition, most of the popular bedding plants, such as impatiens and petunias, are purchased as small plants with flowers. These plants increase in size and continue to flower over a long period of time. Most of the current lisianthus cultivars do not have this characteristic. They have a number of unopened flower buds that may open over time, but generally do not continue to branch and thus do not "grow" larger. PanAmerican Seed Company has introduced a new cultivar, 'Forever White', that has improved longevity in the landscape. Thus, improvements in vigor (which would include disease resistance) and continued improvement for the ability of lisianthus transplants to continue growing and flowering would increase use of lisianthus in the landscape.

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Chapter 25

FREESIA

Freesia x hybrida

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Abstract: *Freesia x hybrida* is a popular cut flower and flowering potted plant. Fragrance, long vase-life, and wide color range make this a versatile floriculture crop. Diagnostic traits of the 11 *Freesia* species are described with reference to their potential commercial value. Temperature and photoperiod are the primary factors controlling flowering. By controlling forcing, conventional and delayed planting, the crop can be kept in production for six consecutive months. Freesias are self-incompatible and exhibit inbreeding depression when inbred parental lines are developed. The crop ideotype consists of the following traits: 7 or more florets/spike, extra-large flower size (tetraploids), 70-80cm long stems, pure and clear flower colors, sweet fragrance, and disease resistance.

Key words: Corms, Cut flowers, Potted Plants, Fragrance, Geophyte, Iridaceae.

1. INTRODUCTION

Freesia is a small genus of southern African *Iridaceae* subfamily Ixioideae, which has been familiar to horticulturists and valued by them for the beauty and fragrance of the flowers. Klatt established this genus in 1866. Species are concentrated in the southern Cape Province area of Southern Africa. Most of the species are found around the 33rd parallel. They are found growing from dry sandy plains to the edges of rivers. In their native habit, freesias sprout in the autumn (February to March), and flower in winter (July to August) at 8C to 10C (Brown, 1935).

Freesia x hybrida is the name of modern *Freesia*. Although *Freesia x hybrida* is a new brand of cut flower in the international flower market, its sale and yield have

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been increased dramatically in recent year. In the Netherlands, about 500,000,000 of cut flower stems are produced each year.

Freesias are excellent cut flowers (Figure 25-1). Their appealing shapes make them suitable line flowering for any arrangement, and their wide range of color increases their versatility. The flowers are popular used for weddings and make fragrant additions to bouquets and body flowers.

Freesia is also uses as a forced pot crop, becoming popular when cultured in cool houses or in hobby greenhouses. As most of the cultivars are highly fragrant, the flowers can also be used to produce floral essences.

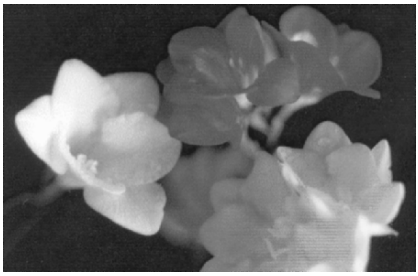

Figure 25-1. Cut flowers of *Freesia x hybrida* (white, pink, yellow cultivars).

1.1 Horticultural History & Domestication

The horticultural history of *Freesia* is probably less well known than other economic plants. The origin of the early cultivars has in fact been documented, though the literature is scattered.

According to available records, *Freesia* was first grown in Europe in the mid-eighteenth century and become one of the more popular plants in horticulture in the last half of the 19th Century. Burman, who described them in 1766, grew collections of both *F. caryophyllacea* and *F. corymbosa,* in Holland. *Freesia refracta* was grown in Vienna later in the century. These species, however, have little to do with the horticultural history of the genus as they were most likely not grown widely, nor were they used in the breeding experiments of the later nineteenth century (Goldblatt, 1982). It was only in 1866 that *Freesia* was described as a distinct genus, when Klatt published a description of *Freesia.* It was not until after the publication of *F. Leichtlinii* in 1874 (Klatt, 1874) that the genus began to attract the fancy of horticulturists, and the cultivation of *Freesia* became more general (Brown, 1935). After 1900 the study of *Freesia* shifted from Europe to South Africa where Louisa Bolus promoted the study of many groups of Cape plants including *Freesia.* She had, by 1933, progressed sufficiently in her understanding of *Freesia* to develop a key to 11 species (Bolus, 1933).

The history of the modern *Freesia*, however, really begins with the discovery by the horticulturist and plant collector, Max Leichtlin, of yellow-flowered plants in the Botanic Gardens at Padua (Jacob, 1909). He obtained some specimens, grew them himself, and distributed material widely. Klatt described this freesia as *F. leichtlinii* in 1874, and it was figured repeatedly in horticultural journals in the following decade. *F. leichtlinii* was evidently a popular ornamental pot plant, and available in the nursery trade (Goldblatt, 1982). The white-flowered, sweet-scented plant called *Freesia refracta* var. *alba* which appeared in the English nursery trade in 1878 is now called *F. alba*, and is known today to be native to the southern Cape Province. Shortly after its appearance in England, *F. alba* spread to the Continent and then to North America. Breeding of *Freesia* began immediately after *F. alba* came on the market (Goldblatt, 1982).

There are few records of *Freesia* breeding until the introduction in 1897 of the rose-pink-colored form of *F. corymbosa*. This lovely flower really provided the stimulus to *Freesia* breeding. Watson (1898) recorded that a Mr. Armstrong, of Port Elizabeth, who found them growing wild on a farm near Humansdorp, sent plants to Kew. A half-dozen specimens bloomed at Kew in 1898, when the plant was named *F. armstrongii* (Goldblatt, 1982).

The Dutch nursery firm of Van Tubergen soon acquired *F. armstrongii*, and this, crossed with *F. alba*, yielded the rose-colored-hybrid marketed in 1905 as *F. "tubergenii"*. Van Tubergen continued breeding with the species and hybrids (Hoog, 1909) and produced a range of tall freesias with colors ranging from blue, mauve, shades of rose, yellow and white. The son of Rodolfo Ragionieri, Attilio, also acquired *F. armstrongii* and bred a strain called *F. "ragionieri"*(Grignan, 1907) by crossing it first with *F. alba* and *F. leichtlinii*, and continuing to breed the offspring of these hybrids (Goldblatt, 1982).

Chapman crossed *F. alba* (*F. refracta alba*) with a plant called *F. aurea*, and then crossed the resulting F1 hybrids back to *F. alba* to produce a range of deep to pale yellow, tall freesias, the finest form of white became known as *F. "chapmanii*." *Freesia aurea* was a name used in horticulture only, and is evidently a deep yellow flowered form of *F. corymbosa* (Brown, 1935) known botanically at this time as *F. odorata*. *Freesia aurea* is thus a second form of *F. corymbosa* to play a role in the history of cultivated *Freesia*. It was also the fourth wild genome that entered the gene pool of horticultural stocks (Goldblatt, 1982).

The subsequent history of freesia breeding does not involve the wild species and is a complex story of the introduction of new varieties, changes in fashion and the resulting succession of named plants finding favor.

Van Tubergen and several other European and American breeders continued to produce new varieties, and the first polyploid "Buttercup" made its appearance in the 1910s. The story of freesia breeding after 1920 has recently been described in some detail by Goemans (1980), a *Freesia* breeder whose knowledge of the genus in cultivation is unparalleled. *Freesia* breeding continues today and varieties including

double flowered types are being produced that bear less resemblance to the original wild species (Goldblatt, 1982).

During the 1900s freesia breeders significantly improved the stem strength, flower size, color range, and disease resistance of greenhouse cultivars. As freesias are essentially self-sterile, the offspring from cross-fertilized parents is somewhat variable. Several diploid, triploid, tetraploid, double-flowered and F_1 hybrid cultivars were introduced. These freesias could be propagated from seed or corm. Beginning in the 1970s breeders have also worked to develop cultivars conductive to year-around flowering rather than just the traditional winter and spring flowering periods (Halevy and Mor, 1969). Today the majority of registered cultivars are polyploid (Mohr, 1958), with diploid strains important mainly in areas where plants are grown every season from seed (Goldblatt, 1982).

1.2 Plant Structure

1.2.1 Plant Morphology

Freesia has the typical several internodes, basal rooting corm, and spicate inflorescence of the group. The significant taxonomic features of the genus are its conical corm, with netted tunics, several equitant leaves, stem flexed below the inflorescence, second flowers, deeply forked style branches and rough surfaced capsule with large seeds.

Freesia is lanceolate with an acute apex, generally erect and arranged in a fan. The rootstock is a tunicate corm of several internodes. There are two types of roots: the thick contractile roots function mainly to support the plant, whereas the numerous thin roots transport materials from the soil to the plant (Ruzin,1979).

1.2.2 Floral Morphology

A freesia inflorescence is a cymose spike with sessile flowers borne along the upper part of the floral scape. The scape bends at a semi perpendicular angle at the base of the inflorescence. Each floret on the spike has membranous or dark green bracts, six tepals that are arranged in a broadly funnel-shaped tube, 6 stamen, and a bifid branched style. The flowering stalk may be branched.

During the flowering season, the plant produces long spike inflorescence with flowers orderly arrayed along one side of the inflorescence on which the flowers start to bloom in succession from the base to top. The plant has small round capsules. After dried, the seeds are dark brown in color, and weight about 50g per 100 seed. The flower produces a very pleasant fragrance and contains aromatic oil that has a certain commercial value.

1.3 Geography

Freesia is endemic in southern Africa and is restricted almost entirely to the Cape Province of South Africa. Species are concentrated along the south coast and adjacent interior valleys and all but *F. andersoniae* occur in an area of significant winter rainfall. *F. andersoniae*, which has as wide a range as all the winter rainfall area species together, occurs in the upper Karoo, Northern Cape, western Orange Free State, and reputedly, although there are no records, in the south-western Transvaal and southern Botswana. This area is a semi-arid steppe and grassland which has predominantly summer rainfall, but also some winter precipitation in the south (Goldblatt, 1982).

F. occidentalis is the only winter rainfall area species with a western rather than southern Cape distribution, and it occurs from the Bonteberg Karoo north to Calvinia along the very dry eastern foothills of the Cedarberg and Swartruggens, and in the Doorn-Tanqua River basin (Goldblatt, 1982).

1.4 Cytology

The basic chromosome number in *Freesia* is x=11. All but two of the 11 species have been counted and all are reported to be diploid, $2n=2x=22$. The first count in the genus was made by Taylor (1926) who counted $2n=2x=22$ in a cultivar which he referred to *F. refracta* 'Fischer'.

Goldblatt (1971) reported the first published counts for wild populations for *F. refracta, F. alba* (=*F. muirii*), *F. caryophyllacea* (=*F. elimensis*) and *F.* cf. *speciosa*. In 1945, Lawrence reported the count of *F. x hybrida*, $2n=2x=22$, $2n=3x=33$, $2n=4x=44$ (Goldblatt, 1982).

Wang and Pu (1988) first reported a karyotypic study of Freesia refracta. There are six pairs of submetacentric chromosomes, four pairs of metacentric chromosomes, and one pair of telocentric chromosomes.

2. SPECIES

Freesia comprises n=11 wild species (Bolus, 1933). The followings are species of *Freesia* arranged in systematic order, by habitats, and features, according to the description by Goldblatt (1982). Another group of *Freesia* is the modern freesia, named *Freesia x hybrida,* which play a key role in the production of cut flower today.

2.1 *Freesia Alba* (G.L. Meyer) Gumbleton

Plants (50-)120-400 mm high. *Corm* conic, ca 10mm in diameter at base, with fine, light brown, reticulate tunics. *Leaves* linear-ensiform, usually erect, occasionally procumbent, tapering, acute, usually the longest about as long as the stem. *Stem* usually erect, rarely inclined, minutely papillate at least towards base, usually branched. *Spike* (2-)3-6(-8) flowered; bracts herbaceous, with hyaline margin, 5-8 mm long, inner usually slightly shorter. *Flower* (25-)35-45(-60) mm long, with a very strong, sweet scent, white, sometimes with purple lines in the throat, and often a purple flush on the reverse of the tepals, fading darker purple, with or without a yellow mark on the lowermost tepal; *tube* (15-)20-40 mm long, narrow basal part 6-12 mm; *tepals* subequal, ± actinomorphic, outspread with upper tepal slightly larger and lower tepals slightly smaller, upper tepal 15-18 mm long, oblong to narrowly ovate, 8-12 mm wide. *Filaments* to 25 mm long; *anthers* 6-9 mm long. *Style* dividing at apex of anthers. *Capsule* ca 10 mm high and to 10 mm wide, rugulose. Flowering time: late June-October.

Freesia alba has been known and widely cultivated in Europe since 1881. It was evidently brought to England from the Cape sometime before 1878. Botanists at first treated this pure white, richly scented form of *Freesia,* as a variety of the very different, and horticultural unattractive *F. refracta*. It was later reclassified as a distinctly different species and named *F. alba. Freesia alba* is probably the most commonly cultivated of *Freesia* species and although its flowers do not compare in size and color with the many cultivars now available, it has by far the finest scent of all species and cultivars. Most, if not all, horticultural forms have *F. alba* in their pedigree and many even today bear a strong resemblance to it in the form of the flower. The superb scent however has been diluted or sometimes lost in some cultivars through breeding for other characters.

2.2 *Freesia Sparrmannii* (THUNB.) N.E.BR.

Plants 120-180 mm high. *Corm* conical, up to 11mm in diameter, with tunics of fine, pale reticulate fibres. *Leaves* erect, linear-ensiform, acute, 100-160 mm long, the longest about as long as the stem apex. *Stem* usually straight, erect or inclined, 2-4 branched, sparely papillate in lower half. *Spikes* horizontal, (3-)5-8 flowered; bracts (3-)4-7 mm long, herbaceous, with hyaline membranous margin; inner bract similar, often slightly shorter. *Flower* (30-)35-40 mm, not scented, white inside with yellow mark on lower tepal, and at apex of narrow part of tube, purple-flushed on reverse of upper tepals and tube; *perianth tube* 20-27 mm, lower part 12-15 mm; *tepals* unequal, upper largest ca 11 mm long, 7 mm wide, obovate, slightly hooded, arching over anthers, upper laterals upright, ca 9 mm long, lower tepals to 9 mm long, 3-4 mm wide, flat. *Filaments* 10-14 mm long; *anthers* to 4,5 mm long. *Style* dividing 1-2 mm beyond anther apex. Flowering time: September.

Freesia sparrmannii is a distinctive, low growing species. It appears to be closely related to *F. alba* but it is easily recognized because of the length of the narrow part of the perianth tube and generally small flowers with narrow tepals and perianth tube. The flowers of *F. sparrmannii*are bilabiate, although this is not readily detected in dried material. The flowers are white inside and purple-flushed outside, with a small yellow mark in the lowermost tepal. Upon drying, the purple coloring is intensified.

2.3 *Freesia Caryophyllacea* (BURM.F.) N.E.BR.

Plants are small, seldom reaching 100 mm high, usually ± prostrate. *Corm* conic, 10-15mm in diameter, tunics medium in texture, pale to light brown. *Leaves* oblong to lanceolate, acute or obtuse, up to 100 mm long, prostrate to inclined or ± erect. *Stem* simple, rarely 1- branched, covered with a dense, minute puberulence, usually flexed close to ground level, and prostrate, occasionally (in shade) suberect or ascending. *Spike* usually prostrate or above ground level and horizontal or not flexed, 3-7 flowered; *bracts* green, herbaceous, usually with a hyaline margin, 4-8 mm long; inner bract similar, but usually smaller. *Flower* 30-45(-50) mm long, sweetly scented or odor-less, white, often fading to brownish or purple, lined with purple within and sometimes purple-flush on reverse of tepals, marked yellow in midline of lower median tepal or all lower tepals; *tepals* unequal, 16-20 mm long, oblong to narrowly ovate, bilabiate , with upper tepal to 20 mm long, and 8-12 mm wide, hooded to erect and upper laterals reflexed outwards when fully open; lower tepals horizontal, smaller. *Filaments* 15 mm long; *anthers* 4-6 mm long. *Style* dividing at apex of anthers. *Capsule* 8-14 mm long, to 10 mm wide. Flowering time: April-June.

Freesia caryophyllacea is a common although poorly known species of the Caledon, Bredasdrop and Swellendam districts where it typically grows on gravelly clay soils in renosterbosveld. It is remarkable in having a flowering period in the late autumn to early winter.

Some of the variation in *Freesia caryophyllacea* may be due to gene flow between related species. *Freesia fergusoniae,* which has broad, obtuse, prostrate leaves and cream and orange flowers, is sympatric with *F. caryophyllacea* to the west of Heidelberg (Goldblatt, 1982).

Hybridization probably also occurs between *Freesia caryophyllacea* and *F. alba* where these grow in proximity. Introgression between *F. caryophyllacea* x *F. alba* hybrids or between *F. caryophyllacea x F. elimensis* would explain the presence of deep purple flower coloring in *F. caryophyllacea* in areas where flowers usually lack purple.

Freesia caryophyllacea makes an inferior horticultural subject although it is of interest for its early blooming habit and the strong scent of some forms. Generally cultivated plants grow poorly. They either produce few flowers, if grown under good

light conditions, or if grown in a greenhouse or in poor lighting, become rank, leafy, or produce flowers, which do not open properly or develop their normal form.

2.4 Freesia Elimensis L. BOL.

Plants are 60-150 mm tall. *Corm* conic, ca 10mm in diameter, tunics of fine, pale fibres. *Leaves* erect or ascending (rarely prostrate), sometimes exceeding the flowers, linear-lanceolate, usually acute. *Stem* inclined, minutely papillate, unbranched. *Spike* usually 4-7 flowered; bracts green, with hyaline margin 7-9 mm long. *Flower* 35-45 mm long, scented, white, flushed light purple on reverse of tepals, and with a yellow-orange mark on midline of lower tepal; *tube*, 25-35 mm long, basal narrow part 8-10 mm long, widening very abruptly; *tepals* unequal, upper largest, 18-22 mm long, 10-12 mm wide, oblong, upper laterals slightly narrow, lower tepals 16-18 mm long, 8-9 mm wide, oblong, horizontal. *Filaments* 18-22 mm long, *anthers* 5-6 mm long. *Style* dividing at apex of anthers. Flowering time: mid May-June.

Freesia elimensis is closely related to *F. caryophyllacea*, and the two are difficult to distinguish. Generally, *F. elimensis* is more robust, has larger flowers of a blue-white color, with a yellow mark only on the lowermost tepal. The stem of *F. elimensis* is also inclined rather than prostrate. The most important difference between the two species is in the perianth tube that is longer, 25-35 mm in *F. elimensis* with the basal part 8-10 mm, which widens very abruptly into a very broad upper part. In contrast the tube of *F. caryophyllacea* is 20-25 mm long, with the basal part 6-8 mm long, and the transition more gradual into the upper part.

2.5 Freesia Leichtlinii Klatt

Plants are 80-200 mm (-500 mm under bushes) tall. *Corm* small, conic 8-10 mm in diameter with tunics of fine, pale reticulate fibres. *Leaves* erect, or inclined (rarely prostrate), usually slightly shorter than the stem apex, but occasionally exceeding the stem (in shade), linear-lanceolate, seldom exceeding 8 mm in width, tapering, acute. *Stem* ± erect, or inclined, sparsely papillate in lower part, often simple or 1-2 branched. *Spike* 2-8 flowered; bracts green, with hyaline margin, 4-7 mm long, inner narrower. *Flower* (25-)30-40 mm long, strongly scented, cream to pale yellow, with lower tepals dark yellow-orange, and upper tepals often brown-purple-flushed on reverse, bilabiate with lower tepals horizontal to down curved; *tube* (15-)20-25 mm long, basal narrow part (4-)6-8 mm, bent at apex of narrow part; *tepals* unequal, upper largest 14-18 mm long and 13 mm wide, ± hooded, broadly elliptical, upper laterals ca 8 mm wide, lower laterals to 14-15 mm long; and 9-11mm wide, ovate, margins curved upward. *Filaments* 10-14 mm long, *anthes* 6-7 mm long. *Style* dividing at apex of anthers. Flowering time: August-September.

Freesia leichtlinii remained in cultivation in Europe for some time, and was used in the production of new cultivars. Now the species is no longer in cultivation, presumably having been replaced by superior horticultural cultivars.

2.6 Freesia Fergusoniae L. BOL.

Plants are (60-) 100-200 mm high. *Corm* conic, to 15 mm in diameter, with pale medium to coarse reticulate tunics. *Leaves* prostrate, oblong, 100-500 mm long, outer, or all obtuse to subobtuse, acuminate, to 15 mm at widest. *Stem* prostrate entirely or only in lower part, and erect, to inclined in upper part, minutely scabrid-puberulent throughout or in lower part; usually 1-2 branched. *Spike* horizontal, 5-10 flowered, bracts herbaceous with hyaline margin, ca 6 mm long, inner similar but smaller. *Flower* 30-45 mm long, sweet-scented, cream-yellow, with dark yellow to orange markings on the lower three tepals, strongly-bilabiate; *tube* 22-26 mm long, slightly curved, narrow basal part 6-8 mm; *tepals* unequal, with upper largest, 12-14 mm long, oval, to 12 mm wide, erect to slightly hooded, outer whorl smallest, to 10 mm long and 8 mm wide, lower tepals ± horizontal, and surface of lower laterals naviculate. *Filaments* 13-16 mm long, *anthers* 5-7 mm long. *Style* dividing just above apex of anthers. *Capsule* 10-12 mm long. ovoid, to 9 mm wide, rugulose. Flowering time: (late June-) July-August.

Freesia fergusoniae is one of the more attractive species of the genus. Well grown individuals can reach 200 mm in height and may bear one or two branches, each carrying several flowers. The flowers themselves are large, creamy-yellow with deep yellow or almost orange markings on all three lower tepals. In seasons with scant rainfall *F. fergusoniae* blooms earlier than usual, late June or July, rather than August, and plants are stunted, only 60-80 mm high and unbranched.

2.7 *Freesia Refracta* (JACQ.) Klatt

Plants are (80-) 200-450 mm high. *Corm* conic, 15-20 mm in diameter, tunics pale, fine, reticulate. *Leaves* linear, tapering above, acute, 150-300 mm (or more)long, usually about two-thirds the length of the stem, but sometimes longer than the stem, 5-8 (-10) mm wide. *Stem* smooth, erect, usually branched. *Spikes* horizontal to decumbent, 5-10 (-12) flowered; *bracts* rather membranous, greenish in young stages, becoming dry and transparent, with brown veins, 5-8(-10) mm long, inner smaller. *Flowers* 25-35(-40) mm long, with a strong spicy scent, bilabiate, usually dull yellowish-brown or green to purple, occasionally pale yellow, with bright orange markings on lower tepals and purple veins in the throat; *tube* 16-24 mm, narrow basal part 5-9 mm; *tepals* unequal, inner cordate, upper largest, 11-14 mm long, 8-11mm wide at base, hooded, upper laterals oblong, acute, 5-7 mm wide, 12-14 mm long, spreading, lower laterals horizontal to downcurved, (8-)11 mm long, 7-9 mm wide, margins curved upwards, obscuring the lowermost tepal. *Filaments*

10-14 mm long, *anthers* 5-6 mm long. *Style* branching at apex of anthers. *Capsule* to 10 mm long, ca 10 mm in diameter, three lobed, sometimes more or less smooth, or rugulose. Flowering time: (late June-) July-September.

Freesia refracta was one of the early species of the genus to become known to botany, having been described in 1795 by Jacquin, as a *Gladiolus*. The nomenclature history of this species gives a clear idea of the confusion that *Freesia* caused plant systematists of the day. Thus *F. refracta* was transferred to *Tritonia* by Ker (1804), then renamed in *Gladiolus* by Persoon, before being finally place in his new genus *Freesia* by Klatt.

The cytological studies demonstrated that the somatic cell of *Freesia refracta* has diploid chromosome, $2n=2x=22$, including six pairs of submetacentric chromosome, four pairs of metacentric chromosomes, and one pair of telocentric chromosomes (Wang and Pu, 1988).

2.8 Freesia Occidentalis L. BOL.

Plants are 90-250(-500) mm tall. *Corm* conic, 13-20 mm wide at base, tunics of brown, medium to coarse fibres. *Leaves* erect, to 150 mm long, usually about two-thirds the of the stem, 6-13 mm wide, usually obtuse to subobtuse, apiculate, (rarely sub-acute). *Spikes* horizontal to slightly decumbent, (3-)6-10 flowered; *bracts* dry, membranous, brown-veined and rust-tipped, 5-6 mm long, inner usually shorter. *Stem* erect, usually branched, smooth. *Flowers* 30-38 mm long, bilabiate, cream-white (rarely pale mauve) with yellow lower tepals and lower tube, lightly sweet-scented or without scent; *tube* 18-25 mm long, basal part 6-11 mm long, strongly curved at apex of narrow part; *tepals* unequal, inner cordate, upper largest, hood-like, 8-11 mm long, 5,8-11mm wide in lower third, upper laterals ovate, 8-10 mm long, 6-7 mm wide; lower laterals horizontal, with margins upcurved, ca 8mm long, ca 7 mm wide. *Filaments* 13-15 mm long, *anthers* 5-6 mm. *Style* branching towards apex of anthers. *Capsule* 6 mm high, 8 mm wide, 3 lobed, surface smooth (evidently). Flowering time: late July to September.

Freesia occidentalis is closely related to *F. refracta*, a species of the Little Karoo and Worcester area, and to *F. corymbosa* of the eastern Cape Longkloof. Differences between these species are discussed under *F. refracta* and *F. corymbosa*. *Freesia occidentalis* is one of the few of the genus not known in cultivation. This is a pity since it grows easily, and is particularly floriferous. The flowers are brightly colored, but comparatively small for *Freesia* (Goldblatt, 1982).

2.9 *Freesia Corymbosa* (BURM. F.) N. E. BR.

Plants are (160) 250-500 mm high. *Corm* globose-conic, to 25 mm in diameter, tunics medium to coarse, light brown, reticulate. *Leaves* linear ensiform, 100-200 mm long, basal, acute. *Stem* erect, smooth, usually much exceeding the leaves,

usually several-branched. *Spikes* (3-)6-10 flowered; *bract* 3-4(6) mm long, usually dry-membranous, brown-veined and rust-tipped (rarely apices transparent), inner bract similar, sometimes slightly longer, or ± equal, bi-apiculate. *Flowers* 25-35 mm long, often without scent, typically pale yellow, with lower tepals bright yellow to orange, occasionally pink with yellow throat; *tube* ca 20 mm long, narrow lower part 4-10 (-15) mm, upper part expanding rather abruptly, *tepals* unequal, upper largest, erect to somewhat hooded, to 12 mm long, and 10-12 mm wide, cordate, upper largest, ca 9 mm long, 5-6mm wide. ovate-lanceolate, lower laterals ca 7 mm, ca 7 mm wide, cordate, ± horizontal, margins curved upward and overlapping lower tepal. *Filaments* ca 15 mm long, *anthers* 4-6 mm long. *Style* dividing just beyond apex of anthers. *Capsule* 6-8 mm long, rugulose, sometimes appearing smooth when ripe. Flowering time: as early as May, but generally late August-October, locally to late November.

Freesia corymbosa is closely related to both *F. occidentalis* and to *F. refracta*, and it sometimes difficult to tell them apart. *Freesia refracta* has pale greenish to membranous bracts without the brown tip characteristic of *F. corymbosa*, as well as rather dull colored flowers with a distinctive spicy scent.

The rare pink-flowered *Freesia*, named *F. armstrongii* by Watson in 1898, is a color form of *F. corymbosa*. The type collection originally came from near Hankey in the Gamtoos River valley and is uniformly pink except for the base of the tube, which is yellow. They have a faint but distinctly *Freesia-like* scent (Goldblatt, 1982).

2.10 Freesia Speciosa L. BOL.

Plants are (80-)120-200 mm high including flowers. *Corm* conic, to 20 mm in diameter, with medium to coarse, light brown, reticulate tunics. *Leaves* straight to falcate, obtuse (-subobtuse), apiculate, usually shorter than the stem, unusually broad, to 20 mm wide. *Stem* erect, usually simple, (or up to 4 branched in cultivated material), smooth. *Spikes* 3-6 flowered; *bract* membranous, greenish below in early stages, becoming dry, brown-tipped, 8-10 mm long, inner bract slightly smaller. *Flowers* large, 50-70 mm long, pale yellow (evidently odorless), lower tepals deep yellow; *tube* 35-50 mm long, long portion 14-20 mm, curved at apex of lower part; *tepals* 13-18 mm long, outer larger, broadly ovate, upper hood-like, 10-12 mm wide, lower laterals naviculate. *Filaments* 25-32 mm long, *anthers* 7-10 mm long. *Style* dividing at mid-anther level or well beyond anther apex. Flowering time: mid-August-September.

Freesia speciosa is the largest flowered species in the genus. It typically has obtuse to subobtuse, and rather broad soft-textured leaves, and yellow flowers usually 60-70 mm long with the tube over 40 mm, and broad, ovate inner tepals 12-15 mm at widest.

F. speciosa was grown for a time in several Botanic Gardens in South Africa, and very successfully judging from the robust specimens made from the cultivated

plants. It is no longer in their living collection, and is not known to be in cultivation elsewhere. With its very large yellow flowers, *F. speciosa* is undoubtedly a desirable garden subject (Goldblatt, 1982).

2.11 Freesia andersoniae L. BOL.

Plants are 100-200 mm tall. *Corm* to 20 mm in diameter, conic, tunics light brown, fibres medium to coarse. *Leaves* several, linear ensiform, acute, (50-)100-180 mm long, usually about as long as the stem. *Stem* erect, smooth, simple or 1-2 branched. *Spikes* horizontal 2-5 flowered; *bracts* 7-10 (-12) mm long, dry-membranous, brown-veined and minutely rust-tipped, inner bract similar, usually ca 1 mm longer, acute or bi-apiculate. *Flower* sweet-scented, white to cream with yellow marking on the lower tepals and purple lines in the throat, 50-60 mm long; *tube* 36-44 mm long, curved towards apex of narrow part, narrow basal part 15-20 mm long; *tepals* unequal, inner 12-15 mm long, ovate, 8-9 mm wide, upper ± erect, outer 12-15 mm long, to 7 mm wide., outspread. *Filaments* 18-25 mm long, *anthers* 8-10 mm long. *Style* dividing near apex of anthers. Flowering time: (late May-) August-early October

Freesia andersoniae is the most widespread species of the genus, having been recorded from sites scattered throughout the upper Karoo, Northern Cape and the western Orange Free State. The narrow and acute leaves together with its large pale flower, make *F. andersoniae* easy to distinguish from other species of the genus (Goldblatt, 1982).

2.12 Freesia X Hybrida

The modern freesia, *Freesia x hybrida*, probably originated from several species but which species are in its genetic background is rather controversial. The height of *Freesia x hybrida* is about 300-400mm. Sword-shaped leaves develop in basal fans.

Freesia x hybrida are noted for their fragrance and bright flower colors. The large-flowered hybrids sold by florists feature sweetly fragrant, funnel-shaped flowers (5-10 per stem) that bloom in one-sided racemes atop leafless, arching, wiry stems to 108 cm (18 in.) tall. The plant has a wide range of flower colors including white, yellow, red, blue, lavender, purple, ivory, pink, orange, bicolor and the flower are available year round. Most of the modern commercial freesia has tetraploid.

3. FLOWERING

There are several factors that impact the flowering of *Freesia*. Two main factors are temperature and photoperiod.

3.1 Effect of Temperature

Temperature is the causal factor in flower bud initiation (FBI) and development in *F. x hybrida.* The apical changes associated with FI and subsequent inflorescence development was described by Hartsema (1962). Heide (1965) reported that the complete differentiation required 6 to 9 weeks at 12C to 15C, depending upon environmental conditions. Freesias will initiate flowers over a range of temperatures (5C to 20C) with 12C to 15C being the optimum (Heide, 1965; Jensen and Bendixen, 1971), while temperature above 21C are no inductive (Gilbertson-Ferriss et al., 1981; Mansour, 1968).

For year round production it is necessary to store corms between lifting and planting date. Storage of dry corms can be done at temperatures of 0.5-17C. At this temperature, the corms can be stored as long as 9 months. At low temperature for very long storage, corms will dry out and die, because of the water stress. At higher temperature, the metabolism of the corms remains active to some degree. The young buds start to swell slowly and develop into new corms on the top of the original ones (DeLint, 1969).

The morphological characteristics of the floral spike are controlled by temperature. A normal floral spike has closely spaced flowers. The portion of the scape bearing the flowers will be perpendicular to the remaining scape. Interruption of FBI by high temperature (above 16C) will cause abnormal scape and inflorescence development, characterized by a flower development some distance below the flowers on the spike (DeLint, 1969; Hartsema, 1962; Heide, 1965; Jensen and Bendixen, 1971; Mansour, 1968). After FI, flower development is hastened at temperatures above 16C but is slowed down at lower temperatures, 10C to 12C. Flower development above the 12C to 15C temperature range, however, usually results in decreased floral stem length, number of flowers per spike, and number of lateral floral spikes. Commercially, entire crops are forced between 12 and 15C, which ensures rapid FBI, development, and flower quality. Freesia will flower when grown under an 8-hr inducing temperature (13C) either during the light or dark span, plus 16 hr of no inducing temperature (24C). The quality is acceptable, but below that of freesia grown at a constant temperature between 12C and 15C (Gilbertson-Ferriss et al, 1981).

3.2 Effect of Photoperiod

Flowering of *Freesia x hybrida* appears to be less responsive to photoperiod than to temperature. Floral bud initiation may be stimulated by short days (SD) (Mansour, 1968) and development enhanced by long days (LD) (Gilbertson-Ferriss and Wilkins, 1978; Heide, 1965; Mansour, 1968). SD increase the number of flowers per inflorescence, flower stem length, and the number of lateral floral stems (DeLint, 1969). In contrast, LD hasten anthesis by 6 to 14 days but result in a decrease in the

above flower quality characteristics. The promotive influence of LD and SD on FBI and development decrease with increasing temperatures (DeLint, 1969; Heide, 1965).

It was observed that an interaction between photoperiod and flower colors by scientists (Heide, 1965; Klougart and Jørgensen, 1962). SD stimulated FBI in yellow and white cultivars while LD stimulated the blue and red cultivars. They suggest that two distinct photoperiodic responses exist originating from two separate species. Perhaps the SD response seen in white and yellow cultivars originated from *F. refracta* while the blue and red LD responsive cultivars originated from *F. armstrongii*. As breeding continues *F. x hybrida* may be losing sensitivity to day length. Gilbertson-Ferriss and Wilkins (1978) observed no flowering differences between colors under LD and SD condition on *F. x hybrida*. The responses of the various cultivars to the increase of light were not identical.

3.3 Effect of Hormone Treatments

Some attempts to promote rapid freesia corm emergence by exogenous growth regulators have been tried. Corms treated with benzylamino purine (BA) on elongated terminal shoots broke apical dominance, but the corms had poor root development and unsatisfactory shoot development (van Bragt, 1974). Resoaking these previously BA treated corms in a gibberellin (GA_3) solution stimulated shoot growth. Addition of naphthaleneacetic acid (NAA) to the BA treatment resulted in maintenance of apical dominance and promoted better root growth.

Freesia corms which were recently harvested and soaked in calcium cyanamide (CA) or BA solution required fewer mean days for shoot emergence with 119.8 days for the control and 101.8 or 115.5 days for the respective treatments. Treatment of CA or BA increased the percentage of corms that flowered by 15% over the water soak control (Nakamura et al., 1974).

The rate or quality of flowering in freesia has not been successfully altered by vacuum infusion or soaking freshly harvested freesia corms in solutions of ethephon, GA, BA, or IAA, or in solution with combinations of these growth regulators (Gilbertson-Ferriss and Wilkins, 1981).

Sytsema (1986) reported that cytokinin, a growth hormone, had a positive effect on improving the quality of the inflorescence of *Freesia*. Cytokinins prolonged vase life in number of days until wilting of the inflorescence as well as the third floret.

3.4 Control of Flowering

Che et al.(1998) reported a method, which can keep the commercial production of freesia in 6 consecutive months in China. By combination of forcing, conventional and delayed planting, freesia could be supplied consecutively from December to May of the following year in areas of Shanghai, China. For forcing plant, the corms

were treated with ethylene for breaking dormancy, then treated with chilling and gibberellin. The plants could continuously flower from December to February. By cooling in the hot season and warming in the cold season, freesia could flower from the end of February to March. In conventional planting, freesias cultivated by normal methods could flower from the end of March to April. For delay planting, corms were stored at normal temperature and planted from December 1 to December 20. The flowering could be delayed to May of the following year.

3.5 Dormancy

There is a physiological dormancy for freesia corms. The dormancy of *Freesia* corms does not directly affect flowering since the flower is initiated after sprouting. However, corm dormancy is important to flowering as corm sprouting is a prerequisite for flowering.

The plant of freesia gradually senesces upon completion of flowering. Traditionally, the corms are then dug and held at 31C to 38C for 10 to 13 weeks to insure rapid shoot emergence when replanted (DeLint, 1969). Storage at 3C to 5C immediately after harvest maintains corm dormancy. When stored at 13C, an interesting physiological phenomenon called pupation occurs in which a new corm forms on top of the old corm. This process takes about 8 months and results in a corm, which is also dormant. It is suggested that freesia corms exhibit a physiological dormancy for 4 to 6 weeks which can be broken by storage at 30C (Gilbertson- Ferriss et al., 1981).

In Japan, the exposure of corms to smoke from burned rice husks for three days during the high temperature storage has been commonly used to uniformly release corms from dormancy. The C_2H_4 and CO present in the smoke is believed to be the effective gaseous components. The exposure of corms to C_2H_4 and/or CO during or after a partial high temperature treatment promoted corm sprouting (Masuda and Asahira, 1980, 1981).

4. PROPAGATION

The propagation of freesia has three pathways: seed propagation, corm propagation and tissue culture propagation. Corm propagation and tissue culture are asexual (vegetative) reproduction, while the seed propagation is sexual.

4.1 Seed Propagation

4.1.1 Seed Collection

Seeds of *Freesia* should be collected immediately after they mature. In southern China, the collecting time is in May or June (Long, 1996). After harvesting, the seeds need to be cleaned and then stored in a seed vault.

4.1.2 Seed Germination

Seeds of *Freesia* should be planted in the soft and easy-to drain soil. Seeds are germinated at 15C to 18C media temperature in the dark for rapid, uniform germination in about 21 days. In the Changjiang area of China, the seeds are seeded directly into cold beds, which can be kept warm in winter (Long, 1996).

Modern tetraploid Freesia cultivars develop quickly from seed, to produce an even crop of superb blooms on long stems. Freesia seed (approx. 80 per g) should be sown at 21C, into large modules or small pots, covered with compost to a depth of 1 cm and germinated in darkness, until signs of germination are visible. Do not allow compost to dry out. After germination reduce temperature to around 14C. Cool, moist conditions are essential for robust growth.

4.1.3 Planting

When seedlings are about 5-6 cm tall, 4-5 weeks after sowing, they can be transplanted into deep flats, benches, or pots for forcing. The temperature at this time should be keep at 21C/18C day/night (Gilbertson-Ferriss, 1985).

When these plants have 5-7 visible leaves, the temperature should be lowered to 13C for FBI and flowering. In this way, the plants from seeds of diploid freesia could flower in two years, but the inflorescence could be shorter and the number of florets may be fewer (Long, 1996). Plants have less disease with seeds than with corms.

4.2 Corm Propagation

The corm propagation of freesia is done by division. New clonal plants are genetically identical to the original plants. Corms of freesia require about 3-4 months from planting to flowering.

4.2.1 Corm Collection

New corms are those, which were produced after 1-2 years culturing by seed, the plantlet of tissue culture or seed corms. As these kinds of corms are relatively young,

they are less likely to harbor pathogens or viruses. They are good candidates for cut flower production, however, if the new corms weigh less than 1g, the quality of cut flower will not meet the need of market.

Corms, when used as maternal corms more than once, are called flowered corms. As these corms are reused, their pathogenic rates increase and they may carry more virus than new corms.

Virus-free corms are termed 'first-class corms'. The second-class corms are the new corms. First and second class corms, which are planted for cut flower production for one year, become the third class corms and the following year become fourth class corms. The fourth class corms should not be grown for cut flower production, but may be used in potted flowering plant culture (Long, 1996).

4.2.2 Planting

Corms can be planted directly in benches or flats, or in pots. Details of planting are discussed in the commercial production section of this chapter.

4.3 Tissue Culture

Freesia can be produced *in vitro* via biotechnology through tissue culture. Tissue culture provides a means of rapid clonally propagation of large quantities of virus-free plants. This is important in commercial production of cut flower *Freesia* where vegetative propagation of corms takes a relatively long period of time and can become infected by viruses. Moreover, flowers regenerated *in vitro* have other advantages, such as more flowers per spike, higher quality flowers, and reduced land usage. Furthermore, it is possible to provide genetically identical flowers on a large through biotechnology. This technique will most likely replace other methods (Mader, 1991) in the production of cut flower of *Freesia*.

Various pathways of *in vitro* morphogenesis can be induced from tissue cultures of *Freesia*. Wang et al.(1990, 1993, 1995, 1996) reported that *Freesia refracta* can be regenerated *in vitro* from various parts of the plant through three pathways by varying the exogenous hormonal conditions in culture media. The three pathways are direct somatic embryogenesis, indirect somatic embryogenesis and organogenesis.

4.3.1 Somatic Embryogenesis

Wang et al (1990) first reported that freesia can be regenerated *in vitro* to whole plants through two pathways of somatic embryogenesis, direct and indirect somatic embryogenesis. In direct embryogenesis, somatic embryos can be induced directly from a single cell on the surface of explants of freesia (Wang et al, 1994). In the indirect pathway, there is a stage of calluses, which initiated from the explants of the

plant. The two pathways can be controlled by varying the combinations and levels of exogenous hormones in culture media.

Explants for inducing somatic embryo. The young inflorescence, young leaf, and zygotic embryo are all suitable explants for induction of somatic embryos. The juvenile tissue and organs are more responsive to the inducing stimuli than the more mature tissue when used as explants (Wang et al, 1990).

Under the culture conditions in the author's lab (Wang et al., 1990), a good efficiency of induction of the somatic embryo was obtained with the explants of young inflorescence segments. The young inflorescence of *Freesia* can be separated into two parts, the flower stalk and the flower bud. Although the flower stalks contained no mitotically active meristems, some of the cells in the epidermis were able to induce direct embryogenesis without callus formation when placed under appropriate culture conditions. The dissected flower buds could continue to develop into flowers on culture medium and somatic embryos were initiated from the basal part of the flower (Fig. 25-2).

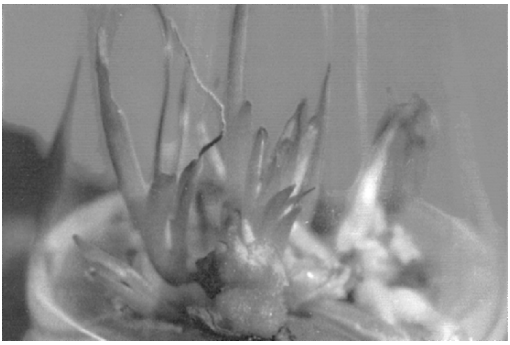

Figure 25-2. Somatic embryo buds directly developed from the young inflorescence segments that were cultured on the modified N6 medium supplemented with 2 mg/l IAA (indole-3-acetic acid) and 3 mg/l BAP (6-benzylaminopurine) without a stage of callus.

A noticeable phenomenon observed in this morphogenetic process was that all of the somatic embryos appeared exclusively at the original morphological lower end of the segments cut from the axes (termed the embryogenic end, EE), while no embryo was formed at the morphological upper end (termed the non-embryogenic end, NEE), irrespective of the gravity of the earth and the position of the explants placed on the medium. Divisions of embryogenic initial cells were observed solely at the EE of the segments in the early stages of culture. SDS-PAGE showed that two polypeptides appeared specifically at the EE of the explanted segments after one day in culture, but they were undetectable at the NEE (Wang et al, 1998).

The segment of young leaf was less regenerative than young inflorescence for embryo induction. Another ideal explant source was the zygotic embryo, which exhibited a high potency of somatic embryo formation (Wang et al, 1996). However,

it is difficult to obtain sufficient embryo material due to the low seed setting rate of freesia.

Induction of somatic embryogenesis and its hormonal control. Explants of freesia cultured *in vitro* can be induced to undergo somatic embryogenesis via two different pathways, i.e., embryos can be formed either directly from epidermal cells of the explants, or indirectly through an intervening callus stage depending on the exogenous hormones included in the media.

Following is an example of inducing direct somatic embryogenesis in *F. refracta*. After three days in culture on the modified N_6 medium (Chu, 1978) supplemented with 2 mg/l IAA (indole-3-acetic acid) and 3 mg/l BAP (6-benzylaminopurine) (Wang et al, 1990), the margins of the explanted segments of young inflorescence began to swell slightly. Subsequent growth and differentiation of the swellings resulted in the formation of globular somatic embryos that became visible within 7-14 days in culture. These embryos arose from the competent cells in the epidermis of the explants via a direct somatic embryogenesis pathway. The most apparent characteristic of this pathway was that the divisions of the pre-existing embryogenic cells in explants gave rise to the embryos without callus formation.

When the segments of young inflorescence were inoculated on MS medium (Murashige and Skoog, 1962) containing 2 mg/l IAA, 0.5mg/l BAP, and 0.5mg/l NAA (naphthalenacetic acid), a translucent, pale-yellow nodular callus formed on explants after 1 week of culture. When this kind of callus was transferred onto MN_6 medium with 2 mg/l IAA and 3 mg/l BAP, globular embryos formed (*Wang* et al,1990).

Table 25-1 summarizes the pathways of somatic embryogenesis in tissue cultures and their dependence on the hormonal conditions. It was possible to manipulate the embryogenic pathways by modifying the medium composition. Among the various factors influencing *in vitro* morphogenesis, the effect of exogenous hormones was most decisive and crucial (Wang et al, 1990).

Table 25-1. Patterns of somatic embryogenesis in explants of young inflorescences of *Freesia refracta* Klatt.

Medium (mg/l)	Pattern of Induction	Number of Explants	Number of explants forming embryos	Induction rate (%)
MN_6 + 2.0 IAA + 3.0 BAP	Direct embryogenesis	50	30-35	60-70
MS + 2.0 IAA + 0.5 BAP + 0.5 NAA ↓ MN_6 + 2.0 IAA + 3.0 BAP	Indirect embryogenesis	50	20-25	40-50

The somatic embryos induced via both the direct embryogenic pathway from explants and the indirect embryogenic pathway from callus cultures were structurally normal, possessing a plumule apex at one end and a radical apex at the other. The development of globular proembryos was marked first by the appearance of a coleoptile, and then simultaneous elongation of the first leaf out of the coleoptile and growth of the root out of the coleorhiza.

Regeneration of plants can be completed without changing the medium composition. For the embryos derived from callus cultures via the indirect pathway, root development was usually delayed when kept in the same culture. Therefore, embryos were subcultured to MN_6 medium without exogenous hormones to promote rooting of the regenerated plants. The established plants were transplanted to pots and were acclimatized in a growth chamber for several days before subsequent transfer to the greenhouse. Generally, 15-20 plants can be obtained directly from a single inflorescence segment of *F. refracta* within a 7-9 week period of culture. More than 20 regenerated plants can be obtained through indirect embryogenesis from an explant segment within about 12-15 weeks (Wang et al., 1990).

4.3.2 Organogenesis

The early work on tissue culture and plant regeneration via organogenesis in *F. refracta* was carried out by Davies and Nichol (1971). Bajaj and Pierik (1974) described the plant recovery from corms, stems, leaves flower buds and anthers under *in vitro* culture conditions in one freesia cultivar. Using buds and roots, Petru et al. (1976) obtained regenerated plants in several cultivars. Other work dealing with freesia tissue culture can be found in the literature (e.g., Hussey, 1975; Mori et al., 1975; Bach, 1984; Bajaj, 1989; Wang, 1996). The results of these studies showed that freesia is very amenable to *in vitro* culture, and the organs and tissues of various parts of the plant have been demonstrated to be capable of regeneration and propagation.

Shoot differentiation of freesia via organogenetic pathways can be induced in callus cultured on MN_6 medium with 3.0 mg/L 2.4-D (2,4-dichlorophenoxyacetic acid), 0.3mg/L NAA, 0.5 mg/L BAP and 0.5mg/L kinetin. Transfer of this callus to the same medium without 2,4 –D resulted in differentiation of green buds, which subsequently developed into shoots. Rooting of the shoots was initiated on hormone-free MN_6 medium.

Yang et al. (1999) reported that the callus of *Freesia* can be stored with the method of cryopreservation. The good candidates for cryopreservation were the calluses, which were subcultured for 15-20 days on medium. The better freezing procedure was to reduce the temperature from 0C to –196C directly. The better thawing condition for the stored calluses was at 4C or in warm-water bath at 40C.

4.3.3 Chromosome Number in Regenerated Plants

Results from cytological studies demonstrated that the regenerated plants from explants of *F. refracta* through direct embryogenesis had a normal diploid chromosome. This could be expected, since here, somatic embryos were formed directly from single epidermal cells in explants without a callus culture stage (Wang et al., 1990, 1994). This characteristics is important for production of *Freesia* in large scale.

The majority of plants obtained through indirect embryogenesis also showed a normal karyotype. Nevertheless, since in the indirect pathway, some of the plants arose from callus that had been kept in culture for up to one year, chromosome variations in regenerated plants were more likely to exist.

Plants recovered from callus cultures via organogenesis showed a considerable variation in chromosome numbers. The examination of the root tip cells of the regenerated plants showed that they were chimeric in chromosome number, with about 60% of the somatic cells being diploid, 10.5% haploid, 8.5% tetraploid, and 21% aneuploid (Wang et al., 1990).

5. COMMERCIAL PRODUCTION

Freesias were first grown commercially in 1873. However it was not until after 1945 that freesias became an important flower crop. About 600 hectares of freesias are currently grown in Europe. An increasing number of cut freesias are being grown in South-America, Asia, and Oceania.

5.1 Greenhouse/Field Corm Production

Freesias can be grown in all moderate climates in either a greenhouse or on field condition. Provided the soil temperature is below 20C for the greatest part of the growing period. The soil temperature should not be below 7C for any length of time. Optimal growing temperature for freesias is between 12C -18C. A day-length of 8 hours during most of the growing period is desirable.

It is important to feed the plants after flowering and until the leaves turn yellow. This enables the bulbs to store enough nutrients to produce flowers the next season.

5.1.1 Soil

All types of soil used for agriculture or horticulture are suitable for growing freesias. The soil should be compost enriched and well drained. Optimal pH. of soil is about 6.5 – 7.5. Never allow the soil to dry out. Moisten soil a few days before planting.

Soil where freesias are to be planted should be free of *Fusarium* disease and weed seeds. It is necessary to pasteurize the soil if *Fusarium* infected freesias have previously been grown. Pasteurizing should be done using steam or chemicals. Do not feed with a fertilizer high in nitrogen, as this will encourage leaf growth at the expense of the flowers.

5.1.2 Planting

Freesias are planted into beds 1 to 1.2 meters wide. The deep of plant for corms is 20 to 40 mm deep. Allow 8 rows per meter, distance between rows 125 mm. A good way to plant is to roll out the support-nets before planting and plant into the squares. Roll out the support-nets soon after planting and fix these well at the ends. With the growth of the freesias these support-nets can be raised.

Cover the soil after planting with 10 or 20 mm. Usage of the peat, pine needles or coarse sawdust as cover-soil will prevent deterioration of the soil and restrict fluctuations in temperature and moisture content.

5.1.3 Water & Temperature

Water. Do not water before freesias are well rooted. After watering, the foliage must be dry again as soon as possible. When cool, water in the morning on a clear or windy day. After harvesting the last flowers, stop watering to prevent Fusarium disease.

Temperature. During the first 6 weeks a temperature between 14^{o}C and 18^{o}C is optimal. During the winter months the temperature from 8^{o}C to 12^{o}C is advisable. During spring and summer keep temperatures as low as possible.

Plants grown from corms can initiate flowers over a wide range of vegetative stages, from 3 to 14 leaves (Gilbertson-Ferriss et al., 1978; Heide, 1965; Mansour, 1968). Freesias raised from corms do not initiate flowers until after sprouting. Plant age at FBI affects the length of the induction period, number of florets, flower stem length, and number of lateral floral. Plants with 3 leaves take 6 to 9 weeks to start FBI, whereas plants with 7 to 8 leaves begin FI in 3 weeks and are well advanced after 6 weeks. However, regardless of the stage when floral induction occurs, date of anthesis varies by only 3 to 5 days, although spike quality increases when more leaves are present at initiation. Consequently, in commercial practice it is desirable to allow from 6 to 8 leaves to develop before starting the floral inducing temperatures of 12C to 15C, as this will ensure high flower quality and yields (Gilbertson-Ferriss, 1985).

During sunny periods in spring and autumn, light shading is usually applied to keep temperatures sufficiently low. In the warm summer months however, depending upon the weather, heavy shading may be necessary from planting until bud formation. The shading will keep the temperature down and prevent the young

foliage from evaporating too much. The soil temperature during the first 6 to 8 weeks determines the time of flowering. After flower bud formation the crop needs more light to develop a good production.

5.1.4 Harvest

Flowers. For the cut flower production, the time of harvest is when the first (lowermost) floret opens. The time between harvesting and placing the flowers in water in the cold store must be as short as possible, certainly during warm weather. Keep buckets clean and fill with fresh water every day. Never harvest too soon. Do not keep flowers in cold store for more than a few days. For market, flowers need to be bunched in 10 or 20 each and boxed carefully.

The procedure of treatment for cut flowers is: 1) cut off the bottom one inch of stem; 2) remove any leaves that will be in the container solution; 3) place the stem into a container of lukewarm water with floral preservation; 4) allow the flowers to condition for about 3 hours and then place in a cooler area (1C -3C).

Corms. After the last flowers have been picked it is still necessary to attend to the crop. As a rule no more water is given because of Fusarium. If very sunny, shading is essential for corms to ripen properly. Usually corms can be lifted four to six weeks after the last flowers are picked.

5.2 Corm Production Using Biotechnology

Biotechnology provides a good way of rapid cloned propagation of large quantities of virus-free plants. However, the regenerated plantlets have to be transplanted to soil for further development and are purchased in this manner.

The author's lab has developed a technique for producing the corms of *Freesia* by tissue culture (Figure 25-3). Corms of the new regenerated plants can be obtained in tubes by varying the combinations and levels of exogenous hormones in culture media (Wang, unpublished data). Corm production in tubes is very important in commercial production of cut flower of *Freesia* since the corm production in soil takes a relatively long period of time and has the problem of virus infection. Moreover, this technique may industrialize the corm production, which can save time and land, increase the quality of corms and decrease the cost of production.

Figure 25-3. Corms of the new regenerated plants of *Freesia x hybrida* can be produced in tubes by varying the combinations and levels of exogenous hormones in culture media.

5.3 Preservation

Before arranging, recut the stems, and remove foliage that will be under water & put in mix of warm water & floral preservative for a few hours or overnight. Place arrangement away from direct sunlight, heat vents, air-conditions and drafts. Add water and remove dying blooms and foliage daily. The average vase life of freesia cut flowers is about four to seven days. To prolong vase life, recut stems every 4-5 days & clean container thoroughly. Rearrange remaining flowers, adding mixture of warm water & floral preservation to your "new" arrangement. Floral preservation is recommended and is available commercially.

The cut-flower preservatives can be classified into three types, preconditioning solution, bud-opening solution and holding solution. Generally, ingredients used in preservative formulas are sugars, germicides, mineral salts, organic acids, vitamins, ethylene inhibitors and antagonists and plant growth regulators. Preconditioning solution for freesia was formulated by Sytsema (1986), 0.2mM STS + 50mg/L BA. Piskornik (1981) reported the ingredient for bud-opening solution as 60g/L S + 250mg / L 8-HQS + 70mg/L CCC + 50mg/L $AgNO_3$.

Not all of the flowers in an inflorescence will open after the stem has been cut. Therefore, a pre-treatment to prolong the life of the inflorescence can be applied directly after cutting. Silver thiosulphate (STS) is most often used in this capacity (Sytsema, 1986).

Freesia is both ethylene- and fluoride-sensitive. They should be treated with anti-ethylene compounds during conditioning/storage. Avoid using fluoridated water throughout the care-and-handling process. Freesias should also not be mixed in a cooler with freshly cut narcissuses or daffodils.

5.4 Problems

Freesia is predominantly grown as a greenhouse cut flower crop. Outdoor growing of some cultivars was popular for a time, but disease problems have substantially decreased the commercial popularity of garden freesias (Gilbertson-Ferriss, 1985).

5.4.1 Diseases

Fusarium disease can occur during the storing of corms. Diseased corms cannot sprout normally or can only generate very small weak plants. The leaves of the infected plant will turn yellow and dried up. Additionally, the contractile roots are brown and rotten. Disinfecting the corms and the soil can prevent Fusarium disease from spreading. Keeping the greenhouse dry and ventilated will decrease the rate of this disease. Destroy affected corms, and drench sound corms in suitable fungicide.

Botrytis, or gray mold, can cause spots on the infected leaves and flowers. During damp periods Botrytis can be a problem. Prevent by spraying with fungicides. Using benomyl and 70% thiophanate to disinfect the greenhouse can prevent the plant from becoming infected with this disease.

5.4.2 Pests

Thrips may attack the blooms and cause silvering and stickiness on leaves. Spider mites wilt the plant, webbing found on highly infested plants. Aphids may eat the flowers and deliver diseases to the plants. Slugs and snails can cause damage to foliage. Spray at regular intervals against thrips with persistent systemic insecticides. A 50% dilution of Fenitrothion is used to kill aphids.

5.4.3 Viruses

As the corms are planted yearly, they are easy to be infected by virus. The main two viruses are cucumber mosaic and tobacco streak virus. The infected plants become stunted, and the spikes are contorted. If viruses infect corms, the quality of cut flowers would not meet the needs of markets.

The following methods can be employed to decrease the infection of virus in *Freesia*: 1) selecting the corms carefully; 2) refreshing the soil of planting bed; 3) disinfecting the greenhouse by suffocating; 4) spraying plants with insecticides; 5) checking the plants frequently and eliminate the infected plants timely. Using tissue culture to product virus-free corms is the best way to avoid virus disease.

5.4.4 Chemicals

Freesias are sensitive to fluorides. Fluorides scorch the leaves of this plant. Moreover, freesias are ethylene sensitive, they should be stored away from fruits and vegetables. Sometimes, ethylene is applied to promote sprouting.

6. BREEDING

6.1 Crossing

As freesias are essentially self-incompatible and are usually cultured in greenhouses, new varieties are usually created by artificial pollination. You can combine sexual and asexual means to create a new variety efficiently. The following is an example of doing this.

First, you can choose two parent plants with two desired traits. The female parent plant was emasculated and carefully bagged. One or two days later, the emasculated flower was pollinated with pollen collected from male parent plant. Collecting the seeds of the cross-pollination. Second, the seeds were planted properly in pots. Once a plant variety with desired characteristics is developed through the sexual reproduction, the vegetative propagation of corms and tissue culture via somatic embryogenesis can be employed. This means has great commercial importance.

We crossed a pink-flower plant (*Freesia x hybrida*) as the female to a male freesia (*Freesia x hybrida*) with white flowers. The new verity has lavender flowers with pleasant scent (Fig. 25-4).

Figure 25-4. Crossing a pink- x white-flowered parent (*Freesia x hybrida*) produced a hybrid with a different color from either parent.

6.2 Crop Ideotype

Selection of superior genotypes is important for commercial production. For *Freesia x hybrida*, breeders usually look for some noteworthy characteristics in the crop ideotype. These characteristics can be summarized as:

Number of florets per spike: 7 or more florets per spike
Flower size: extra large flowered (tetraploid Freesia)
Stem quality: long straight stems (70-80cm)
Flower color: extra clear color
Fragrance: sweetly fragrant
Resistance: disease resistant

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Chapter 26

ROSE

Rosa x hybrida

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Abstract: Roses, *Rosa*, are native to diverse habitats within the Northern hemisphere and over 130 species are recognized. Only seven to ten species, however, are in the background of most modern rose cultivars, leaving vast untapped genetic resources. Cultivars are almost exclusively asexually propagated. Roses are cross-pollinating, woody shrubs, and progeny can segregate widely for traits due to heterozygosity. Most cultivars are tetraploid, while most species are diploid. Limited fertility, reproductive barriers, germination challenges, relatively few founding cultivars among elite germplasm, and the need for large progeny sizes often pose significant challenges to breeders. Increasing knowledge of the inheritance of traits and molecular genetics techniques are valuable tools aiding modern rose breeders. The need for new cultivars remains strong as divergence among rose market types increases, production systems become more specialized, and new markets develop. Breeding objectives with high priority include cut flower cultivars adapted to emerging production regions, blooming potted florist roses with long display life, and lower-maintenance landscape roses.

Key words: Breeding, cultivars, hybridization, inheritance, rose, phylogeny, polyploidy.

1. INTRODUCTION

1.1 Value and Use of Roses

Roses have gained the title of the world's favorite flower in part due to their vast diversity in plant habit and floral characteristics (Cairns, 2001). They have been bred and selected to serve a number of niches including flowering landscape shrubs,

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formal garden specimens, cut flowers, blooming potted plants, and sources of perfume and vitamin C. Roses rank among the top three cut flowers and are worth approximately US$11 billion per year in worldwide retail cut flower sales (International Trade Centre, 1987; Short and Roberts, 1991). In addition, over 200 million roses are planted annually with a value of approximately US$720 million in retail sales (Short and Roberts, 1991).

1.2 Commercial Propagation of Cultivars

Roses are woody shrubs and cultivars are almost exclusively asexually propagated by bud-grafting or from softwood or semi-hardwood cuttings (Hartmann et al., 2002; Krüssmann, 1981). Modern rose cultivars possess high levels of genetic variability (Debener et al, 1996), and progeny tend to segregate widely for traits. Variability in progeny and difficulty in seed germination are key reasons asexual propagation of elite genotypes predominates. Some specialty seed catalogs, however, sell open-pollinated seed of species or variable seed-propagated varieties such as 'Angel Rose' or 'Angel Wings' (*R. chinensis minima* (Sims) Voss). Sexual propagation of roses is primarily used for the development of new cultivars and for the production of some rootstocks.

1.3 Rose Species

The genus *Rosa* contains over 130 recognized species which are native to diverse climatic regions (Cairns, 2000). Roses are native to the Northern hemisphere (20-70^{o} N. lat.) and have been introduced and naturalized throughout the world (Krüssmann, 1981). Within the genus there are three subgenera (Eurosa, Hesperhodos, and Platyrhodon). The subgenus Eurosa contains greater than 95% of rose species and is divided into ten sections (Cairns, 2000; Krüssmann, 1981). Species roses contain an array of desirable traits that breeders can introgress into modern cultivars. Many species roses and early generation hybrids are quite ornamental themselves and should not be overlooked.

1.4 Early Hybridization

Before the 20th century, controlled pollinations were not widely used in rose hybridization (Krüssmann, 1981). New cultivars were generally developed by finding or raising seedlings from open-pollinated flowers of existing cultivars. Few records were kept. As trade increased between Europe, the Middle East, and Asia, various rose species with unique traits were exchanged. This afforded new opportunities for inter-specific hybridization. Cultivars sharing traits which were new and distinct from previous cultivars were frequently grouped as a new horticultural class (Table 26-1). Horticultural classes give consumers an idea of the

characteristics a cultivar possesses and may or may not infer a particular genetic background. Horticultural classes that were in existence before 1867, the year of introduction of the first rose of the hybrid tea class, 'La France', are considered old garden roses (Cairns, 2000; Krüssmann, 1981). Classes of roses recognized from this date to the present are termed modern roses.

Table 26-1. Horticultural rose classes developed by the World Federation of Roses and the American Rose Society (Cairns, 2001).

Species or Cultivar	Class
SPECIES ROSES	Species
Old Garden Roses (OGRs; <1867)	Alba
	Ayrshire
	Bourbon, Climbing Bourbon
	Boursault
	Centifolia
	Damask
	Hybrid Bracteata
	Hybrid China, Climbing Hybrid China
	Hybrid Eglanteria
	Hybrid Foetida
	Hybrid Gallica
	Hybrid Multiflora
	Hybrid Perpetual, Climbing Hybrid Perpetual
	Hybrid Sempervirens
	Hybrid Setigera
	Hybrid Spinosissima
	Misc. OGRs
	Moss, Climbing Moss
	Noisette
	Portland
	Tea, Climbing Tea
Modern Roses, (>1867)	Floribunda, Climbing Floribunda
	Grandiflora, Climbing Grandiflora
	Hybrid Kordesii
	Hybrid Moyesii
	Hybrid Musk
	Hybrid Rugosa
	Hybrid Wichurana
	Hybrid Tea, Climbing Hybrid Tea
	Large-Flowered Climber
	Miniature, Climbing Miniature
	Mini-Flora
	Polyantha, Climbing Polyantha
	Shrub

1.5 Development of Modern Cultivars

Interestingly, only about seven to ten rose species are found in the background of most modern rose cultivars (Figure 26-1). Molecular techniques are being employed to decipher the species contributing to the various horticultural classes and piece together a more complete story of the evolution of cultivated roses. Iwata et al. (2000), for instance, investigated the origin of the Damask roses (*R. damascena*), a class of fragrant roses popular in Europe in the 19th century and still used in the perfume industry today. It was determined through various molecular techniques that *R. moschata* Herrman, *R. gallica* L., and *R. fedtschenkoana* Regal contributed to the four Damask cultivars sampled. The chloroplasts were of *R. moschata*, identifying this species as the cytoplasmic parent. In addition, molecular techniques are being employed to clarify the phylogenetic relationships among species (Debener et al., 1996; Fernández-Romero et al., 2001; Jan et al., 1999; Martin et al., 2001; Matsumoto et al., 1997).

Most species roses have been used in breeding to only a limited degree or not at all. The multiple generations necessary to incorporate traits from species before widely accepted cultivars can be obtained makes introgression from species a long-term objective and has deterred many commercial breeders. However, the most innovative breeding programs, such as the breeding program of Ralph Moore at Sequoia Nursery (Moore, 1978 and 1990), tend to invest at least a small portion of their resources in longer-term introgression efforts.

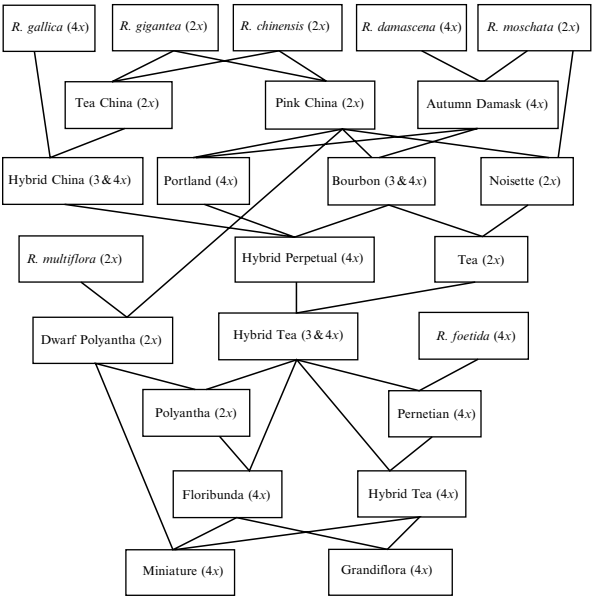

Figure 26-1. The origin of the modern cultivated rose adapted from Stewart (1969).

1.6 Horticultural Rose Classes

Thirty-five horticultural classes have been developed to describe rose cultivars that share general characteristics related to their growth habit, floral display, or genetic derivation (Table 26-1). Characteristics of roses within a class, however, can vary greatly (Julien, 2004). Breeders often make inter-class crosses and assignment of new cultivars to horticultural class is subjective. The most popular horticultural class of modern roses today according to cultivar number is the hybrid tea with over 10,000 registered cultivars (Cairns, 2000). Hybrid tea roses typically have medium to large blooms borne singly or in small clusters and is the primary class used for the cut flower industry (Table 26-2, Figure 26-2). The next most popular horticultural classes of modern roses are floribundas with over 4,500 registered cultivars and miniatures with over 3,000 registered cultivars. Floribundas differ from hybrid teas in that they tend to produce slightly smaller blooms in relatively larger clusters on more compact plants. Miniature roses are proportionately dwarfed in size and can resemble dwarf versions of roses from any horticultural class.

Table 26-2. Descriptive features of the most popular horticultural rose classes.

Horticultural Class	Plant height	Flower Size	Flowers/Stem	Popularity[1]
Hybrid Tea	Medium – tall	Large	One to a few	1
Floribunda	Medium	Medium	Several	2
Miniature	Short	Small	Variable	3
Shrub	Medium – Tall	Variable	Variable	4
Grandiflora	Tall	Medium – Large	Few	5

[1]Based on number of registered cultivars (Cairns, 2000).

1.7 Hybridization

1.7.1 Interspecific

Sexual hybrids are possible among most rose species and horticultural rose classes, although various symptoms of hybrid breakdown and incongruity routinely occur. For instance, Svejda (1974) reported low fertility (only 25 out of 3,562 seedlings were female-fertile) and reciprocal differences in interspecific crosses between diploid hybrid rugosa and hybrid china cultivars (Svejda, 1976). Other common symptoms of hybrid breakdown include early embryo abortion, poor seed germination, albinism, greater than two cotyledons, weak growth, chlorosis, dwarfism, and distorted leaves/blooms.

Figure 26-2. Examples of horticultural classes of roses: (a) hybrid tea (upper left and moving clockwise, 'First Prize'), grandiflora ('Queen Elizabeth®'), miniflora ('Honeybee™'), miniature (1B43, seedling raised by David Zlesak), polyantha ('The Fairy'), floribunda (Day Breaker™'), and (b) shrub roses (left to right, 'Carefree Beauty™', 'George Vancouver', 'Baby Love™', and 'Scarlet Meidiland™').

1.7.2 Intergeneric

One of rose's closest relatives is *Hulthemia persica*, a xerophytic species native to the Middle East and once classified as *Rosa persica* (Cairns, 2000). Breeders have long wanted to introgress the dramatic crimson petal bases of *H. persica* into *Rosa*. Introgression of this trait from *Hulthemia persica* into *Rosa* has been challenging due to reproductive barriers and difficulty growing *H. persica* and hybrids in cultivation (Harkness, 1977). Obtaining backcross hybrids with *Rosa* has been difficult, and less than one in 50 backcross seedlings typically possess the distinctive petal base (Chris Warner, 2002, pers. comm.). 'Tiggles' has yellow flowers with a crimson petal base and is the first recurrent blooming *Rosa* / *Hulthemia* derived cultivar (Figure 26-3b). 'Tiggles' was bred by Chris Warner of England and won a 2001 Royal National Rose Society of Great Britain certificate. In addition, intergeneric hybrids using protoplast fusion with *R. hybrida* 'Frensham' / *Prunus avium x pseudocerasus* 'Colt' and 'Frensham' / *Rubus laciniatus* 'Thornless Oregon' have been attempted and putative hybrids have been regenerated (Mottley et al., 1996).

1.8 Mutation Breeding

Spontaneous color mutations and mutations producing climbing versions of bush roses (or visa versa) are a constant source of new cultivars, but hybridization remains the primary source. Efforts to increase the rate of mutation through radiation, chemical mutagens, and somaclonal variation using callus culture have demonstrated increased mutation rates are possible (Arene et al., 1993: Arnold et al., 1998; Kaicker and Sqarup, 1978). However, inducing mutations for cultivar development has not been popular. Mutations affecting traits less obvious than dramatic changes in petal color and plant habit are likely accumulating over time within cultivars and can lead to subtle, yet detectable differences in cultivar performance across clonal lineages. However, little has been done to identify, control, or take advantage of such variation in roses (Nobbs, 1984). In Easter lily, for instance, the North American market relies primarily on one clone that is >50 years old ('Nellie White'), and bulb producers consciously select and reselect superior somaclones for propagation to control undesired variability (Zlesak and Anderson, 2003).

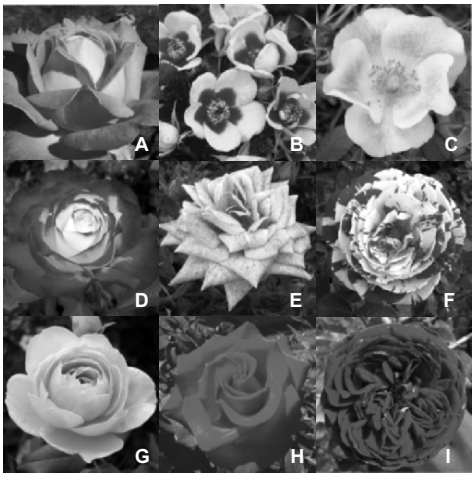

Figure 26-3. Examples of rose flower color patterns include (a) bicolor ('Chicago Peace®'), (b) halo ('Tiggles™', photo provided by Chris Warner), (c) handpainted ('Carefree Delight™'), (d) light induced anthocyanin production ('Double Delight™'), (e) stippling (1T38, seedling raised by David Zlesak), and (f) striping ('Scentimental™'), and examples of rose flower forms include (g) cupped ('Heritage®'), (h) high-centered and pointed ('Timeless™'), (i) quartered ('The Prince™'), and (b,c) single.

2. CYTOGENETICS

2.1 Chromosome Number

Rose species range from diploid ($2n$=$2x$=14) to octoploid ($2n$=$8x$=56) with most being diploid (Cairns, 2000). The majority of species have been documented at only a single ploidy level, while a few form polyploid series (Cairns, 2000). *Rosa acicularis*, for example, can be found from the diploid to octoploid level with increasing ploidy levels at higher latitudes (Krüssmann, 1981). Aneuploidy, incomplete sets of chromosomes, is rare in roses, as is the presence of supernumerary or B chromosomes (Erlanson, 1933; Lata, 1982; Rowley, 1961; Shahare and Shastry, 1963). Most modern classes of roses are tetraploid and many breeders choose to work exclusively with elite germplasm at the tetraploid level (Krüssmann, 1981). Some older horticultural classes of roses are primarily diploid such as hybrid Chinas and hybrid multifloras. Crosses between diploid and tetraploid roses result primarily in triploid progeny. Triploids generally have reduced fertility and can become a bottleneck for the development of additional generations (Rowley, 1960).

2.1.1 Polyploidization

Mitotic polyploidization of diploid germplasm before crossing with tetraploids has been accomplished through colchicine, oryzalin, or trifluralin application to the apical meristem of young seedlings and apical or axillary meristems of established clones (Basye, 1990; Kermani et al., 2003; Ma et al., 1997a; Roberts et al., 1990; Semeniuk and Arisumi, 1968; Zlesak et al., 2002). Careful identification, isolation, and propagation of tetraploid tissue are necessary due to the high frequency of chimeras with topically applied chromosome doubling agents. Meiotic polyploidization is another method to bridge ploidy levels, however, the use of 2n gametes in roses has been limited due to minimal diploid germplasm known to possess them and lack of understanding of their inheritance in roses. Consistent 2n gamete production in diploid rose genotypes has recently been reported and offers new possibilities to breeders (Crespel et al., 2002b; El Mokadem et al., 2000 and 2001).

2.1.2 Haploidization

Diploid roses derived from modern tetraploids would allow for multiple research and cultivar development opportunities. Crosses can be made between haploids and diploid species or older diploid cultivars while staying at the diploid level (Crespel et al., 2002b). Inheritance studies are more easily performed at the

diploid level due to more manageable segregation ratios (Debener, 1999). In addition, inheritance studies using diploids having sought after traits found in the most advanced tetraploid cultivars would aid in the study of such traits, especially if they are not easily found in present diploid germplasm. Haploidization through anther culture has not been successful in roses due to challenges in callus induction and shoot regeneration (Tabaeizadeen and Khosh-Khui, 1981; Wissemann et al., 1998). Recently reported methods to optimize *in vitro* adventitious shoot regeneration from leaf explants is worth exploring for haploid regeneration using anther or microspore culture (Zlesak et al., 2004). Haploids have been obtained using irradiated pollen and embryo rescue from tetraploid cut flower cultivars including 'Sonia' (syn. 'Sweet Promise') and 'Leonidas®' (Crespel et al., 2002b; El Mokadem et al., 2002; Meynet et al., 1994). Additional methods of haploid extraction commonly used in other crops could be explored in roses and include parthenogenesis using males with a dominant selectable marker, twin seedlings where periodically the second embryo has developed from a synergid and is haploid, and inter-generic crosses with genome elimination of the paternal parent (Chase, 1969; Laurie and Bennett, 1986; Rowe, 1974). One dominant, single gene marker that could be useful in the identification of haploids from recurrent blooming tetraploid roses is non-recurrent bloom, a condensed flowering period in late spring / early summer induced by vernalization (Semeniuk, 1971a, 1971b). Crosses of recurrent blooming, tetraploid females with males which are homozygous dominant for non-recurrent bloom should produce non-recurrent progeny needing vernalization to stimulate flowering. Rare, recurrent blooming progeny (flowering without vernalization) may be haploid and can be confirmed for ploidy level.

2.2 Meiosis

Meiosis in roses varies greatly depending on ploidy, homology between genomes of ancestral species, and structural changes and rearrangements in chromosomes. Shahare and Shastry (1963) suggest that changes to chromosomal structure may be a significant factor explaining to the high rates of univalents and heteromorphic bivalents they observed. They found that such meiotic abnormalities were associated with decreased fertility. Erlanson (1933) found multivalent configurations even in diploids which she suggested may be due to duplications or translocations. Recently, Ma et al. (2000) observed meiosis in tetraploid progeny of a cross having four species as recent ancestors. Variability was observed among and within seedlings for meiotic configurations, suggesting both disomic and tetrasomic inheritance depending on homology between homoeologous chromosomes. Ma et al. (2000), however, did not find significant correlation between pollen stainability and multivalent pairing or pollen stainability and frequency of chiasma binding one or both chromosome arms. Complexities in meiosis can offer challenges to the breeder through effects on fertility and inheritance.

Species in the Caninae, or dog rose, section are polyploid, and unequal distribution of chromosomes to gametes occurs within this rose section that is gender dependent (Täckholm, 1920). In addition, apomixis has been documented among Caninae species (Werlemark, 2000). During typical gametogenesis in section Caninae roses, seven bivalents occur with the rest of the chromosomes as univalents. The female gamete retains the univalents plus one chromosome set of the bivalent pair. In the male, the univalents are lost and each gamete receives one set of seven chromosomes. Due to unequal meiosis, progeny involving crosses with these species tend to be skewed to the maternal parent (Werlemark et al., 1999), and ploidy of inter-specific hybrids can be manipulated depending on the direction of the cross. Many propagators favor seedling-raised Caninae section species for the production of uniform, virus-free rootstocks.

3. FERTILITY

3.1 Breeding Practices Affecting Fertility

Fertility varies considerably among rose cultivars and has led breeders to strongly favor the more fertile genotypes for use as parents. The variable and often low fertility of modern rose cultivars may be due to a number of factors including incongruity due to inter-specific derivation, meiotic abnormalities, and the accumulation of deleterious recessive alleles through generations of crossing heterozygous, polyploid parents (Erlanson, 1931; Ogilvie et al., 1991). In general, it is more difficult to find amenable female parents than male parents. This may be due in part to low fertility in males being overcome through heavy pollen application and pollen competition. The practice of breeders frequently using a limited number of fertile cultivars that produce above average progeny has led to considerable inbreeding in modern rose classes (de Vries and Dubois, 1996), and inbreeding can lead to reduced fertility by increasing the frequency of gametes (n=2x) homozygous for deleterious or lethal recessive alleles (Ogilvie et al., 1991). Many breeders are concerned that the genetic base of modern rose classes (i.e. hybrid teas and floribundas) is too narrow (de Vries and Dubois, 1996).

3.2 Environment and Gamete Viability

Visser et al. (1977a) found that the environment during microgametogenesis affects pollen viability and, in general, lower viability was found at higher temperatures. In addition, physiological changes affecting fertility also occur throughout the growing season (Gudin, 1992; Gudin et al., 1991). Gudin (1992) found that bloom production and fertility differed among plants of the miniature rose

'Orange Sunblaze™' grown under different environments before being brought into a common environment. One group was held at 4C for four weeks and the other group was kept in active growth during the four week period.

Both groups were pruned and allowed to regrow. After regrowth, those given the vernalization treatment had more blooms per plant (12.25 versus 5.62) as well as better pollen viability, hip production, and seed production. A hip, or rose fruit, is a developed hypanthium, a cup-shaped extension of fused floral parts (Figure 26.4). Increased fertility in vernalized plants continued in the second bloom cycle, but to a reduced degree.

3.3 Self Incompatibility

Gametophytic self incompatibility is present in the rose family (Heslop-Harrision and Shivanna, 1977). Rarely has self fertilization been reported in diploid rose species, and self-fertilization is limited, but more common, in polyploid species (Cole and Melton, 1986; Ueda and Akimoto, 2001). Self fertilization, however, is common among tetraploid cultivars (Morey, 1959; Rajapakse et al., 2001; Zlesak, 1998), and emasculation before anthesis is routinely practiced to avoid contamination of controlled crosses. Gametic self incompatibility has been overcome in a number of crops through methods including high temperature treatment to flowers before or at pollination, stylar treatments (i.e. cut styles and chemical application), polyploidy, and selecting for pseudo self-compatibility (Ascher, 1976). Exploring these methods to induce self fertilization in normally self-incompatible roses can aid in developing inbred lines for genetic studies and the development of uniform F_1 seed-propagated hybrids. High temperature treatment is one method which may be effective in roses. In a warm summer greenhouse (>37C for daytime highs) the author has had seed set on diploid *R. chinensis minima* genotypes which have not set seed in the greenhouse without controlled cross-pollination when grown under cooler conditions. To test if the seed was from self-fertilization, seedlings of the sole genotype were raised that were homozygous recessive for two monogenic traits, petal color (white) and prickles (thornless) (Debener, 1999). All of the >30 seedlings were white and thornless, suggesting self fertilization.

3.4 Pollen Storage

Rose pollen can be used fresh or preserved for later use at temperatures near or below freezing (Khosh-Khui et al., 1976; Rajesekharan and Ganeshan, 1994; Visser et al., 1977b). Rajesekharan and Ganeshan (1994) report that after one year, cryopreserved pollen had comparable germination to fresh pollen, and fertilization as measured by hip and seed number was generally comparable between cryopreserved and fresh pollen. Pollen stored with desiccant at -18C has proven to

be effective in fertilization after at least two years (Kathy Zuzek, 1999, personal communication). Some breeders pollinate immediately following emasculation, while others wait a day or more until a sticky exudate is present on the stigmas. One application of pollen is routine for most breeders. De Vries and Dubois (1983) found that repeated pollinations at daily intervals significantly increased seeds per pollinated flower up to five pollinations. The increase in seeds per pollinated flower from one to two pollinations was more than two-fold (4.1 to 9.5), but was less than two-fold (9.5 to 15.6) between the second and fifth pollinations. An application of putrescine to styles and stigmas at emasculation extended the duration during which fertilization can occur and in some cultivars increased the rate of hip formation (Gudin and Arené, 1992). Gibberellic acid (GA_3) (250ppm) applied 10 days after pollination was found to increase rate of hip formation, but in some cultivars decreased number of seeds per hip (Ogilvie et al., 1991). Dubois and de Vries (1986) found a trend for both increased hip and seed set as GA_{4+7} concentration increased from 0 to 1,250 ppm tested at 0, 7, or 14 days after pollination.

3.5 Cultural Management of Maternal Parents

Commercial rose breeders typically perform pollinations on plants growing in pots and use greenhouses or shade houses to have greater environmental control. De Vries and Dubois (1987) grew plants of 'Sonia' at five constant temperatures (10, 14, 18, 22, and 26C) and found that the optimum temperature for rate of hip maturation was 18C for a cross of 'Sonia' x 'Hadley'. However, 22C was the best temperature for number of seeds per pollinated flower as well as subsequent germination. After pollination, hips typically mature in three to five months depending on genotype and environment. Breeders using females in outdoor beds typically make crosses with the first or second cycle of bloom to allow enough time for maturation. Upon ripening, hips turn a lighter shade of green, yellow, orange, red, or purple. Number of seeds per hip can range from zero to greater than 50.

4. GERMINATION

4.1 Erratic Germination

Achieving uniform and high rates of germination has long been a challenge for rose breeders. Rose seeds are achenes, individual fruits, consisting of a pericarp (epi-, meso-, and endocarp), testa, and embryo (Figure 26.5). Low and non-uniform germination from variation in achene viability and dormancy limits progeny sizes and lengthens generation intervals (germination occurs within weeks of harvest to years). Since roses are native to northern, temperate climates (20-70^o latitude),

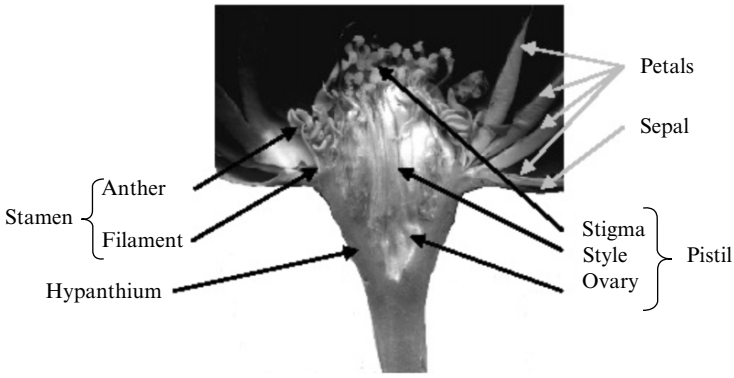

Figure 26-4. Morphology of a rose flower (cv. Lipstick).

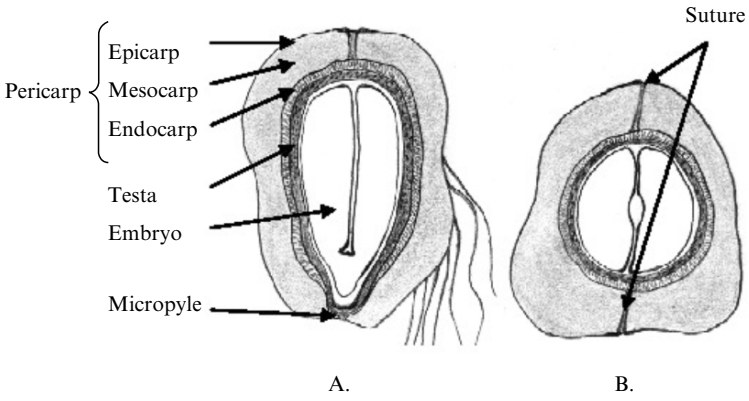

Figure 26-5. Morphology of a rose achene. (A.) longitudinal view and (B.) transversal view adapted from Jackson (1968) and Rowley (1956).

periods of chilling (cold stratification) to overcome dormancy has evolved as a fundamental mechanism to avoid premature germination and seedling death (Hartmann et al., 2002). The degree of dormancy as well as time and temperature necessary to overcome dormancy varies according to germplasm, maturity at harvest, time of seed extraction, temperatures during seed development, and temperature and duration of stratification (Buckley, 1985; Gudin et al., 1990; Rowley, 1956; Semeniuk and Stewart, 1962). Germination challenges have led breeders to indirectly select parents with greater fecundity due to increased fertility along with reduced germination barriers and to investigate an array of parameters to overcome dormancy and promote early, uniform, and high rates of germination.

4.2 Chemical Dormancy

Water soluble germination inhibitors are produced in the pericarp and testa, but not the embryo. Excised embryos readily germinate when the pericarp, testa, and hip are completely removed (Barton, 1961; Blundell, 1965; Bo et al., 1995; Jackson and Blundell, 1963; Yambe et al., 1992). The key germination inhibitor known in roses, and many other plant species, is abscissic acid (ABA) (Cornforth et al., 1966; Hartmann et al., 2002). In a survey of three rose species, increasing ABA concentration followed increasing dormancy, and within *R. rugosa* Thunberg, excised embryos treated with varying levels of exogenous ABA showed increased dormancy with increased concentrations (Jackson, 1968). Bo et al. (1995) examined achenes from the hybrid tea 'Crimson Glory' and determined that the highest ABA concentration was in the testa (1.38 ug g^{-1}), followed by the pericarp (0.85 ug g^{-1}), and then the embryo (0.18 ug g^{-1}). Concentration of ABA in the hip was not reported. Although excision of embryos results in consistently high and uniform germination, it is rarely practiced due to difficulty of pericarp removal and risk of embryo injury. Maturity at hip collection and time of achene extraction also affects germination. Rowley (1956) found that the best germination of *R. canina* L. seed occurred when hips were collected after full coloration, but before the hypanthium began to soften. Waiting beyond initial coloration to collect hips and remove achenes appears to be associated with greater dormancy and may be associated with increased ABA levels.

Plant hormones have been explored to aid rose seed germination, but with mixed results. Jackson (1968) found that GA and benzylaminopurine (BA) help to overcome dormancy and improve seed germination, but others, including the author, have not found them beneficial. The effects of auxin, ethylene, and other plant growth regulators on rose germination have not been sufficiently investigated.

4.3 Physical Dormancy

The pericarp has been thought to limit germination due to being a physical barrier to water penetration and embryo expansion. Bo et al. (1995) compared imbibition time of rose, Chinese cabbage, and cucumber and found the time for imbibition to be >24, 4, and 6 hours respectively. Scarification with macerating enzymes has been shown to loosen bonds among cells in the middle lamella in the pericarp and aid germination by allowing for easier splitting of the pericarp along the suture (Yambe and Takeno, 1992). Other forms of scarification have also been investigated. Blundell and Jackson (1971) found that treatment with sulfuric acid before stratification was beneficial, but Morey (1956) found it to be detrimental. Perhaps variation in duration of acid application, concentration of acid, and pericarp morphology among germplasm may have led to mixed results.

Gudin et al. (1990) investigated the effect of temperature during seed development and subsequent germination and found that increased endocarp thickness reduces germination and endocarp thickness is affected by temperature during development and by paternal parent. They found that thicker endocarps developed when female plants were grown at cooler temperatures (15C versus 18-24C). In addition, embryos with relatively slower growth rates had significantly thicker endocarps and embryo growth rates can be explained in part due to paternal parent. Interestingly, even though endocarp thickness was increased at cooler temperatures, overall pericarp thickness was unaffected. Final endocarp thickness appears to be more dependent on temperatures early in achene development rather than later. More experiments are warranted to determine if the negative effect on germination from a thickened endocarp is due to reduced water penetration, increased ABA synthesis, or both.

4.4 Hip Harvest

Standards for determining harvest time vary by breeder. Some breeders collect hips when hypanthium tissue just starts to change color in an effort to avoid accumulation of growth inhibitors. Others prefer to collect hips when the hip has completely changed color, but before it softens. Those doing the latter believe excessive accumulation of germination inhibitors in the achenes is avoided, yet additional photosynthate translocation to the embryo occurs and aids in germination. Hips are typically collected before a hard freeze by breeders in cold climates that do not have the benefit of a greenhouse. Freezing before full seed development may result in lower seed germination, especially in more cold sensitive germplasm. Dionne (1993) observed that if cane injury has not occurred due to cold temperatures, achenes contained in hips on such canes generally remain viable.

4.5 Achene Extraction

Achene extraction is most commonly performed manually, however, some breeders working with large seed lots prefer to use food blenders with dulled cutting blades, water, and decanting. The frequency and degree of embryo injury must be closely monitored when using the blender technique. After extraction from hips, achenes are typically kept moist because drying of achenes is generally thought to reduce germination, although drying is commonly used for longer-term storage of seeds of rose species for restoration work (Gill and Pogge, 1974). A comprehensive study looking at the effects of drying has not been reported. After achene extraction, some breeders use a float test to separate those with viable embryos (they are denser than water and typically sink) from those with aborted embryos (air pockets typically cause such achenes to float). The float test is not completely accurate as more achenes tend to sink over time. In addition, achenes with viable embryos can have air pockets between the embryo and pericarp, and some species like *R. rugosa* are buoyant and typically float (Svejda, 1972).

4.6 Stratification

Cold stratification has been the most commonly and widely used means to stimulate germination in *Rosa*. The benefit of a warm stratification treatment before cold stratification has also been reported, especially for species exhibiting a relatively long dormancy period like *R. canina* and *R. rugosa* (Rowley, 1956; Svejda and Poapst, 1972; Werlemark et al., 1995). Warm stratification periods typically are administered at room temperature (20C) for a period of 4-12 weeks with the achenes kept moist in sand, peat, vermiculite, perlite, or paper. Rowley (1956) found that a greenhouse with elevated temperatures (>20C) worked better than a constant 20C for *R. canina*, but does not report actual temperatures attained in this greenhouse. Warm stratification before cold stratification is thought to benefit germination by allowing better imbibition before cold stratification by facilitating pericarp softening and decay (Blundell and Jackson, 1971).

Cold stratification is typically provided by commercial breeders at temperatures between 2-5C for 6-12 weeks. Semeniuk and Stewart (1962) found that secondary dormancy can be induced in some species by warming chilled (4.4C) rose achenes to 15-18C before their chilling hours were fully satisfied, necessitating achenes to begin accumulating chilling hours anew. Once dormancy requirements are met, achenes may start to germinate during cold stratification. Raising the temperature to 13-21C after cold stratification allows germination to proceed at a faster rate and allows for better growth of emerged seedlings. If temperatures rise above 27C, germination often ceases and secondary dormancy is induced in non-germinated achenes (LeGrice, 1965).

4.7 Light

Achenes of *R. multiflora* Thunberg are photoblastic (Yambe et al., 1995). Exposures to only two minutes of red light (2 Wm^2) stimulated germination and was reversible with exposure to far red light. The photoblastic response was strongest soon after harvest and was less effective over time. Future experiments are warranted to determine the presence of a photoblastic response in other species and rose cultivars as well as possible interactions between light and temperature. Because periods of only two minutes of red light are needed for a positive effect on germination of *R. multiflora*, rose breeders may unknowingly be providing an effective light treatment to rose seed with the use of florescent light or natural light during handling.

4.8 Commercial Germination Protocols

Most large-scale commercial rose breeders plant achenes in greenhouse beds of soilless media ~5cm apart (Krüssmann, 1981). Warm and cold stratification are either administered to the whole greenhouse after planting or to seed lots before planting in containers with moist medium. Stratifying seed lots in containers before planting can save valuable greenhouse space, fuel costs, and allow for the use of growth chambers where temperature can be controlled more accurately. Fungicide drenches are routinely used on seed beds to prevent pathogens that lead to damping-off, and fungicide sprays are typically used on seedlings to prevent powdery mildew outbreaks in the greenhouse. Seed beds are routinely discarded after the first season, although additional germination can occur in subsequent years.

5. SELECTION

5.1 Early Roguing

Most recurrent blooming roses flower within several weeks of germination, allowing selection for floral traits to be made relatively early compared to most woody species (Zimmerman, 1972). Many factors are considered in the breeder's decision to rogue. Seedlings with weak growth, disease, colors that are "muddy" or fade unattractively, spent petals that do not fall cleanly, and blooms that are grossly misshapen are relatively easy to identify and remove. Practicing heavy selection at the first bloom tends to emphasize floral traits, allowing for more rapid progress in these traits. Most commercial breeding programs typically rogue 75-95% of seedlings at first bloom. Gain from selection is generally slower for traits like disease resistance and winter hardiness, where more resources are needed to assess a

seedling's performance. Strong emphasis and early selection for floral traits leads to the possibility that population sizes may be so strongly bottlenecked that there may be little variation left to make gain from selection for non-floral traits which take more time to express themselves.

Strong early selection for floral traits is evident in the wide range of flower forms and colors present in modern roses (Figure 26-3). Selection objectives for floral traits such as color and form shift over time as consumer preferences change. For instance, in the mid 20th century there was intense selection for blooms with uniformly tall petals (high centers) that are wide and spiral open from a pointed center. However, due to the efforts of breeders like David Austin (breeder of the popular English roses), there has been a growing trend for modern roses with flower forms reminiscent of many old garden roses (i.e. high petal counts with petals cupped upward or condensed in the center of the flower in quadrants) and fragrance. Roses with nostalgic or "old-fashioned" form have always been among seedling populations, but now breeders are selecting for them instead of against them. Because floral characteristics sway consumers heavily in purchasing decisions at both the florist shop and garden center, heavy selection for floral characteristics is commonly practiced. Cultivars that have average or below average floral characteristics and possess traits that may not be evident in the retail environment, such as disease resistance and greater stress tolerance, often need additional marketing to communicate such benefits and stimulate demand. Without exceptional floral characteristics to command the attention of shoppers, a rose tends to be much less desirable to the average consumer.

5.2 Effectiveness of Early Selection

Little is known about the stability and expression of traits in roses as they transition from young seedlings to mature plants and across different environments. De Vries and colleagues performed a number of experiments to determine the efficiency of early seedling selection. Their objective was to breed for hybrid tea florist roses suitable for winter production in the Netherlands under low irradiance and short photoperiods. Reduced cut flower productivity during winter is typical in the Netherlands due to a combination of reduced shoot numbers per unit area as well as an increased percentage of "blind" shoots, shoots where the flower bud aborts early in development.

5.2.1 Selection for Low Irradiance Tolerance

When varying irradiance level (4-24 Wm^{-2}) during an eight hour photoperiod, de Vries and Smeets (1978a) found that seedlings grown under lower light intensities had fewer petals and grew more slowly than those at higher irradiance, but produced the same number of nodes before flowering. De Vries and Smeets (1978b) report

that at the lowest light intensity (4 Wm^{-2}), there was 80% mortality and the lowest ratio of blooming to non-blooming seedlings. As irradiance increased, survival and the percentage of blooming seedlings increased. Family x environment interaction was not significant for blooming across irradiance levels, and the authors suggest the best selection environment is 8 Wm^{-2}.

De Vries et al. (1978) clonally propagated a sample of young seedlings selected for a high percentage of blooming shoots and a high percentage of blind shoots under low irradiance and monitored bloom production over time in a simulated commercial setting. They found that clones selected for a high percentage of blooming shoots under low irradiance continued to produce a higher percentage of blooming shoots and slightly more shoots overall compared to those selected for a high percent of blind shoots under low irradiance. This trend was observed throughout the whole growing season, but especially in winter, demonstrating that early seedling selection for blooming under low irradiance is effective. De Vries et al. (1980a) suggest that additive gene action is the main component for ability to bloom under low irradiance.

5.2.2 Selection for Flower Yield

De Vries (1976) found that flower yield can reliably be selected in young hybrid tea populations. Seedlings that flowered quickly continued this trend in subsequent bloom cycles, increasing overall flower yield per plant. Unfortunately, the stems were generally shorter for clones with higher bloom yields which limits the applicability of this selection tool for cut roses, because increased value is given to blooms with longer stems. Reduced time to bloom and shorter stems, however, can be an asset in roses used in the landscape or for the flowering potted plant market where compact, floriferous plants are desired.

5.3 Increased Progeny Size

There is a recent trend for hundreds of thousands of seedlings being raised annually by each of the leading commercial breeders. Herb Swim, a 20th century American rose breeder, never raised over 12,000 rose seedlings per season and developed 25 cultivars that won the prestigious All-America Rose Selection (AARS) award (Swim, 1988). This is more AARS award winners than any other breeder to date. Swim kept a higher ratio of seedlings past first flower than many current breeders and believed that the more he critically observed maturing seedlings, the more desirable qualities he saw emerge. Resources are limited and each breeder needs to find a compromise in resource allocation between raising large progeny sizes to increase the probability of having superior genotypes among their populations and evaluation of each genotype so superior genotypes can be identified.

5.4 Marker Assisted Selection (MAS)

Crespel et al. (2002a), Debener and Mattiesch (1999), and Rajapakse et al. (2001) constructed genetic linkage maps for diploid and tetraploid rose populations using AFLP, RAPD, and SSR markers. Markers were found that segregate with the qualitatively inherited traits petal number (double / single flowers), flower color (pink / white), recurrent bloom (+ / -), and prickles (+ / -) (Crespel et al., 2002a; Debener, 1999; Rajapakse et al., 2001). Molecular markers can be useful to aid in early seedling selection and physical mapping through linkage to traits of interest and for selection of progeny possessing multiple, desirable genes for gene pyramiding (Debener, 1999; Ma et al., 1997; von Malak and Debener, 1998). In addition, MAS can aid in introgression projects by helping identify progeny possessing relatively less of the donor parents genetic background, yet still express the trait of interest from the donor parent (Debener et al., 2004). Before MAS in roses will have greater applicability for cultivar development, more markers need to be found which are linked to traits of economic importance and especially markers linked to traits that are more costly to select using conventional, phenotypic selection.

5.5 Rootstocks

5.5.1 Propagating Selections-Grafting Versus Own-Root

Utilizing commercial propagation practices allows breeders to assess the selection's ease of propagation and its performance as the consumer will see it. Rootstock-scion interactions are difficult to predict and rootstocks can affect the vigor and floral traits of the scion (Lindstrom and Kiplinger, 1955). A vigorous rootstock typically enhances scion performance. However, this is not always the case, such as with some greenhouse cut rose cultivars grown in rockwool (Miller, 1985). There is a trend in the United States towards own-root production (propagation of cultivars from softwood and semi-hardwood cuttings), although, in Europe such a shift is not occurring.

5.5.2 Rootstock Breeding and Selection

Although the majority of modern rose cultivars are sold bud-grafted to rootstock, it is surprising that the same major rootstocks have been dominant during the past 50 years (Halevy, 1986). Commonly used rootstocks include *R. canina*, *R. indica major*, *R. multiflora*, 'Manetti', and 'Dr. Huey'. The major traits important for in rose rootstocks are compatibility with a wide array of cultivars, ease of bud-grafting over an extended season, well-branched, flexible root systems, tolerance to various

soil types, vigor, disease resistance, reduced thorn number, reduced suckering, and ease of propagation (Buck, 1978). Although universally adaptable rootstocks would be preferred by growers, they may not be feasible. Different target environments require rootstocks with unique qualities. For example, gardeners in Florida favor the nematode-resistant rootstock, 'Fortuniana', gardeners in climates with low winter temperatures favor *R. mulitflora* and *R. canina*, and greenhouse growers favor 'Manetti' because of its reduced dormancy requirements for year round production. With the trend in some regions towards increased own-root production, resources devoted to rootstock development may decrease. However, one high priority that remains is long-lived and strong rootstocks or interstocks for the trunks of rose standards.

5.6 Advanced Trials

After superior seedling selections are made and genotypes are asexually propagated, many nurseries send selections to off-site collaborators for advanced trials. These trials are often conducted in partnership with public or private gardens. Genotypes that prove to be widely adapted and consistently high performers are retained and further propagated. The best genotypes are then typically entered into independent trial/award programs which are generally open to all commercial breeders before the final decision regarding commercial introduction is made. The time from germination to cultivar introduction is typically seven to ten years.

6. TRAIT INHERITANCE

6.1 Aiding Breeding Decisions

Understanding the inheritance of key traits allows breeders to better identify and achieve their objectives (Table 26.3). Depending on the combination of traits and relative importance given to each, different breeding methods and parents may be more efficient. For instance, if a breeder wants to emphasize quantitative traits, recurrent selection may be an appropriate approach. Whereas to obtain the expression of recessive qualitative traits, some form of inbreeding (i.e. backcrossing, selfing, and sib-mating) and progeny tests would be appropriate.

6.2 Disease Resistance

Roses are susceptible to a number of diseases (Horst, 1983), and increasing host resistance is becoming a higher priority for many rose breeders. The public's demand for low-maintenance, "environmentally-friendly" roses is growing as more

gardeners are becoming less willing to expose themselves and their families to pesticides, legislation restricts pesticide use, and the costs of pesticides increase. In addition, disease resistance saves production costs as well. The primary fungal diseases of rose are black spot (*Diplocarpon rosae*), powdery mildew (*Sphaerotheca pannosa* var. *rosae*), rust (*Phragmidium* spp.), downy mildew (*Peronospora sparsa*), spot anthracnose (*Cercospora* spp.), and verticillum wilt (*Verticillium albo-atrum* or *Verticillium dahliae*) (Horst, 1983). The primary bacterial disease of rose is crown gall (*Agrobacterium tumefaciens*) (Horst, 1983). The greatest amount of documented resistance breeding in roses has been with black spot. Perhaps this is due to the potential for rapid defoliation, most cultivars are susceptible, the pathogen is amenable to *in vitro* culture, and the ability to more easily monitor infection and lesion growth than with other diseases. Host resistance to *Diplocarpon rosae* can be race specific (Bolton and Svejda, 1979; Debener et al., 1998; von Malek and Debener, 1998; Yokoya et al., 2000) or non-race specific (Xue and Davidson, 1998).

6.2.1 Race-Specific Resistance

Although race-specific resistance is often more easily selected for and introgressed due to its generally high expression and typically monogenic, dominant nature, there is a constant threat of the resistance breaking down. For instance, many breeders have recently used 'Baby Love™' as a source of extreme black spot resistance. However, this resistance has recently been shown to be race specific and has already been compromised (Yokoya et al., 2000). Sudden susceptibility among once highly black spot resistant roses has also occurred within hybrid rugosa cultivars which are used primarily in the landscape (Bolton and Svejda, 1979). The hybrid rugosas 'Hansa', 'Martin Frobisher', 'Scarlet Pavement', and 'Pristine Pavement' have shown severe black spot defoliation during recent years in the Northern Midwest of the United States. Since most rugosa hybrids display a strong phytotoxic response to fungicides (Olson and Whitman, 1998), cultivars that have severe infections of black spot have limited value as long as active virulence allele(s) remain in local pathotypes.

To increase the probability of durable host resistance, one approach breeders can take is to stack or pyramid multiple race specific resistance alleles. The only characterized gene for black spot resistance (*Rdr1*) and gene for powdery mildew resistance (*Rpp1*) are race specific (Linde and Debener, 2003; von Malek and Debener, 1998). Given this, pyramiding *Rosa* derived resistance genes and alleles is limited until more are identified. Molecular markers tightly linked to resistance genes can aid in the selection of individuals with multiple resistance factors (Von Malek et al., 2000). Even if multiple resistance genes and alleles can be stacked, the threat still exists that the corresponding virulence alleles can be acquired by a single pathotype allowing for a breakdown in resistance.

6.2.2 Non-Race-Specific Resistance

An approach that should have greater durability is non-race-specific or horizontal resistance, an incomplete form of resistance typically due to multiple genes having an overall additive effect (Simmonds, 1991). Horizontal resistance typically is effective against all races of a pathogen and is difficult for the pathogen population to overcome. Xue and Davidson (1998) screened 11 cultivars and breeding lines and found varying levels of partial resistance to black spot. Five components of partial resistance were scored; incubation period (IP), leaf area with symptoms (LAS), number of lesions (NL), lesion length (LL), and sporulation capacity (SC). Strong linear correlations were found among LAS, IP, NL, and LL, however, correlations between SC and the other components were negligible. The authors suggest that when resources are limited, LAS and SC are the most efficient components to score due to ease of scoring and information gained. Partial resistance can be difficult to assess since the epidemic must be strong enough, but not too strong to be able to discriminate subtle differences in host susceptibility. In addition, horizontal resistance can be masked in the presence of race specific resistance. Using recurrent selection to build levels of partial resistance has been effective for other diseases and crops (Díaz-Lago et al., 2002) and is worth exploring in roses.

6.2.3 New Resistance Sources

A transgenic approach to resistance breeding is another option whereby a resistance mechanism not naturally present within roses can be added or an existing mechanism can be transferred or enhanced. The potential for durability of such an approach must be considered independently for each mechanism considered. Marchant et al. (1998a) transformed the floribunda 'Glad Tidings' with a chitinase transgene and found transformants having a 13-43% reduction in black spot. Li et al. (2003) transformed the shrub 'Carefree Beauty™' with *Ace-AMP1*, an antimicrobial protein gene, and found enhanced resistance to powdery mildew. In addition, somatic hybridization is being explored by developing fusion hybrids of normally difficult to intermate black spot resistant species and modern roses (Schum and Hofmann, 2001).

6.3 Double Flowers

Most rose species have blooms with a single row of five petals. Most cultivars, however, are double-flowered with an increased number of petals. Roses have one whorl of five true petals and double-flowered cultivars have additional petals or "petaloids" which are converted stamens or converted stamens and pistils (Morey, 1959). Petal number in double flowers appears to be determined by a relative

proportion of floral initials. Early season flowers and primary flowers in clusters tend to have more floral initials than later season blooms and secondary and tertiary flowers and therefore have more petals. In addition, some rootstocks increase scion vigor and can increase petal number slightly (Morey, 1959).

Morey (1959) self-pollinated the tetraploid cultivar Golden Scepter (approximately 30 petals) and observed a wide distribution in petal number among the seedlings, suggesting that petal number among double-flowered roses is additive. Debener (1999), working with diploid populations derived in part from *R. multiflora*, found that segregation for double flowers versus single flowers fit a one gene model with the allele for double being dominant to single. This confirms the hypothesis of Lammerts (1945). There appears to be a qualitative gene governing double versus single flowers, and within genotypes having the double-flowered allele, additional additive genes regulate degree of doubleness. Optimum petal number varies and is based on cultivar use, petal size and substance, flower form, and opinion.

6.4 Color

6.4.1 Pigments Systems

Modern roses come in a wide array of colors and color combinations with the notable exception of blue (Krüssmann, 1981). Dubois and de Vries (1980) examined rose cultivars representing many color classes for their relative quantity of carotenoids, anthocyanidins (cyanidin and pelargonidin), and flavonols (quercetin and kaempferol). They found that all cultivars screened contained quercetin and kaempferol. The white roses examined had only flavonols, and the yellow roses had only flavonols and carotenoids. Pink, red, orange, and remaining color classes had various quantities and combinations of flavonols and anthocyanins or flavonols, anthocyanins, and carotenoids. Flower color was able to be approximated given pigment constitution, but the opposite was more difficult.

De Vries and Dubois (1980) and de Vries et al. (1980b) used cultivars which varied in pigment composition for breeding studies and found that the gene action of pigment genes in modern roses is generally additive. They suggest that breeders should consider color as the interaction between pigments and breed for desired colors accordingly. Marshall et al. (1983) examined three anthocyanidins (cyanidin, pelargonidin, and peonidin) and their heritability in roses. They found relatively high narrow-sense heritability estimates using mid-parent / progeny regression (h^2=0.58 to 0.69) and a small inverse relationship between peonidin and pelargonidin.

Yellow color in modern cultivars traces back to one source, ‘Soleil d’ Or’ (Paris and Maney, 1944), a yellow blend cultivar deriving its yellow color from *R. foetida*

persiana (Lemaire) Rehder. There are other sources for yellow including *R. ecae* Aitchison, *R. hugonis* Hemsley, and *R. primula* Boulenger. Unfortunately, difficulty exists in using such species as sources for yellow color in that either the color is weak and difficult to intensify or viable hybrids between such species and modern roses are difficult to obtain. In an attempt to re-access the strong yellow color of *R. foetida* through new breeding lines, de Vries and Dubois (1978) developed F_1 and BC_1 generations between white hybrid teas and the *R. foetida* cultivars Persian Yellow and Austrian Briar. White hybrid teas were chosen to avoid anthocyanin pigments which can mask carotenoids. They found that yellow coloration was easily transmitted in the F_1 as well as the BC_1 progenies (white hybrid teas as the backcross parent).

6.4.2 "Blueing"

Some red or pink roses develop a purple cast as flowers mature. This is known as "blueing" and is associated with a rise in vacuole pH as the petals age (Oren-Shamir, 2001). Lammerts (1960) reports that this is due to a dominant allele at a single gene he called M for magenta. Depending on the specific combination of pigments and color transitions, this effect may or may not be desirable. In general, breeders have selected against this color transition in pink and scarlet-red roses and in favor of it in crimson and purple roses. Studies are warranted to understand the interaction of pH with each anthocyanin on color expression and inheritance of vacuole pH to better breed for desired colors and color transitions.

6.4.3 Color Breaks & Bicolors

The inheritance of color breaks (stripes and stipples) within a petal and bicolored petals (upper and lower petal surfaces differ in color) in roses is poorly understood. Lammerts (1945) suggests that bicolors (Figure 26-3. A) may be recessive. Striped roses have dramatic color breaks along the petals in vertical patterns (Figure 26-3. F). Stippled roses have small, vertical regions of intensified red or pink coloration (Figure 26-3.E, i.e. 'Dorcas', 'Freckles', and 'Spanish Rhapsody'). Striped roses have been grown for centuries, with striped cultivars available among many old garden rose classes in various combinations of red, pink, and white. However, striping has not been heritable from widely grown striped old garden roses or the rare, subtly striped sports of modern roses. This has led some to think that striping may be due to virus or transposable elements, though this has not been confirmed. Ralph Moore (1990) made a breakthrough when he identified that striping from the hybrid perpetual 'Ferdinand Pichard' (introduced 1921, unknown parentage) is heritable. This discovery has led to many striped roses from several breeders during the past decade in a wide range of color combinations. Striped rose cultivars descended from 'Ferdinand Pichard' appear to have at least one direct

striped parent, and the frequency and degree of expression of striping is variable among crosses.

6.5 Foliage

Attractive foliage not only provides a complimentary backdrop for blooms, but also ornamental interest even when the plant is out of flower. Foliage among roses varies greatly in size, texture, and color. Breeders are placing greater emphasis on ornamental foliage, especially for roses selected for use in the landscape. Some roses, like the hybrid rugosa 'Frau Dagmar Hartopp', even have dramatic golden fall color. Foliage with a shiny cuticle is generally favored and is dominant to a dull cuticle (Lammerts, 1945), and the season-long, reddish-purple coloration of the foliage of *R. glauca* Pourret is readily transmitted to progeny (Wright, 1947).

6.6 Fragrance

Many rose species are fragrant to attract nectar collecting pollinators, while others like *R. setigera* Michaux do not produce nectar and attract pollen feeding insects (Kevan et al., 1990). The characterization of fragrance can be challenging due to the expression of scent being variable based on environment, maturity of the bloom, and human perception of scent (Mouchotte, 2001). Breeding for fragrance can be elusive. Two very fragrant rose parents can produce scentless offspring and visa versa. There are many different types of scents and complicated scent producing pathways found in roses (Guterman et al., 2002). Popular rose fragrance categories include traditional old rose which is commonly used in perfumes (damask), fruity (citrus, apple, raspberry…), myrrh, tea (crushed green tea leaves), and musk. Rose cultivars can fit into one scent category or be combinations of multiple categories. Many breeders choose to include strongly scented parents in their breeding program and hope that as advanced generations are produced, seedlings with exceptional fragrance emerge.

A linkage between strong damask scent and short vase life has been widely accepted (Mouchotte, 2001). Mouchotte (2001) suggests that for successful cut rose cultivars possessing a damask scent, a compromise must be reached between vase life and fragrance intensity. If simple genetic recombination could separate this tight linkage and it is not due to a physiological condition, techniques such as congruity backcrossing which allows for repeated opportunities for recombination between parental genomes may be worth exploring (Haghighi and Ascher, 1988). Cultivars with a moderately strong damask fragrance and a minimally acceptable vase life (~9 days) may be best suited to production areas with significant local markets able to pay a premium price. Fortunately, correlation does not appear to present between short vase life and strong fragrance for other, although less popular, rose scents.

6.7 Miniature Stature

Miniature roses are dwarfed in size and can resemble hybrid teas, floribundas, shrubs, and even climbers. Dubois and de Vries (1987) report that dwarf stature is due to a dominant allele at one major locus. Miniature roses have descended from a few founding diploid cultivars of *R. chinensis minima* (Shepherd, 1954). Through repeated backcrosses to tetraploid modern rose classes, most miniature cultivars are also tetraploid. Although the miniature stature is due to a dominant allele at one locus, minor genes may also be involved regulating expression due to the variation in degree of miniature stature. Miniatures range from what are popularly known as micro-minis, the smallest of all roses, to mini-floras, the largest miniature roses recently recognized as their own horticultural class (Julien, 2004).

6.8 Moss

Moss roses got their name due to the glandular "mossy" protrusions on the flower buds. When bruised, these ornamental protrusions exude a strong resinous pine scent or sweet scent. Moss roses are suspected to have originated as a mutation of *R. centifolia* L. in the late 17th century (Hurst and Breeze, 1922), and during the following two centuries many cultivars were developed. Moore (1978) distinguishes two distinct classes of moss roses- the "thorn type" (*R. centifolia muscosa* (Aiton) Seringe) which owe its moss to modified thorns and in general have an abundance of thorns all along the canes and the "crested type" (*R. centifolia cristata* Vibert) that has modifications on the sepals, without necessarily having an abundance of thorns on the rest of the plant.

De Vries and Dubois (1984) determined that thorny moss is due to a single locus with a dominant allele. Minor genes are suspected to be involved in regulating the degree of expression. Ralph Moore, an American breeder, introgressed thorny moss into modern, recurrent-flowering roses, used various forms of inbreeding to intensify the expression of this trait (Moore, 1978). The inheritance of the crested type moss has not been reported and, according to Moore (1978), is more difficult to introgress into modern roses due in part to low fertility.

6.9 Prickles

The appendages on rose stems and petiole undersides commonly called thorns are in botanical terms prickles because they are outgrowths originating from the epidermal layer. True thorns are modified stems originating from an axillary bud. Unarmed or "thornless" roses do offer the advantage of a reduced risk of injury. Thornless roses and roses with reduced numbers of thorns are especially valuable where frequent handling occurs such as in the case of rootstocks and cut flowers. Thornless garden rose cultivars currently marketed to the public include the Smooth

series of hybrid teas bred by Harvey Davidson as well as various miniature and some old garden rose cultivars.

Thornlessness is expressed in varying ways and degrees. The thornlessness common within *R. blanda* Aiton has prickles on the canes at the base of the plant and then canes become thornless near the top (Hansen, 1947). A second form involves thornless canes, but thorns under leaf petioles (rachis). This type of thornlessness is common among *R. multiflora* descendents. Finally, there are roses like the Smooth series of hybrid tea roses and some miniature cultivars that are thornless on the stems and rachis. Most modern, recurrent blooming hybrid teas and miniatures that are thornless on the stem and rachis trace back to a common floribunda parent introduced in 1956, 'Little Darling' (Cairns, 2000). It is typical to obtain a low frequency of thornless progeny in a wide array of crosses using 'Little Darling' as either a maternal or paternal parent. Interestingly, 'Little Darling' itself has thorns on both the stem and rachis.

Thornlessness, as expressed in *R. multiflora* descendants, appears to be due to a single recessive gene (Debener, 1999), however, the inheritance of complete thornlessness found in tetraploid cultivars may be more complicated. Rajapakse et al. (2001) report segregation ratios in an F_2 population from a cross between the completely thornless tetraploid cultivar Basye's Blueberry and a thorny tetraploid selection. Their data suggests that thornless stems and a thornless rachis segregate independently and a thornless rachis may be due to a single recessive gene. Thornless stems in this germplasm, however, may be controlled by multiple genes. Crespel et al. (2000a) report two quantitative trait loci (QTLs) explaining 66.4% and 13.8% of the phenotypic variability for thorniness in a diploid population derived from a cross of a haploid of the modern tetraploid rose Zambra® and the diploid species *R. wichuraiana* Crépin. The two QTLs were found to be linked (39.7cM apart) and flanking the major gene governing recurrent bloom (Crespel et al., 2002a). Although most thornless cultivars are derived through standard hybridization, somaclonal variation has also been used to repeatedly select smoother areas of stems for propagation until nearly thorn-free plants are obtained (Nobbs, 1984).

6.10 Recurrent Bloom

Non-vernalization requiring roses that flower continually over the entire growing season are preferred by most rose growers. The market for roses that bloom prolifically for a short season in spring (once blooming), only after flower initiation is triggered by vernalization, has become increasingly small. Fortunately, the inheritance of recurrent bloom is relatively straightforward and is controlled by a major locus with recurrent bloom expressed in homozygous recessive genotypes (de Vries and Dubois, 1978 and 1984; Semeniuk, 1971a, 1971b). Complementation reveals that the same major gene governs recurrent bloom in hybrid china / hybrid

rugosa crosses and crosses between the hybrid china descendant 'Goldilocks' and a recurrent flowering variant of *R. wichuraiana* (Semeniuk, 1971a, 1971b; Svejda, 1974). In addition, minor genes appear to be involved that regulate the expression of recurrent bloom. Some recurrent blooming roses tend to produce distinct cycles of bloom with relative synchrony (e.g. 'Therese Bugnet'), while others tend to be more free-flowering with plants having at least some blooms open at most times during the growing season (e.g. 'Nearly Wild'). A more free-flowering, recurrent habit is favored by most gardeners and commercial growers.

6.10.1 Juvenility

The juvenile period in modern recurrent-blooming roses, as defined by time from germination to visible bud, is relatively short compared to most woody crops (Zimmerman, 1972). The recessive allele for recurrent bloom in most cultivars came from tea china / hybrid china roses, and in particular the "four stud Chinas"; 'Hume's Blush Tea-scented China' (1809), 'Old Blush' (1793), 'Park's Yellow Tea-scented China' (1824), and 'Slater's Crimson China' (1792) (Filiberti, 2001). The origin of these Asian rose cultivars is unclear, but they are suspected to be descendants of the once blooming species *R. chinensis* Jacquin and *R. gigantea* Collett. In hybrid tea seedling populations, the average juvenility period typically is between four and five weeks under favorable conditions. Duration of juvenile period was found to be a quantitative trait (de Vries, 1976). A comparatively short juvenility period is true for other hybrid china descendants such as miniatures, floribundas, grandifloras, and many shrub roses. Seedling populations deriving recurrent bloom from *R. rugosa*, however, typically have juvenility periods that last months or years, although I have observed precocious seedlings that bloom within several weeks of germination. In once blooming roses, the juvenility period can last from one to several years; plants bloom after reaching physiological maturity, a critical size, and receiving adequate vernalization.

6.10.2 Gibberellic Acid & Recurrent Bloom

Roberts et al. (1999) suggest that the major gene controlling recurrent bloom in roses may be a GA gene. They compared the levels of GA_1 and GA_3 in 'Félicité et Perpétue' (once blooming), and its recurrent blooming sport, 'Little White Pet'. The two GA's followed a similar trend. Both roses have a relatively low GA concentration in early spring when flower buds are initiated. Soon after flower initiation the GA concentration of 'Félicité et Perpétue' quickly rose, but the GA concentration of 'Little White Pet' remained relatively low. A threshold of GA above which flower initiation is arrested and below which recurrent bloom is expressed is suggested. High GA concentrations can inhibit flower bud formation in apples and various stone fruits, and GA sprays are commercially used in these crops

to reduce flower density and fruit set (Byers et al., 2003; McArtney and Li, 1998). Expanding the work of Roberts et al. (1999) would be valuable. GA binding chemicals could be investigated for their potential to promote precocious flowers in once blooming hybrids to speed along selection and generation intervals. Additional questions include if GA concentration is associated with flower initiation in other rose germplasm and if differences in GA concentration or type is influencing the vastly different juvenility periods in recurrent flowering hybrid rugosa and hybrid china descendants.

6.11 Winter Hardiness

Selection for roses that are able to withstand winters in cold climates allows for greater use of roses as low-maintenance landscape and garden plants in such regions. Fortunately, there are many rose species native to cold climates from which cold hardiness can be introgressed. Rajashekar et al. (1982) screened acclimated canes of reputedly hardy rose species and cultivars to determine the temperature at which tissue damage occurred and the mechanism of survival. They found several species possessing extreme cold hardiness. Supercooling was found to be the main mechanism by which hardy roses are able to resist damage, limiting breeding for cold hardiness in roses to about -40C.

Svejda (1979) examined the inheritance of winter hardiness in various cultivars and their progeny by monitoring percent cane survival in field trials over years. She estimated broad sense heritability of cane survival to be high (0.51 to 0.92), suggesting the potential for rapid improvement. Karam and Sullivan (1991) developed a controlled freezing assay that can be used to differentiate genotypes for cold-hardiness and accelerate breeding efforts.

7. COMMERCIALIZATION OF NEW CULTIVARS

7.1 Award Programs

There are many independent, rose trials worldwide and different trialing programs focus on different classes of roses and emphasize different traits in the selection process. The strengths of each advanced selection, resources needed to submit an entry in a trial, and relative odds of a genotype winning are all considered in the decision of which, if any, program to enter an advanced selection. In general, award winning cultivars are widely publicized, increasing demand and sales. Trials are typically located in municipal gardens and are open for public viewing. Breeders often visit trial sites to not only monitor their own entries, but to observe the competition.

7.2 Registration of New Cultivars

The American Rose Society has served as the International Registration Authority for Rose (IRAR) since 1955. Rose cultivars are registered with the IRAR for eligibility in rose shows and for historical and botanical purposes. Information regarding flower and plant characteristics as well as a suitable name are required for registration. There are guidelines for name selection. Breeders generally choose both a code or variety denomination (consisting generally of the first three letters of the breeder's last name or company name plus some unique combination of letters) and a recognizable, often trademarked name under which the rose is sold to the public. Registration information and forms can be obtained from the American Rose Society on-line at http://64.78.40.53/irar/irar.htm or by standard mail at P.O. Box 30,000, Shreveport, Louisiana 71130-0030 USA.

7.3 Intellectual Property Rights

Patents and / or plant breeder's rights are often sought for new cultivars to provide a legal means by which to control propagation and obtain royalties. Different regions of the world (i.e. Canada, Europe, Japan, and the United States) have their own protection systems and protection can be obtained in multiple regions on the same cultivar. Care must be taken to understand and comply with the regulations for each regional protection sought to not forfeit eligibility due to technicalities. In addition, common law or registered trademarks are often used on the name(s) under which a rose is marketed. Molecular markers (AFLP, RAPD, and STMS) and electron microscopy of leaf surfaces have been useful in fingerprinting rose genotypes and can aid in the application of patents / breeder's rights and settling disputed infringements (Debener et al., 2000; Esselink et al., 2003; Gallego and Martinez, 1996; Krause, 1981; Torres et al., 1993).

8. TRENDS & FUTURE PROSPECTS

8.1 Trends in Miniature Roses

Miniature roses are the most versatile modern class of rose. They resemble dwarf versions of roses from other classes and can readily be incorporated into urban gardens and home conditions where space is limited. Many professional and amateur breeders are working with miniatures which is leading to accelerated divergence within this class as cultivars are selected for specialized niches. Due to the miniature factor being dominant (Dubois and de Vries, 1987), using full-sized cultivars already suited to desired niches in crosses with miniatures can speed the

development of adapted miniature cultivars. Dwarfed roses suitable for pot culture (including hanging baskets), cut flowers, the traditional rose garden, and a low-maintenance outdoor landscape situation are four key directions I envision miniature roses diverging.

8.1.1 Cut flower Miniature Roses

A trend among amateur breeders that take pride in exhibiting roses over the past few decades has been to cross miniatures with exhibition hybrid teas possessing exceptional flower form. This approach has led to many modern miniatures with exhibition, hybrid tea-like flower form and thick petals with extended vase life. Some modern, exhibition-type miniatures such as some cultivars in the Scentsation® series even have intense fragrance. Exhibition miniatures provide a great resource from which to begin breeding and selection for productive miniatures with relatively long stems suited to the cut flower market.

8.1.2 Potted Florist Miniature Roses

The potted miniature market requires cultivars with quick re-bloom on a compact plant with relatively short stems and long lasting flowers. In addition, miniatures for the potted market must tolerate low light as well as elevated ethylene levels without dropping leaves and petals during transit, retail conditions, and the home environment. Early selection for flowering under low light conditions and quick re-bloom on short stems was shown to be effective in hybrid tea cut rose populations and can be investigated in potted miniature rose populations (de Vries, 1976; de Vries et al., 1978). Variation among miniature rose cultivars was found for longevity of flowers and ability to retain leaves and flowers under elevated ethylene levels (Cushman et al., 1998; Müller et al., 1998). Müller et al. (2001) identified 'Vanilla®' from the Kordana™ series as a superior genetic source for breeding long display life due to the lack of an autocatalytic response to exogenous ethylene. Genotypes that pass initial selection criteria for blooming under low light and having quick rebloom can be propagated and tested for ethylene sensitivity and display life under simulated production, shipping, and retail conditions. In addition, cultivars such as 'Front 'N Center™' (2002 introduction) have been identified as having a reduced water requirement (Anonymous, 2001). This trait may be especially valuable to potted miniature rose growers in regions where water resources are becoming increasingly limited.

8.1.3 Garden Miniature Roses

Miniature roses for garden use have the greatest range for diversity and have been the primary market under which most breeding of miniature roses during the

Table 26-3. The principal mode(s) of inheritance of selected traits in *Rosa*.

Trait	Qualitative		Quantitative	Reference
	dominant	recessive	additive	
Black spot resistance	x		x	von Malek and Debener (1998), Xue and Davidson (1998)
Double flowers	x		x	Debener (1999), Morey (1959)
Flower color-flavanoids			x	de Vries et al. (1980b), Marshall et al. (1983)
Flowering under low irradiance			x	de Vries (1980a)
Glossy foliage	x			Lammerts (1945)
Juvenile period			x	de Vries (1976)
Male fertility			x	Visser et al. (1977a)
Miniature stature	x			Dubois and de Vries (1987)
Moss	x			de Vries and Dubois (1984)
Prickles	x		x	Crespel et al. (2002a) Debener (1999)
Powdery mildew resistance	x			Linde and Debener (2003)
Recurrent bloom		x		Semeniuk (1971a, 1971b)
Winter hardiness			x	Svejda (1979)
Yield (flowers)			x	de Vries (1976)

Table 26-4. Genes of horticultural interest transferred to rose via genetic transformation.

Objective	Gene category	Gene	Reference
Black spot resistance			
	Chitinase		Dohm et al., 2001
		RCH10	Marchant et al., 1998a
	B-1, 3-glucanase		Dohm et al., 2001
	Ribosome inhibiting protein		Dohm et al., 2001
Enhanced adventitious rooting			
	Root loci *(Agrobacterium rhizogenes)*		Van der Mark et al., 1990
		ROLA	Van der Salm et al., 1997
		ROLB	
		ROLC	
Petal color modification			
	Chalcone synthase		Firoozabady et al., 1994 Souq et al., 1996
Powdery mildew resistance			
	Antimicrobial protein	*Ace-AMPI*	Li et al., 2003
Prolonged vase life			
	Antibacterial protein	Nos*NptII*	Derks et al., 1995
		CecB	

past century has been accomplished. Miniatures bred for outdoor garden performance are gaining popularity and are eligible for a number of international trial awards. However, they are more difficult for the average consumer to find for sale. Miniature roses marketed for pot culture are sold by major propagators at economical prices and retailers often choose to sell these cultivars for both the bedding plant and decorative potted plant markets. In general, miniatures best suited for garden performance often make less than optimum candidates for pot culture because they do not remain as compact and require more growth regulators or pinching to look full and in proportion to the small pots in which young plants are typically sold. In general, when miniature roses adapted to pot culture are planted in the garden, they remain relatively compact and do not perform as well as miniatures selected for garden performance. Similarly, hybrid tea florist roses selected for superior greenhouse performance often do not have superior garden performance (Barry, 2000). Sources for miniature roses selected for good garden performance are primarily small specialty nurseries that rely heavily on mail order sales. There has been a growing trend of field production of miniatures that are good garden performers by major bareroot rose suppliers. Bareroot field grown miniature roses are potted and forced for spring sales in nursery pots, much like bareroot hybrid teas and floribundas.

8.1.4 Landscape Miniature Roses

Miniature roses can be used as low-maintenance landscape shrubs filling a niche often occupied by compact herbaceous perennials. Traits such as strong disease resistance, winter hardiness, shade tolerance, and a free-blooming habit are favorable characteristics for wide adaptation to the landscape setting. Many of the smaller roses advertised for the landscape rose or shrub rose market have a miniature parent and could have been assigned to the miniature or mini-flora horticultural rose classes (e.g. 'Baby Love™'). Great progress can be made within this arena as early generation selection among seedlings is accomplished using low light intensities (shade tolerance) and fast rebloom as selection criteria, similar to the protocol proposed for potted miniature cultivars. In addition, strong emphasis needs to be placed on selection for disease resistance, winter hardiness, and an attractive, floriferous plant habit.

8.2 Cut Rose Trends

8.2.1 Changing Regions of Production

Considerable investment is devoted to the development of cut flower rose cultivars. Such cultivars typically yield three to five times the royalties generated by

cultivars of other rose categories (de Vries and Dubois, 1996). With a shift in production of cut roses from greenhouses in Northern latitudes to more economical greenhouse and outdoor production systems in locations such as Israel, India, and South America, the need for cultivars adapted to these new production regions is growing. Relatively low production costs in emerging cut rose growing regions affords the culture of higher demand, larger-flowered cultivars that are typically lower yielding. Many traditional cut rose growers in Northern temperate regions have difficulty competing in the marketplace due to rising labor and fuel costs and faster and more efficient transportation available from new production regions to key markets.

8.2.2 Breeding Cut Rose Cultivars

When outdoor cut rose production is initiated in a new region, cultivars identified as being adapted to the climate and that may make suitable cut flowers are trialed. They are trialed for their production potential as well as their potential as germplasm from which to build a breeding program (Hassan et al., 1982). In addition to developing cultivars adapted to new production regions that mimic the floral traits of predominant cut rose cultivars, the development of novel cultivars for the specialty market will continue to grow. There will be a growing demand for spray roses (multiple blooms per stem), cut roses with fragrance, and flowers with unique color combinations and "old-fashioned" or nostalgic flower form. Selection for long vase life, yield, and disease resistance will continue to be important factors for all cut rose cultivars no matter what market or production region is targeted. In addition, many cut rose breeders are seeing the potential of developing a dual market for their cultivars. Although cut rose cultivars typically are not selected for their garden performance, many grow satisfactorily and are in demand for garden use by rose enthusiasts (Barry, 2000).

8.3 Genetic Transformation

Transforming roses with genes coding for traits they do not naturally possess or genes not readily available in elite germplasm offers opportunities for rapid advancement. Tissue culture regeneration systems for both organogenic and embryogenic rose calli can be challenging, but are not impossible to optimize (Castillón and Kamo, 2002; Ibrahim and Debergh, 2001; Mathews et al., 1991; Noriega and Sondahl, 1991; Rout et al., 1999; van der Salm et al, 1996). Both *Agrobacterium*-mediated transformation and biolistic transformation have been successful in trans-gene integration in rose (Dohm et al., 2001; Firoozabady et al., 1994; Marchant et al., 1998b; Souq et al., 1996; van der Salm et al., 1997). Kim et al. (2004) found higher transformation frequencies in rose using *Agrobacterium*-mediated transformation when multiple copies of *virE* and *virG* were present. In

addition, they determined that the green fluorescent protein gene was a useful reporter gene in roses and had advantages over β-glucuronidase.

Relatively few genes with commercial potential have been introduced into rose to date (Table 26.4), and currently no transgenic rose is being marketed. The development of a stable, blue-flowered rose cultivar through transgenic means has been a long time goal and is yet to be realized. There can be inconsistency in the expression of blue coloration in flowers due to factors such as unfavorable vacuole pH, presence of co-pigments, secondary modifications of delphinidin, and interactions of anthocyanins with metal ions (Courtney-Gutterson, 1994). Public perception of transgenics will be a key factor for the success of transgenic roses in the marketplace.

8.4 Additional Breeding Directions

8.4.1 Introgression of Novel Ornamental Traits & Stress Tolerance

There are many desirable traits in wild rose species and older cultivars that can be explored further for introgression into modern cultivars. Potential traits include dioecy from *R. setigera* (male morphs tend to have more flowers and do not produce hips) (Kevan et al., 1990), fragrant foliage from *R. rubiginosa* L. (apple-scented) and *R. pomifera* Herrmann (evergreen-scented), and ornamental prickles from *R. sericea pteracantha* Franchet. Traits that allow roses to adapt to a wider range of environments and require less resources will also continue to be investigated. In addition to disease and insect resistance, breeders can pursue such traits as increased nutrient use efficiency and increased tolerance to abiotic stress including drought, pH and temperature extremes, salinity, and shade.

8.4.2 Rose Hips as an Alternative Crop

The market for rose hips is expanding due to a growing interest in new products for floral arrangements and natural or organically-grown dietary supplements and foods. Rose hips are high in vitamin C and can be made into high-valued jellies, preserves, and soups. Key traits in the development of cultivars for culinary and nutritional purposes include high individual hip weight and overall plant yield, high vitamin C content, and pleasing flavor (Simanek, 1982; Uggla and Nybom, 1999). In addition, branches with clusters of ripe rose hips can make colorful additions to fall and holiday bouquets. Little has been done to select superior cultivars for this niche market. Roses in the Synstylae section may be especially well-suited for use in the cut hip floral trade because of numerous, brightly-colored hips per stem and a relatively thin hypanthium which allows the hips greater resistance to decay and be more amenable to drying. Characteristics that would aid in the development of rose

hips as an alternative crop for either market include lack of or reduced thorns, uniform ripening, and high pest tolerance.

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Chapter 27

STAR OF BETHLEHEM
Ornithogalum

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Abstract: Star of Bethlehem are grown as cut flower and potted plant crops throughout the world. A relatively new crop, *Ornithogalum* is favored for its unusual flower shapes, coloration, and use for holiday (Christmas) floral designs. The leaves are variable, ranging from grass-like to strap-shaped. While the inflorescence is a raceme, its appearance changes due to varying floret peduncle lengths. Most flowers within the genus are primarily white or yellow with a midrib stripe. Flower breeders have created new colors with interspecific hybridization including orange and orange-red. Phenotypic traits requiring future improvement include plant height, flower color, disease and virus susceptibility.

Key words: Cut flowers, geophytes, polyploidy, potted plants.

1. INTRODUCTION

The genus *Ornithogalum* L. belongs to the subclass Monocotyledonae and was originally classified in the family Liliaceae (Van Scheepen 1991), but was later transferred to Hyacinthaceae (Du Plessis & Duncan 1989, Meerouw 2002). About 200 species are known from Africa, Europe and Asia (Obermeyer 1978). *Ornithogalum* are closely related to *Albuca* and *Lachenalia*, of which the latter have been developed as flowering pot plants (Kleynhans et al., 2002). Only a few species of *Ornithogalum* are used in the floriculture trade (De Hertogh & Le Nard 1993). *O. umbellatum*, *O. pyramidale* and *O. nutans* are used in gardens. *O. arabicum*, indigenous to the Middle East, is used as cut flower. *O. saundersiae*, a species from the High Veldt region of South Africa and Swaziland used as a cut flower, produces exceptionally long stems. *O. thyrsoides*, the first fresh cut flower to be traded internationally from South Africa, is the most widely grown cut flower type

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(Pertwee 2000). More recently *O. dubium*, a short stemmed species from the Southern Cape area of South Africa, has been developed in Israel as cut flower (Luria et al. 2002) and in South Africa as a pot plant (Littlejohn & Blomerus 2000). *O. conicum* var. *strictum* from the Nieuwoudville semi-desert area of South Africa is also used as a cut flower, traded by Israel as *Ornithogalum* 'Nova' (Philosoph-Hadas et al. 1998). Approximately 40 million stems of cut *Ornithogalum* are sold through the Dutch Auction system annually, with sales occurring in all months of the year (Pertwee 2000).

The name *Ornithogalum* dates back to antiquity, the Greek *Ornithogalen* meaning Bird's Milk. The name may originate from the whiteness of the flower. *O. umbellulatum*, a Mediterranean region species, is referred to in the Bible as Bird's Dung, as the scattered flowers in a field resemble birds droppings. Common names for *Ornithogalum* abound (De Hertogh & Le Nard, 1993), in some cases referring to more than one species. Star of Bethlehem is the common name for *O. arabicum*, and *O. umbellulatum*. South African *O. thyrsoides* are commonly known as tjienkerintjie or chincherinchee, an anomatopeic attempt to imitate the characteristic squeaky sound produced when the fresh peduncles are rubbed against one another. *O. dubium* are known as Dirkies in the region where they are endemic.

Ornithogalum used in the flower trade are generally easily grown, requiring full sun and well drained soil. The natural habitats of the wild species, however, ranges from dry semi-desert areas to marshy wetlands and riverbanks, to mountain tops. All species produce fleshy, tunicate bulbs, mostly deciduous, although some South African species are evergreen. *O. saundersiae* behave as evergreen plants when in cultivation in Kenya where daylength differs little throughout the year (Kariuki & Kako 1999). The bulbs can comprise of scales, leaf bases and non-emerged leaves of three seasons (Rees 1985). The leaves are highly variable, depending on the species. They can be grass-like, lance-shaped or strap-shaped. The scape height can be over a 1 m, but generally less. The inflorescence is a simple raceme, but may take different shapes due to the length of the peduncle of the florets. The flowers consist of six petals in two whorls. The flowers are generally not scented, although some scented wild species are found in South Africa (Obermeyer 1978). The majority of the flowers within the genus are white or yellow, with a green/brown stripe on the midrib of the petals (Figure 27-1). The species used as fresh cut flowers, and more recently as pot plants have saturated colored petals with no midrib stripe, and range in color from white, through yellow, to orange and orange-red.

Figure 27-1. Ornithogalum secundum indicating the dark midrib on the petals, a trait found in many of the wild species.

2. CYTOGENETICS

The chromosome numbers within the genus range from $2n = 3$ to $2n = 26$ (Darlington & Wylie 1955, Pienaar 1963, Pastor & Diosdada 1993, Van Scheepen 1991). Chromosome studies were made of the subgenus *Aspasia* within *Ornithogalum* (Leighton 1944a, b; 1945). The basic chromosome number of this group, which incorporates *O. thyrsoides* and *O. dubium*, was found to be x = 12, with one aberrant genotype of *O. conicum* var *strictum* with x = 10. Karyotypic differences between the species and between ecotypes within species were observed. Use of confocal microscopy to study chromosomes of *O. dubium* and *O. thyrsoides*, revealed the major difference in the chromosomes to be associated with the nucleolar organizer region (NOR), located on chromosome 2 in *O. dubium* and chromosome 5 in *O. thyrsoides* (Griesbach 1998). The study of Van Niekerk (1965) indicated that the pairing of chromosomes during meiosis in hybrids between the *O. dubium* range of ecotypes and the *O. thyrsoides* range of ecotypes depended on the ecotypes used in making the cross. In some interspecific hybrids no meiotic chromosome pairing was observed, while in others six bivalents were observed. The results of these studies were confirmed by Littlejohn et al. (1992), indicating the need to delineate species differently to that proposed by Obermeyer's 1978 revision of the South African *Ornithogalum.* Heterochromatin studies of some *Ornithogalum* species from South Africa indicated a correlation between high heterochromatin quantities in the chromosomes and adaptation to extreme habitats (Vosa 1997).

Chromosome studies in *O. umbellatum* have indicated different ploidy levels within populations in Poland (Czapik 1993) and Spain (Pastor & Diosdada 1993), ranging from stable diploids to full tetraploids and all combinations in between. Morphological and flowering patterns were not associated with the ploidy level differences in a study conducted over ten years (Czapik 1993).

3. INTERSPECIFIC HYBRIDIZATION

Large differences in the ability to produce progeny seed from interspecific as well as intraspecific crosses using different ecotypes of the same species have been observed in the *Aspasia* sub genus (Pienaar 1963, Littlejohn et al. 1992). *O. thyrsoides* functioned well as a pollen parent in crosses with *O. dubium*, but *O. dubium* pollen did not germinate on the stigma of *O. thyrsoides*. In crosses using *O. thyrsoides* as pollen parent, pollen germination occurred, pollen tubes effected fertilization and the developing embryos were normal up to 15 days after pollination (Niederwieser et al., 1990). Endosperm failure after 15 days necessitated the in vitro rescue of the developing embryos, which resulted in normal progeny. Griesbach et al. (1993) observed that *O. dubium* crosses reciprocally with *O. thyrsoides*, and could easily produce backcross and F_2 progeny. It is possible that the *O. thyrsoides* parent in these hybrids was an ecotype of *O. dubium* from the west coast area of the Cape Province in South Africa, a confusion highlighted by Littlejohn and Blomerus (1997a). This variant produces long stemmed, white flowers and hybridizes well with all *O. dubium* ecotypes, but poorly with *O. thyrsoides* ecotypes. It was classified as *O. dubium* by Obermeyer (1978) and confirmed by Littlejohn and coworkers (1992). Experience with other attempts to hybridize *O. dubium* ecotypes with *O. thyrsoides* ecotypes produce very few successful progeny, even with embryo or ovule rescue techniques (Littlejohn & Blomerus 1997a, b). The interspecific hybrids produced are usually poorly fertile and produce backcross progeny or F_2 progeny with difficulty.

The *Ornithogalum* used for garden purposes, namely *O. umbellatum*, *O. pyramidale* and *O. nutans* leave no record of having been hybridized for the purpose of producing new cultivars. These species all have white flowers with a green stripe on the petals. The plants used are selections from the wild, mostly propagated by seed, although in vitro propagation is possible in *O. umbellatum* (Nayak & Sen 1995).

O. arabicum and *O. saundersiae* can be propagated by seed, or by bulbil formation on scales or by bulb division. No references to special selection of these species are recorded. The flowers of both species are clear white, with a black ovary. The petals have no coloration other than white. No hybrids have been produced when using *O. arabicum* ($2n = 51$) or *O. saundersiae* ($2n = 14$) in combination with the *Aspasia* sub genus ($2n = 24$), nor with other *Ornithogalum* that have a dorsal stripe on the petals.

4. BREEDING OPPORTUNITIES

The most interesting group of *Ornithogalum* for breeding purposes is the sub genus *Aspasia* of the Southern African *Ornithogalum*. This group includes *O.*

thyrsoides, O. synanthifolium, O. conicum, O. conicum var *strictum, O. dubium, O. pruinosum, O. multifolium* and *O. maculatum*. There are three commercially used species within this group. The observable variation for some traits of importance in breeding *Ornithogalum* was measured for a large range of species and ecotypes within species of some of the *Aspasia* sub genus (Littlejohn & Blomerus 1997a, b). The measurements indicated differences in plant height, leaf shape, attitude and color, floret size and color, inflorescence shape and density, in vitro propagation rate and ability to self pollinate or hybridize within species as well as between species. Therefore, variation required to successfully breed for new traits is available within the species of interest. Hybridization undertaken at the Agricultural Research Council in South Africa has produced the hybrids indicated in the polygon in Figure 27-2.

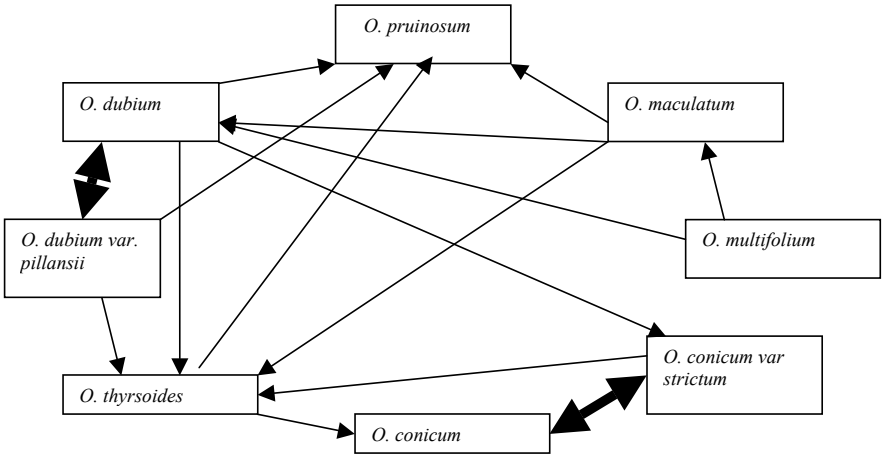

Figure 27-2. Crossing polygon indicating successful interspecific *Ornithogalum* hybrids produced in the *miniatum-maculatum* group. Wide arrows denote the cross produces many seeds, reciprocally; thin arrows indicate few seeds are produced with the seed parent at the arrow point.

The development of cultivation techniques (Luria et al., 2002) coupled with mass selection of seed populations of *O. dubium* resulted in this species becoming an important export cut flower crop from Israel since the early 1990's (Pertwee 2000). The attraction of this species is primarily the range of flower colors available,

namely white, through cream, yellow, orange and dark orange (Figure 27-3). Only ecotypes of *O. maculatum* (Figure 27-4) could rightfully be called orange-red. A variant of *O. conicum (O conicum* ssp. *strictum)* (Figure 27-5), previously known as *O. lacteum*, is currently being marketed by Israel as *O.* 'Nova' (Philosoph –Hadas et al., 1998). It has a long spike of white flowers with pale yellow ovaries, therefore presenting a very white inflorescence. This variant hybridized fairly easily with *O. dubium* (Figure 27-6) producing progeny with pastel shades of yellow and orange, with long stems (Littlejohn et al. 1992). Backcrossing to either parent is possible, although at a low frequency. F_2 progeny are difficult to obtain due to poor fertility of the interspecific hybrids and no selections with saturated color combined with stem length have yet been obtained (Figure 27-7). A problem observed in these hybrids is the fading of the petals.

It is well-known that chromosome doubling of infertile interspecific hybrids can improve the fertility and enable crossing to continue (Van Tuyl 1997). A range of interspecific hybrids and species selections of *Ornithogalum* of the *Aspasia* sub genus were treated with colchicine in vitro to produce tetraploid plants (Blomerus 2002). The tetraploid plants were hybridized with one another in many combinations (Table 27-1), but no greater level of success in crossing the tetraploids was obtained above using diploids. In some cases the tetraploids produce larger flowers and more robust plants, which may be a useful trait.

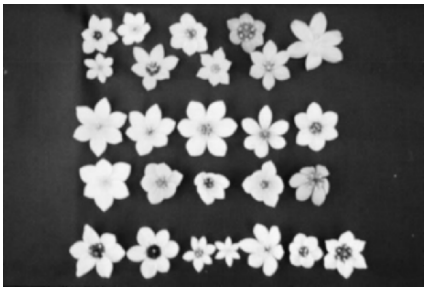

Figure 27-3. Flower color, shape and size variation observed in different ecotypes of *Ornithogalum dubium* collected over their habitat range in Southern Africa.

Figure 27-4. The orange–red inflorescence color of some ecotypes of *Ornithogalum maculatum.*

Figure 27-5. The inflorescence of *Ornithogalum conicum var. strictum.*

Figure 27-6. Flowers of *Ornithogalum dubium* 'Namib Sunrise', a pot plant cultivar developed by the Agricultural Research Council in South Africa.

Figure 27-7. Ornithogalum conicum var. strictum x Ornithogalum dubium.

Table 27-1. Results of crossing tetraploid and diploid genotypes of *Ornithogalum* interspecific hybrids and species.

Ornithogalum seed parent	Ploidy	*Ornithogalum* pollen parent	Ploidy	Seed Set
dubium x thyrsoides	*4x*	*thyrsoides*	*4x*	+
		conicum var. strictum x thyrsoides	*4x*	+
conicum var. strictum x thyrsoides	*4x*	*thyrsoides*	*4x*	+
dubium	*4x*	*dubium*	*4x*	+
conicum var. strictum x dubium	*4x*	*conicum var. strictum x dubium*	*4x*	+
		dubium x conicum	*4x*	--
		dubium x thyrsoides	*4x*	--
dubium x thyrsoides	*4x*	*dubium x conicum*	*4x*	--
dubium	*4x*	*conicum var. strictum x dubium*	*4x*	--
thyrsoides x dubium	*4x*	*conicum var. strictum x dubium*	*4x*	---
thyrsoides x dubium x thyrsoides	*4x*	*thyrsoides*	*4x*	--
dubium	*4x*	*dubium*	*4x*	--
		thyrsoides	*2x*	--
	2x	*dubium*	*2x*	--
	4x	*dubium x thyrsoides*	*4x*	--
	4x	*dubium*	*2x*	--

4.1 Opportunities

4.1.1 Plant Height

The average stems length of *O. thyrsoides* sold on the Dutch auction system is 50 cm (Pertwee 2000), while *O. dubium* range from 10 to 25 cm in length (Luria et al., 2002). The aim of hybridization within the *Aspasia* subg genus is primarily to combine longer stem length, robustness and greater hardiness with the vibrant flower colors of the *O. dubium*. The median height to the base of the inflorescence

measured in 151 ecotypes of *O. thyrsoides* was 52±1.22 (Littlejohn and Blomerus, 1997a), while in the colored *O. dubium* it was 25±1.61. The *O. dubium* of the *pillansii/leipoldtii* (Obermeyer 1978) ecotypes often confused with *O. thyrsoides* (Littlejohn & Blomerus 1997a), had a mean stem height of 57±5.95. Thus there is greater variation for stem height in *O. dubium* than *O. thyrsoides*, however, tall *O. dubium* selections tend to produce rather lax, spindly stems, especially under low light conditions.

At the other end of the spectrum, the short stature of *O. dubium* is advantageous for the production of flowering potted plants (Littlejohn & Blomerus 2000). Hybrids between *O. dubium* and *O. multifolium* have produced cultivars suitable as potted plants (Griesbach & Meyer 1998).

4.1.2 Flower Color

The petal color range in *O. thyrsoides* is from pure white to creamy white, with no coloration at the proximal end of the petal, to almost half of the petal being dark-green to black in the ecotype formerly known as *O. ceresianum.* The latter type, a very rare form from the Ceres district of the Western Cape Province, South Africa, hybridizes easily with a range of other *O. thyrsoides* ecotypes and the large dark centre is easily transferred to the progeny (Figure 27-8).

The petal color range in *O. dubium* is from pure white with only a black/brown ovary (Figure 27-9), to deep orange with a brown ovary and brown proximal regions of the petals, or with a yellow ovary. Progeny from crosses between the extreme color variants produce a rainbow of flower colors between the two extremes, indicating a polygenic genetic control of flower color. Backcrossing to either parent, or intercrossing the F_1 hybrids or selfing to produce an F_2 result in regaining the color extremes of the parents. Although Griesbach et al. (1993) reported this type of behaviour from *O. dubium* crossed with *O. thyrsoides* and backcrossed or selfed, our experience has been different, in that we have not been able to regain the saturated colors of the *O. dubium* when hybridizing with *O. thyrsoides*. *O. conicum* var *strictum* produces excellent pastel-shade hybrids with *O. dubium*, but poor fertility hampers the production of backcrosses and F_2 progeny to regain the stem length and saturated colors of the flowers.

Figure 27-8. The flower color of *Ornithogalum thyrsoides* ecotype (=*O. ceresianum*), indicating the large dark coloration of the proximal end of the petals, creating an interesting black and white flower.

Figure 27-9. The flower color of *Ornithogalum dubium* ecotypes with large dark centres and long stems (= *O. leipoldtii*; = *O. pillansii*). Note that the filaments are black, whereas in *O. thyrsoides* (Figure 27-8), the filaments are white.

4.1.3 Pathogens

Ornithogalum are infected by the following diseases (De Hertogh & Le Nard 1993): *Botrytis, Erwinia carotovera, Penicillium, Sclerotinia bulborum,* Virus, especially ornithogalum mosaic virus (OMV), and *Puccinia hordei* (Wallwork et al. 1992). No records of resistance to these diseases are available. Personal observation indicates that there are differences in the severity of symptoms exhibited by OMV

infected plants, both between and within species. Particularly in *O. thyrsoides* ecotypes plants exhibiting no or minor symptoms are observed.

Ornithogalum of the *Aspasia* sub genus and *Lachenalia* were observed to be infected by virus particles in 1976 (Klesser & Nel 1976). The virus was identified as a potyvirus and named ornithogalum mosaic virus (OMV) (Burger & Von Wechmar 1989). It causes stunted growth, mosaic symptoms on the leaves and stems and often fraying of the margins of flower petals. It is transmitted by aphids or mechanical damage. Many wild populations of particularly *O. thyrsoides* are infected by this virus (pers. obs.). The coat protein gene of this virus was sequenced (Burger & Von Wechmar 1989) and it was postulated that the coat protein gene could be used as a source of resistance to the virus. Ornithogalum mosaic virus coat protein sequence isolated in Israel, proved to be 95 % compatible with that isolated in South Africa (Zeidan et al., 1998). Wangai and Bock (1996) observed very rapid re-infection *of O. thyrsoides* plants free of OMV once planted in a field next to infected stock. OMV has also been isolated from *Alstroemeria carophyllea* (Bowen & Vlugt 2000). Successful transformation of *Ornithogalum* thin slice epidermal layers of tissue using biolistics and regeneration of transformed plants has been achieved (De Villiers et al., 2000).

5. PRODUCTION AND FORCING

One of the major production factors in flowering bulbs is determination and control of the temperatures during bulb storage and growing so as to force the bulbs to bloom when required. The temperatures at which the bulbs are stored after harvest not only influence the initiation and development of the next season's inflorescence, also influences the timing of flowering, the quality of the inflorescence, stem and leaves. Each *Ornithogalum* species requires a slightly different schedule of temperatures and time frames to produce the best quality cut flower, flowering pot plant or garden specimen under the prevailing cultivation conditions (De Hertogh & Le Nard 1993).

O. nutans, O. pyrimidale and *O. umbellatum* are planted in late fall, flower in late spring and are harvested during mid summer. They are best stored at 20° C and given a short pre plant cooler treatment. *O. arabicum*, *O. thyrsoides* and *O. dubium* are grown in the temperate climates as spring planted bulbs producing a cut flower in mid summer and bulb harvesting in fall. In Mediterranean climates they are planted in fall, flower in spring and bulbs are harvested in mid summer.

Forcing of *O. arabicum* in temperate regions is done by storing harvested bulbs at 30° to 35° C for five or more weeks, then storing for 11 weeks at 20° C to initiate the inflorescence (Shoub et al., 1971). They are subsequently grown at 12° C until flowering. Using this method flowering can be obtained throughout the year.

Langeslag (1989) recommends storing *O. thyrsoides* at 23° to 25° C for prolonged periods, giving four weeks at 17° C pre-plant and cultivating at 10° to 15° C. When planting earlier it is recommended to use larger bulbs. Van Vuuren (1997) recommends higher storage temperatures for forcing of *O. thyrsoides* for South African conditions. However, great care must be taken to keep the bulbs at high relative humidity when storing at 30° C.

O. dubium can be manipulated to flower year round by storing the bulbs at 24° to 30° C for prolonged periods, then treating at 15° to 17° C for four weeks prior to planting. Cultivation at 15° to 25° C results in longer stems for cut flower production (Luria et al., 2002) and produces better quality flowering pot plants (L.M.Blomerus, personal communication).

O. saundersiae, cultivated primarily in Kenya, an equatorial country, grows under these conditions as a perennial, synanthous geophyte, behaving as a monopodial plant producing lateral inflorescences bearing stems, until after a few seasons of flowering a terminal inflorescence bearing stem is produced at the bulb apex (Kariuki & Kako 1998). Flowers are produced throughout the year.

6. FUTURE OPPORTUNITIES

Although *Ornithogalum* are a relatively minor flower bulb crop, the genetic resources available within the genus are still largely unexploited. It is clear when new interspecific hybrid cultivars are produced, or even new selections within species, that physiological studies to understand the bulb storage requirements and control of flowering will need to be done to support commercialization of the cultivars. Ornithogalum mosaic virus is a major threat to field grown, cut flower production, particularly with the ever increasing pressure to reduce chemical insect control. It is probable that coat protein mediated virus resistance and selection for symptomless cultivars will be used to limit the damage caused by the virus. Expansion of the product range into the flowering pot plant market provides new challenges that are on the road to success.

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HERBACEOUS PERENNIALS

Chapter 28

MONARDA, BEE-BALM
Monarda didyma

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Abstract: Approximately 15-17 species and hybrid swarms of *Monarda* are native to North America. *Monarda didyma* is a fragrant herb in the Lamiaceae, which attracts many pollinators. While commonly grown for its flowers, it is also an important food, flavoring, and medicinal crop. Most hybrids on the market are derived from intra- and inter-specific crosses of *M. didyma* and *M. fistulosa.* Cultivars vary for flower color, stature (plant height), habit, and disease resistance. Powdery mildew and rust are the most common diseases noted in landscape plantings. Recent efforts have focused on increasing disease tolerance or resistance to these pathogens. Future directions for improving this crop include flower colour (true red, white, bi-colours), increased flowering duration, larger inflorescences, floral/foliar/root disease resistance, dwarf plant stature, increased stem strength, and essential oils.

Key words: *Monarda fistulosa,* propagation, reproductive biology.

1. PREAMBLE

"Eighty-five years after Columbus reached the America's, a notable volume dealing with the medical plant products from the New World and entitled *"Joyfull Newes out of the Newe Worlde*", was published in Seville, Spain. The author was Nicolas Monardes, a physician, also known to us from his "*Historia Medicinal of the Indies Occidentales*", published in Seville in 1569, 1574 and 1580. It was in his honor that Linnaeus named the genus *Monarda* (1737, 1753)." (Scora, 1967).

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2. INTRODUCTION

Monarda, commonly known as horsemint, bee balm, or wild bergamot, belongs to the mint family, Lamiaceae (Labiatae). It is an erect aromatic annual, biennial or perennial herb widely distributed throughout North America (Bailey, 1977). Several of the species of this genus have been utilized as ornamental plants, food, flavoring additives and for medicinal purposes. Essential oil components in the leaves have been helpful to determine genetic relationships between species (Scora, 1967a and b).

Although recognized as an asset in the garden landscape, *Monarda* is often overlooked as a culinary herb and a tisane (herbal tea). To create a mock-Earl Gray tea, steep 2 tablespoons of dried Monarda flowers with a good black tea for 5-7 minutes. A single cup can be made by pouring one cup of boiling water over 2 teaspoons of dried flowers in a tea “infuser” or strainer. Do not boil the flowers since boiling can evaporate the oils, which produce the flavor. It is best to use the flowers for tea, the leaves have a hotter, more oregano-like flavor.

The common name most frequently encountered in the herbal literature is "bergamot". The scent of the leaf and other plant parts resembles the small, bitter Italian bergamot, *Citrus aurantium* var. *bergamia*, which produces the oil of bergamot used in aromatherapy, perfumes, and cosmetics (Scora, 1967a and b). John Bartram of Philadelphia was instrumental in introducing *Monarda* into England. Bartram collected seeds near Oswego, New York, in 1743 and sent them to Peter Collinson. They first bloomed in Collinson's garden in 1745, and he named the plant "Oswego Tea" based on the point of collection in North America. The Oswego Indians infused *Monarda* as a drink, and it became a popular tea substitute in New England following the Boston Tea Party. Several native American tribes used *Monarda* to ease colds and bronchial complaints. The common name, Bee Balm, comes from the folk use of the flowers which, when pounded into a poultice, can be used to ease the pain of bee stings.

3. TAXONOMY AND DISTRIBUTION

Family - LAMIACEAE (Labiatae) - Mint Family

Genus: *Monarda* L. Gen. Pl., ed 1, 6, 1737; ed 5, 14, 1754

Type species: subgenus *Monarda* - *Monarda fistulosa* L. - Species Plantarum 1: 22. 1753.

Type species: subgenus Cheilyctis - *Monarda punctata* L.

3.1 Chromosome Numbers

Monarda fistulosa	*2n=2x*=36 (Scora, 1967), one report of *2n=4x*?=32 (Bushnell, 1936)
Monarda didyma	*2n=2x*=32 (Bushnell 1936), *2n=2x*=36 (Scora 1967)
M. x media	*2n=2x*=32?,36?; a putative sterile? hybrid between *M. didyma* and *M. fistulosa* *2n=2x*=36 (Fernald, 1950; Gleason, 1963, Gill 1977)
M. punctata	*2n=2x*=21 (McClintock and Epling, 1942, Scora 1967); *var*. *villicaulis* 2n=2x=24 (Scora 1967).

3.2 Diversity and Range

The genus *Monarda* is comprised of 15-17 species with numerous subspecies, depending on the authority (Table 28-1). All are limited to the North American continent, ranging from the Canadian prairies (north to near the Arctic Ocean) through to Texas and Mexico and easterly to the Atlantic ocean (Straley, 1986; Turner, 1994). *M. fistulosa* is the farthest ranging species and is often observed in native prairie remnants and roadsides. Scora (1967a and b) suggests the center of origin for the genus *Monarda* was in the area of northern Mexico and Texas. This hypothesis is based on the diversity of species in these areas and the range of diversity he observed in detailed taxonomic studies.

McClintock and Epling (1942), in their treatise on the genus, subdivided the group into two sub-genera: Eumonarda (*2n=2x*=36)and Cheilyctis based on floral formation (e.g. terminal verses an interrupted spike) (Table 28-1). Subsequently, Scora applied *monarda* as the sub-generic name (Scora, 1967). Additionally, Scora (1967) recognized two sections in the subgenus *Cheilyctis* (section Aristate *2n=2x*=18 and section *Cheilyctis 2n=2x=22*) The sub-genus *monarda* contains the two most frequently encountered species in the ornamental industry, *M. didyma* and *M. fistulosa*. One hybrid swarm is commonly recognized in the genus. *M. x media* is reported as a putative hybrid between *M. didyma* and *M. fistulosa* (Fernald, 1950 and Gleason, 1963) and is a more or less variable grouping of plants resembling *M. didyma*. Both authors recognized *M. x media* as a putative naturally occurring interspecific hybrid. Scora (1967) in his review obtained viable pollen from two interesting inter-specific (artificially developed) hybrids; *M. clinopodia* x *M fistulosa* (2 percent viability) and *M. clinopodia* x *M. didyma* (5 percent viability). Gill (1977) reported a sterile (seed and pollen) natural hybrid of *M. didyma* x *M. fistulosa*.

Table 28-1. Listing of the genus *Monarda* (after Scora, 1967 ; McClintock and Epling, 1942).

Subgenus	Species	Corolla Color	Comments
Monarda[1]	*M. braduriana*	white to flesh color	*2n=2x*=36, may hybridize with *M. russliana*
Monarda	*M. russeliana*	heavily pink spotted	*2n=2x*=36, may hybridize with *M. braduriana*
Monarda	*M. pringlei*	crimson	*2n=2x*=36, Mexico
Monarda	*M. eplingiana*	purple	*2n=2x*=36, Mexico
Monarda	*M. didyma*	crimson	*2n=2x*=36, wide ranging
Monarda	*M. dressleri*	rose-purple	*2n=2x*=36, Mexico
Monarda	*M. bartlettii*	magenta	*2n=2x*=36, Mexico
Monarda	*M. lindeheimeri*	white to cream colored	*2n=2x*=36, may introgress with *M. fistulosa (var. menthifolia)*
Monarda	*M. fistulosa*	lavender to rose to cream to white	2n=2x=36, wide ranging; varieties of fistulosa include: *M. rubra, M. brevis, M. fistulosa menthifolia and M. mollis*)
Monarda	*M. malloryi*	crimson to crimson-magenta	*2n=2x*=36, Mexico, borders of jungles
Monarda	*M. x media*	reddish to crimson to rose-purple	*2n=2x*=36? 32?, natural hybrid of *M. fistulosa, didyma (and clinopodia?)*
Monarda	*M. clinopodia*	whitish-greenish with purples spots on lower lip	*2n=2x*=36
Cheilyctis[2]	*M. mexicana*	white to pink	*2n=2x*=22?, Mexico
Cheilyctis	*M. punctata*	white-cream to yellow to pink	*2n=2x*=22?, wide ranging; varieties of *M. punctata* include *occidentalis, fruticosa, immaculata, standfieldii, intermedia, coryi, villicaulis-(2n=2x=24) , lasiodonta, arkansana and punctata*
Cheilyctis	*M.citriodora*	white to reddish to purple	*2n=2x*=36? n=9 rarely 18?, subspecies and varieties *austromontana, citriodora, attenuata, parva*
Cheilyctis	*M. clinopodioides*	white to pale pink	*2n=2x*=36?, Mich-Kansas to Texas
Cheilyctis	*M. pectinata*	white to pinkish	*2n=2x*=36?, wide ranging

[1]Subgenus *Monarda* - Glomerules usually solitary, appearing terminal; dilated portion of the corolla tube equal or exceeding the unexpended portion, stamens exerted; leaves ovate to ovate lanceolate; perennial

[2]Subgenus Cheilyctis - Glomerules several verticillastrate dilated portion of the corolla tube shorter than unexpended portion; stamens usually included; leaves elliptical to linear, annual, biennial and rarely perennial

3.3 Generalized Description of *Monarda Fistulosa* and *Monarda Didyma*

3.3.1 Vegetative Characters

Foliage is opposite and decussate. The leaf blades are lanceolate to ovate or deltoid-ovate. The largest leaves are generally the median ones. The leaf apices are acute to acuminate while their bases are acute to rounded. Petioles may be up to 45 mm but are generally much shorter, often appearing to be sessile or clasping. Stems are green and four-sided. Roots are shallow, fibrous and stoloniferous. Plants can spread (not aggressively) by the underground stolons.

3.3.2 Reproductive Characters

A "head-like" inflorescence or glomerule (two types - compact and sessile cymes) is subtended by bracts. The outer bracts generally occur in two's and are somewhat petiloid, opposite, and decussate in relation to the next lower leaves. The glomerules appear as solitary or terminal verticillastrate. The glomerulate disc is more or less round and is covered with calyxes borne on short pedicels. The calyx is a five lobed tubular structure and usually greens, although reddish tinges are known. Calyxes with the reddish tinge can be "showy" and add to the overall floral appeal of a flower.

The corolla is tubular but becomes progressively more open, acropetally (centrifugal) (Cruden et al., 1984). Pigmentation may be of one color or several colors unevenly distributed and range from white, rose, and purple to lavender. The nectaries are specialized glands located in the throat usually on the upper side of the corolla tube. These produced nectar which is sought after by different insect species (e.g. *Bombus*) (Cruden et al., 1984, Ayers et al., 1994).

Stamens are attached to the throat near the opening but generally below the junction of where the upper and lower portions of the corolla lip meets. Two groupings of anthers are often visible, one slightly shorter (2-4 mm) than the other. The anther-bearing stamens are exerted with slender and usually white filaments. The anthers are narrowly oblong (linear) and two parted (2mm). Pollen grains are spheroidal.

The ovary is deeply four-parted. The style is two-cleft at the apex. The styles, usually white and may exceed the upper corolla lip (exerted). The fruit consists of four ovoid nutlets that generally remain attached to a stock until they are disseminated. Seeds may stay in the calyx over winter and fall out when the calyx deteriorates the next season.

3.4 Phytochemicals

M. fistulosa usually yields an essential oil high in geraniol content (Marshall and Scora, 1972; Marshall and Chubey, 1983; Mazza et al., 1987). When this species was crossed with *M. didyma*, the hybrid also yielded essential oils rich in geraniol, linalool, thymol, carvacrol, 1,8-cineole, and other terpenes (Mazza et al., 1993). Dried leaves of *M. fistulosa* inhibit oviposition by several grain storage insects (Dunkel et al., 1994, Weaver et al., 1995). The dried leaves, when added to grain storage bins, likely caused behaviourial differences due to the added particulate matter and volatile components from the plant material. Hybrids of *Monarda* are potential sources of geraniol, linalool, thymol, and carvacrol. There is some evidence that the essential oils are produced via glandular hairs (Heinrich et al., 1983) and are synthesized from terpene glycosides. Essential oil yield varied from about 0.65 to 1.2 g/100 g of fresh plant material (Mazza et al., 1987), but different geographical regions may yield different oils (quantity and quality) due to environmental influences on plant growth and development (e.g. temperature and moisture) as well as genetic make-up. It may be possible to develop specific high oil yielding "chemo-types" if a concerted breeding effort was made.

Geraniol is used in perfumery for its rosy scent and in food products as a flavor ingredient. An oxygenated terpene, 1,8-cineole, has also been extracted from *Monarda*. 1,8-cineole, also known as eucalyptol, is used in pharmaceutical preparations and food products such as beverages, ice cream, candies, baked goods, and chewing gum (Mazza et al., 1987). The flavor of "Earl Gray" tea is often attributed to *Monarda*, yet the taste actually originates from the oil of *Citrus aurantium bergamia*. The two species are unrelated but have similar flavors.

4. REPRODUCTIVE BIOLOGY

Cruden et al. (1984) suggested that successful cross-pollination of *M. fistulosa* involves a complex interaction of events; starting with the opening of the flower and ending with seed set and maturation. Flowers open centrifugally over a period of several weeks. Approximately 18-20 hours after opening, a flower will have both anthers and stigmas visible. If pollination does not occur immediately, the style continues to grow, ultimately reaching a length of greater than 30 mm, hence increasing the exposure of the stigmatic surface to flower visitors (stigmas can remain receptive for up to 4 days after opening). After fertilization, the style reflects (up and backward). The corolla lasts 4-5 days depending on the time of fertilization (Whitten, 1981).

Cross pollination is generally more successful than self-pollination. According to Gill (1977) and further supported by Cruden et al. (1984) as well our observations and trials at Agriculture and Agri-Food Canada, Morden Research Station (MRS),

the breeding behavior, based on bagged studies, suggests that *M. fistulosa, M. didyma* and related hybrids are partially self-compatible and self-pollinating but seed set and seed germination are higher under open-pollinated conditions. Under natural conditions, bees are more effective than butterflies or hummingbirds in transferring pollen amongst flowers (Scora, 1967, Whitten, 1981). The ruby-throated hummer was the most frequently observed avian visitor to wild *M. didyma* in Tennessee-North Carolina area (Whitten, 1981, Temeles and Rankin 200).

Removal of opened flowers or flowers that have been previously fertilized did not impact the potential success of pollination of the remaining flowers (Cruden et al., 1984). Emasculated flowers do not set seed, indicating that parthenogenesis does not occur (Whitten, 1981).

4.1 Genetic Enhancement & Breeding Strategies

4.1.1 Prior Cultivar Development

Two species, *M. fistulosa* and *M. didyma,* historically have been important in the landscape industry. These species and hybrids form the basis for numerous cultivars (> 60?) (Hawke, 1998) (Table 28-2). In most cases, the exact parentage of the cultivars is not known or reported. Based on limited trials at the MRS, the majority of the cultivated plants appear to be derived from *M. didyma*, *M. fistulosa* and their hybrids. A detailed taxonomic assessment is needed to better delineate the relationship amongst the various garden varieties.

A large number of the cultivars in the market place were selected for flower colour, disease resistance and plant stature. The majority of these are clonally propagated but the potential for seed-derived cultivars is present. A series of tests would be required to determine if the parents used in the cross would produce progeny suitable for the commercial market place. Flower colors of the cultivated types range from pale pink to mauve to purple to red. White flowered types are also known, but they are generally not as vigorous as the other named cultivars. By far, most cultivars are greater than 75 cm in height (Table 28-2) and many can reach 1.25 m. Two recent introductions, ‘Petite Delight’ and ‘Petite Wonder’ (Collicutt and Davidson, 1999; Davidson, 2002), are dwarf, being less than 30 cm. Many of the cultivars are powdery mildew susceptible, which can reduce plant vigor and landscape survival. Recent efforts have been aimed at increasing disease tolerance or the development of resistance, especially to rust and mildew (Collicutt and Davidson, 1999).

Table 28-2. A partial listing of *Monarda* cultivars and their morphological characteristics.

Cultivars	Flower Color	Mildew / Disease Resistance Comments[1]	Height[2]
Adam	Red	poor to fair	t
Alba (syn Albescens?)	White	poor	t
Aquarius	Mauve-Purple	poor	t
Beauty of Cobham	Purple, Purple-pink	poor	t
Blaukrantz (Blue Wreath)	Dark Mauve	fair to good	t
Blue Stocking	Violet-blue	fair	t
Cambridge Scarlet	Crimson	poor	m
Claire Grace (fistulosa?)	Lavender	poor	t
Colrain Red	Red	good	t
Croftway Pink	Rose-pink	poor	t
Donnerwolke (Thundercloud)	Violet-purple (61a)	poor	m
Falls of Hill Creek	Red	fair	t
Fistulosa Variegated	Purple-mauve	poor	t
Feurschopf (Firecrown)	Rose Red	fair	t
Gardenview Scarlet (Gardenview Red, Gardenview)	Red	fair to good	t
Granite Pink	Pink	poor	t
Kardinal	Red	fair	m
Jacob Kline	Red	reported resistant	t
Mahongany	Dark Red	poor	m
Marshalls Delight	Pink	good	t
Mrs Perry	Red	poor	m
Ohio Glow	Dark Pink-purple	fair to good	m
Panorma	Purple-red	poor	t
Petite Delight	Purple-rose	good	d
Petite Wonder	Pink	fair to poor	d
Prairie Night	Purplish-violet , Purple Red	poor	t
Pink Tourmaline	Dark Pink	?	t
Prairie Night	Dark Purple	poor	t
Purple Mildew Resistant	Purple	good	t

Cultivars	Flower Color	Mildew / Disease Resistance Comments[1]	Height[2]
Purpurkrone	Purple	poor	t
Raspberry Wine	Wine Red	good	t
Rose Queen	Rose Red	good	t
Rosy-Purple	Red-purple	good	t
Scorpio	Purple	?	t
Scorpion	Red - Dark Purple	?	t
Snow Maiden	White	poor	m
Snow Queen	White	poor	t
Snow White	White	fair	t
Souris	Red-purple	fair	m
Squaw	Red	fair to good	?
Stone's Throw Pink	Pink - Medium	fair	m
Sunset	Purple	fair	m
Twins	Pink-dark	?	m
Violet Queen	Violet	good	t

[1]after Hawke 1998, Perry, 1997 and Davidson unpublished

[2]t=tall (>75 cm), m=medium (40-75 cm), d=dwarf (<40 cm)

Clearly defined goals for a breeding program are critical to help direct the strategic use of limited resources. The breeding program within MRS has been on-going since the mid-1980's, but originated with the late Dr. Henry Marshall while he was at the AAFC-Brandon Research Station. The goal of the program today is to develop well adapted plants in a range of flower colors and resistant to the major pathogens. Concerted breeding efforts on *Monarda* are few. Most asexually propagated cultivars have resulted from growers identifying superior plants in seedling populations. Reports of controlled crossing are very limited (Collicutt, 1989). Controlled crossing is time consuming and generally yields few seedlings. Controlled pollination is useful and is often utilized for specific characters that have proven otherwise difficult to combine. There are no mutation breeding programs known to the author. Small point mutations often lead to interesting plants types (M. Tristam, 1999 per com). For example, in large populations of seedlings, variegated foliage types have been observed but these have not been stable after subsequent propagation.

More recently, the breeding program at MRS has moved towards a population improvement approach where large populations of open pollinated seedlings are grown to better capture the variability and diversity in the populations. Detailed information is captured on the parental lines and this information, coupled with

performance based assessment of seedling progeny, is used to further develop the populations. Both asexually propagated and seed propagated lines can be selected from this type of strategy. The open pollinated seedling populations we have used often function similar to a poly-cross (Allard, 1960). Parents can be selected and planted in close proximity to one another. Bees and other insect pollinators work the flowers and result in good seed set (Ayers et al., 1994). It would be possible to expand this concept and isolate parental lines in cages and introduce pollinator insects.

4.1.2 Propagation

Monarda can be propagated from seed harvested in the fall or early spring as well as clonally by a variety of techniques including rhizome and softwood stem cuttings, division and tissue culture.

Seed propagation. Seed can be harvested from mature, usually brown colored seed heads in late fall or in early spring. The seed heads need to be rubbed against a course surface to dislodge seed. The resulting debris is then sifted through various sized screens to capture the seed. Seed do not appear to have any after-ripening requirement and will germinate shortly after sowing without further pretreatment. Seed spread on moist peat based media will usually emerge in 7-21 days, although germination is not always uniform. Germination percentages vary depending on the seed parent and environmental conditions. Two to four weeks post-emergence, plants can be transplanted to larger containers and grown in a greenhouse or another similar protected location. After 6-12 weeks, seedlings can be acclimated to outdoor conditions and transplanted in the field. Both spring and fall transplanting has been successful at MRS.

Asexual Propagation. Rhizome cuttings are very successful and appear to be a very rapid means for increasing plant numbers. A primary rhizome gives rise to side branches, which grow about 2-10 cm below the surface and produce lateral buds. These side branches have a diameter of 1-2 mm, are succulent (especially in early spring), and are a milky-white color. The rhizomes and their branches give rise to numerous small roots, which form a shallow root system. Because spreading occurs rapidly, plants should be spaced 18-24 inches apart. A piece of edging placed around the plant in the bed can also keep it from wandering via the shallow, underground rhizomes. Approximately 30 rhizome cuttings per plant have been obtained from two to three year old plants (Davidson, 2002). Rhizome pieces, 2.5 to 5 cm in length, should be harvested in early spring or in the fall with 0.5 to 1 cm of terminal growth, inserted into potting media, then grown in the greenhouse or outdoors under protection. This technique will not be successful if rhizome cuttings have too much vegetative leaf growth.

Stem cuttings of approximately 10 to 12 cm in length and all but two leaves removed, including the shoot tip, can be rooted but at a variable frequency (cultivar

basis). The bases of the cuttings should be dipped in 1,000 ppm IBA and placed under intermittent mist (approx. 10 sec every 10 minutes) in moist peat-based media. Drainage of the media is important. If the media is too wet for an extended period of time, basal root rots develop and greatly reduce success rates. Cuttings taken in late-May to early-June, when the plants are actively growing, usually produce root initials within one week and should be ready for transplanting within 15 to 20 days.

Propagation of *Monarda* can also be achieved through crown divisions. Two-year-old plants can often yield 6 to 8 divisions. Propagation by tissue culture has also been successfully carried out in our laboratory using leafy cuttings as explants.

Specific goals for plant improvement programs include both reproductive (floral) and vegetative/physiological characteristics. While it is obvious that the whole plant needs to be evaluated for use in the landscape, floral attributes are considered some of the more important attributes for landscape utilization. Observations at MRS suggest that the inheritance of these characteristics appears to be under both qualitative and quantitative genetic control.

4.2 Selecting for Reproductive Characters

4.2.1 Flower Color

Pigmentation of corollas may be uniform over the entire surface or several colours distributed uniformly or irregularly (margins, spots, speckles, streaks, etc.) (Figure 28-1) (Scora, 1967). Coloration of segregating populations can be fairly continuous from white through pink to red and mauve to purple (Figure 28-1).

There appear to be two major avenues for colour expression: increasing red tones and increasing blue. Anthocyanins are the most frequent suggested coloring pigment (Scora, 1967). White flowers are known as well. In a breeding program, a relatively simple operational scoring system is needed to assess large populations due to the potential for confusion in scoring (Grant, 1975). At MRS, we use the following classes to score seedlings - pink, purple, mauve, red-purple and white (Table 28-3). This classification is arbitrary at best, and in reality, there is a fairly significant continuum. Chromatographic or HPLC examinations of corolla extracts could assist in determining better delineation of flower colours, but this is difficult in larger scale breeding programs due to the significant labour input (Scora, 1967; Deroles et al., 2000). Thin layer chromatography and HPLC data have been used to distinguish the pigments in other species such as petunia (*Petunia hybrida*) where three cultivars representing the standard colours, ‘Purple Pride’ (purple), ‘CWA

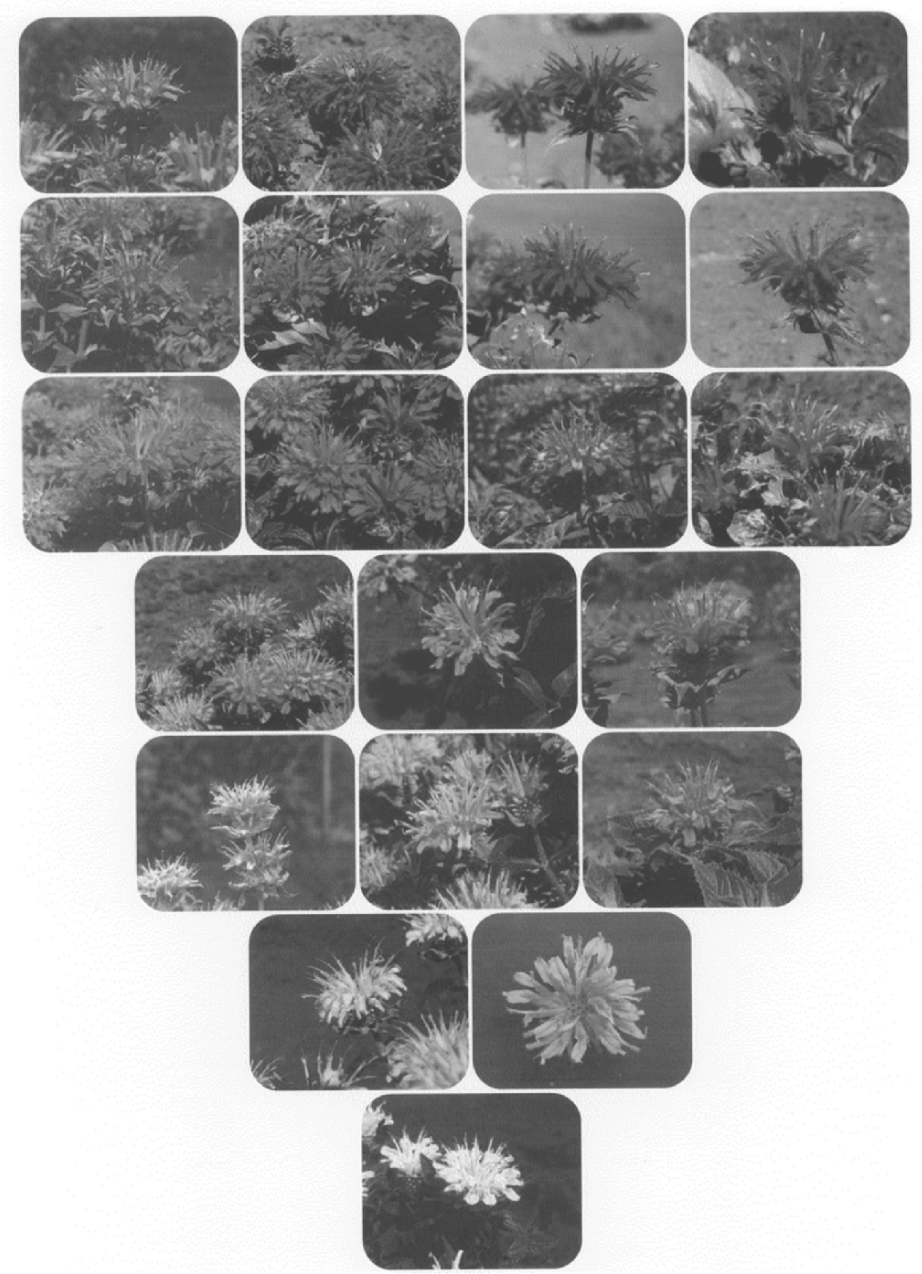

Figure 28-1. Flower colour segregation in *Monarda*.

Pink' (pink) and 'Alba' (white), could readily be separated (Deroles et al., 2000). Grant (1975) in a general overview of inheritance of flower colour, suggested that background colours often related to flavones (ivory, cream and yellow) and foreground pigments (generally anthocyanins) work in combination with each other to produce a large range of flower colours. Further he suggested that the interaction of two flavone-producing genes and two anthocyanin-producing genes determines the mixture of pigments and types of colour expression (Grant 1975). Additional effects may be caused by colour-inhibitor gene(s). Detailed controlled crossing experiments are required to fully explore the inheritance of colour in *Monarda,* but such studies are further complicated by the out-crossing nature of the plant.

Inheritance of Flower Color. Analysis of segregating populations for flower colour provides a window to understand the complex genetic controls in place (Table 28-4). Open pollinated seedling populations from over 30 different parents representing the major flower colour classes were scored over a period of three years at MRS. In these populations, the female parent flower colour had a strong influence on the flower colour of progeny but over 80% of the seedlings observed were scored in either the pink or purple flower classes. However, every major colour class was observed in segregating seedling populations regardless of the original female parent color.

White and red colours were the least frequent colour groups observed (11.2% and 7.9%, respectively) (Table 4). Not unexpected, both pink and purple flower parent classes gave rise to a higher percentage of progeny in these two classes than in either red or white. Red parents did produce a higher frequency of red progeny than other parents, but purple and pink classes were well represented. Pink, red and rose colours are often recessive to dominant purple and/or violet (Grant, 1975). The white parent class had over 80% of the observed seedlings in non-white classes, suggesting that white is recessive to other colours. However, there were relatively few seedlings scored and only one parental population was available for analysis in this colour class.

Obtaining a true "pure" red, blue or white colour is difficult. These colours are often found in combination with other pigments. Undoubtedly, modifiers that influence pigment location (fringed, spots) as well as "diluters" and "intensifiers" are operational (Davidson and Lenz, 1989; Grant, 1975). Future breeding efforts will require large seedling populations in order to identify the desired flower colour in combination with other attributes such as disease resistance and plant stature.

4.2.2 Duration of Flowering

There is considerable variability that could be exploited for duration of flowering (Davidson, unpublished observation). Hawke (1998) scored flower duration in a large number of cultivars and found that flowering ranged in duration from approximately 4 to 8 weeks. Flowering generally starts (at MRS) in mid- to

late-July and continues for approximately 4-6 weeks. Plants have been observed starting earlier and ending later but the room for extension is about 1-2 more weeks. Selection for increased flowering duration is possible, but efforts would be needed to assess this character over a range of years and environments.

Table 28-3. Flower colour classes used to categorize *Monarda* flower colours.

White	Pink	Red	Purple
white	rose-pink	red-purple	lavender
white/lavender tips	rose-mauve	red-mauve	mauve
white/mauve outline	coral-pink	coral-red	lavender-mauve
white/pink outline	coral	bright red/pink	mauve-pink
white/pink tips	rose-purple		mauve-red
white/mauve tips	rose-red		
white/pink outline			

Table 28-4. Observed frequency (%) of *Monarda* flower colour segregation based on four different maternal parent flower colour classes.

	Pink Female Parent	Purple Female Parent	Red Female Parent	White Female Parent	
Seedling Colour Classes[1]	Frequency (%)	Frequency (%)	Frequency (%)	Frequency (%)	Overall Means/Totals
Pink	46.8	25.1	26.4	42.9	35.3
Purple	39.0	63.7	45.5	34.3	45.6
Red	9.7	6.0	26.3	2.9	11.2
White	4.5	5.3	1.8	20.0	7.9
Total # of seedlings rated	413	483	271	35	1202
# of female parents	12	10	8	1	31

[1]See Table 28-3.

4.2.3 Inflorescence Size

Hawke (1998) presented data on inflorescence size for a wide range of cultivars. The majority of cultivars have inflorescence diameters of 2-3 inches. In seedling populations, plants with both larger and smaller diameters can be observed, but without the benefit of testing in a range of environments, it is difficult to identify true genetic effects from environmental (e.g. soil fertility) influences. Most have similar sizes and it is hard to determine environmental effects. Occasionally one

may observe an inflorescence arising from within inflorescence. The secondary inflorescence is smaller than the original (colloquially referred to as "piggy back" flowers. There may be potential to select for this attribute to increase the flowering capacity, but further research is needed to determine the exact origin (elongation of a new shoot or an extension of the existing inflorescence) is required.

4.3 Vegetative /Physiological Characteristics

4.3.1 Plant Stature

The majority of existing cultivars are approximately one meter in height (Table 2). The first dwarf cultivars, 'Petite Delight' and 'Petite Wonder', were observed in populations derived from 'Marshalls Delight' (Collicutt and Davidson, 1999; Davidson, 2002). To better understand plant stature, we have developed a scoring system for plant height: "dwarf" less than 40 cm, "mid-size" between 40 and 80 cm, greater than 80 cm being classified as "tall". It takes several years to clearly determine plant stature, but this must be examined due to environmental differences (soil moisture and nutrition). Studies are underway to determine inheritance of plant stature, but preliminary results suggest a complex inheritance (quantitative). Segregation of seedlings tends to follow a "bell shaped curve".

4.3.2 Foliage

In seedling populations one is able to identify different "tones" of green. Colours range from "light" green to "dark" green. Selecting darker foliage colours often adds to the overall appearance of the plant. Light foliage colouration may be interpreted by the public as weak and unhealthy. Very dense foliage types are known, but this may predispose them to greater disease pressures due to restricted airflow. Differences in foliage colour within and among genotypes, needs to be determined in replicated trials to determine the influence of soil nutrition and other environmental variables.

4.3.3 Other Vegetative Characters

The vigor of the plant is another important vegetative character (regardless of size class). Plants need to be quickly established and grow to maximum size with minimal interruption (especially in the spring). Stem strength (lodging resistance) is important, especially for taller growing cultivars. Plants need to be able to stand upright for the entire season. Flopping types need to be avoided due to their potential for greater disease problems (soil contact) and "untidy" appearance. Another component of the overall plant performance is re-growth post flowering. In

areas with long growing seasons, post-flowering vegetative appearance and growth is important and many leaves are frequently lost due to pathogens (leaf spots and rust). Foliage that develops post-flowering, should establish quickly and be vigorous to maintain the overall landscape value of the plant. Under shorter growing seasons (such as MRS) this attribute is not as critical.

4.3.4 Plant Hardiness and Adaptability

In long-lived herbaceous perennials, adaptability and plant hardiness are important attributes. Field tests are an effective means of screening large seedling populations, but once selections have been made further testing is needed to identify the range of adaptability. In trials at MRS, hardiness has not been a significant problem. Losses that can clearly be identified as cold or hardiness related have been minimal possibly due to removal of tender types earlier in the breeding or to protected locations and good snow cover. Hawke (1998) recognized winter injury while rating plants in the Chicago area and observed a reduction in plant vigour during the trial period.

4.4 Pathogens & Disease Resistance

Monarda sp. are susceptible to a number of diseases. Pirone (1978) listed leaf spots (*Cercospora sp., Phyllosticta decidua, P. monardae, Ramlularia brevipes and R. variata*), rust (*Puccina angustata* and *P. menthae*) and crown gall (*Pellicularia rolfsii*) to be present but not important enough to warrant control. Nagy (1977) reported on a new species of mildew (*Erysisphe monardae* Nagy) occurring on *M. didyma*. Conners (1967) and Ginns (1986) reported *Puccinia menthae* Pers., *Mycosphaerella tassiana* de Not., *Erysiphe cichoracearum* DC.: Merat and *Fusarium oxysporum* Schlecht. as infecting *M. didyma* in Canada. Farr et al. (1989) lists an extensive number of fungi that have been observed on a range of different *Monarda* species. In their native habitat, *Monarda* occur more frequently on well drained soils. In some poorly drained, fine textured soils at MRS, various “root rots” have been observed. These rots caused a sudden wilting of the foliage and ultimately the demise of the plants. Preliminary examination of infected plants indicated that a complex of “root rot” fungi (*Phytophora, Fusarium* and others?) may be the causal organisms. Additionally, in the greenhouse environment, over-watering of plants in peat based potting mixes can result in similar symptoms and ultimately the death of the plants.

Hwang et al. (1997) reported an “Aster Yellows” disease of *M. fistulosa* L. caused by a phytoplasma. The organism was subsequently characterized (Khadhair et al., 1997) via molecular techniques based on PCR amplification and RFLP analyses of 16S rDNA sequences. Symptoms include a leaf reddening, chlorosis, stunting and phyllody of inflorescence. The authors suggested that leafhopper

transmission from nearby potato fields might have been the source of the phytoplasma.

In general horticultural publications, rust and mildew are frequently listed a major pathogens of many *Monarda* cultivars. Leaf spots are also common and often cause defoliation late in the growing season. Mildew is the most frequently cited pathogen which may limit production in nursery environments and use in the landscape (Table 28-5) (Howard et al., 1995). The powdery mildew fungus is an obligate parasite and is characterized by the appearance of spots or patches of white to grayish powdery colonies/mycelium on leaves and other foliage parts. In time, the entire plant may be covered by the fungus. Powdery mildew is widespread and total losses on all crops (food and ornamentals) probably surpass the losses caused by any other single pathogen (Agrios, 1997).

During the period 1994 -1997, Perry (1997) examined mildew incidence (based on frequency of infections) on a variety of cultivars in Vermont. The bee balm cultivars most resistant to powdery mildew were 'Blue Stocking', 'Violet Queen', and 'Marshalls Delight'. The most susceptible cultivars, generally with 80-100% of foliage showing mildew each year, included 'Adam', 'Cambridge Scarlet', 'Croftway Pink' and 'Souris'. Evaluations at the Chicago Botanical Garden (Hawke, 1998) included 39 accessions (cultivars and species/subspecies) assessed over the period between 1993-1998. Of these only 15% were scored as having good resistance to mildew ('Colrain', 'Marshalls Delight', 'Purple Mildew Resistant', 'Raspberry Wine', 'Rose Queen', 'Rosy Purple', 'Violet Queen', and *M. fistulosa* var. *albescens*).

Research at MRS has focused on the development of resistance to this and other pathogens (Collicutt, 1989, Collicutt and Davidson, 1999; Davidson, 2002). The first major advance in this program was with the release of the cultivar 'Marshalls Delight' (Collicutt, 1989).

Disease resistance is a key long term goal for sustainable production and planting programs. Integral with this is the development of screening programs for pathogens such as powdery mildew, rust and leaf spot. In larger populations of field planted seedlings, selection for putative resistance is possible at a relatively young age. In trials at MRS, culling of susceptible seedlings is conducted often within the first two years. Greenhouse screening is also possible particularly for diseases such as powdery mildew.

Table 28-5. Checklist of fungi reported on *Monarda sp.* (Farr et al., 1989).

Chytridiomycetes	Basidiomycotina - Other
Synchytrium holwayi	*Ceratobasidium anceps*
Ascomycotina	Deuteromycotina - Hyphomycetes
Diaporthe arctii	*Cercospora* sp.
Diaporthopsis sp.	*Cladosporium monardae*
Erysiphe cichoracearum	*Ramularia brevipes*
Leptosphaeria conoidea	*R. variata*
Leptosphaeria sp.	
Lophiostoma caulium	Deutoeromycotina - Coelomycetes
Massarina gloeospora	*Ascochyta* sp.
Mycosphaerella tassiana	*Phoma exigua*
Ophiobolus anguillidus	*Phyllosticta monardae*
	Septoria brunellae
Basidiomycotina - Uredinales	*Septoria* sp.
Puccinia angustata	
P. menthae	Deuteromycotina - Other
P. monardae	*Sclerotium rolfsii*

4.4.1 Powdery Mildew

Natural infections can be effectively used to screen for resistance/tolerance. Since powdery mildew is so widely distributed, it is usually prevalent wherever larger numbers of plants are grown. Disease nurseries are easily established by planting susceptible cultivars/genotypes in close proximity to candidate genotypes. Limiting air movement by close spacing and combined high humidity will help to encourage rapid disease development. Observations of disease incidence are required over several years to ensure correct ratings are obtained. In addition, disease ratings often change during the course of the growing season. In the first year of planting, disease incidence is often limited. In the second and third years, disease frequencies generally increase due to a build up of the disease-causing organisms (unpublished data). In addition to the year to year variability, there are differences in disease incidence during the course of the growing season. Mildew is usually first visible in early July and slowly develops so that by the end of August, the disease is at epidemic levels. At MRS, we score disease ratings three times during the growing season to follow disease progression. This enables a comprehensive assessment of resistance during the entire growing season. Very susceptible genotypes are classed as ones that have a high early score followed by continuing high scores. High scores early in the growing season are much more damaging than late-season increases.

Average disease frequencies of powdery mildew recorded from 3-year old open-pollinated seedling populations derived from resistant, partially resistant and susceptible female parents are presented in Table 28-6. Mildew frequency scores in seedling populations is highly dependent on the parent genotype. Susceptible

parents yielded a high percentage (>70%) of susceptible or partially susceptible progeny (Table 28-6). Mildew resistant or partially resistant parents yielded a higher percentage (approx. 30%) of putatively resistant offspring. This type of screening program is useful in the "first round" of selection. Since environmental conditions can effect the expression of the disease, further trials are needed, preferably at two or more locations over several years. At MRS, selections have been apparently disease free for up to eight years and then succumb to this pathogen. Whether the environmental conditions were such to promote severe disease development or a new "pathotype" invaded remains under study. Regardless, screening for disease resistance is a multi-year, multi-location exercise.

Table 28-6. Overall average frequency of powdery mildew (PM) disease ratings at MRS on open pollinated *Monarda* seedlings derived from three different parental groups.

Disease scores[1]	Resistant PM Parent	Partially Resistant PM Parent	Susceptible PM Parent	Overall Means
0 to <2	56.4	49.3	72.5	59.4
2.0 to 2.5	14.3	18.9	18.8	17.3
2.5 to 3.0	29.3	31.8	8.71	23.3
Total number of seedlings rated	1039	1733	368	3140
Number of parents	12	16	4	32

[1]Where 3=resistant, 2=partially resistant, 1=susceptible, 0=dead

4.5 Rust & Other Pathogens

Natural rust infections at MRS are usually consistent from year to year; thus, selecting for resistance/tolerance under field conditions is feasible. We have not attempted to work with this pathogen in the greenhouse or growth room. There are several rusts that have been identified on *Monarda*, *Puccinia angustata*, *P. menthae* and *P. monardae*. These are either heteroecious or autoecious. The heteroecious type, *P. angustata*, are listed as requiring members of the Cyperaceae as an alternate or telial host (Farr et al., 1989). At MRS, we have not identified which species is most common and tend to treat all rust(s) in a similar fashion. Rust disease rating schemes follow a similar format to those used for powdery mildew. Plants are rated several times during the growing season and over several years. In a population (approx. 1400 seedlings) developed from rust resistant and rust susceptible female parents, over 50% of all the seedlings scored had a rating of higher than 2 (where 0=dead, 1 very susceptible and 3= no symptoms), indicating resistance is generally available in the plant populations.

Root rots in *Monarda* can be a problem, since this species is generally not well adapted to moist or poorly drained soils. Planting seedling populations in clay soils

can be used to induce root rots. In trials at MRS, the ability to overcome this disease is minimal. When root rots are observed, they tend to spread rapidly and infect all plants in the area. Leaf spots caused by various pathogens (*Cercospora sp., Phyllosticta decidua, P. monardae, Ramlularia brevipes and R. variata*) are also common but do not show up until later in the season (August). We have not identified which species are common in our field or initiated screening programs. Damp cool weather at flowering time may cause deterioration of flowers at a faster than normal rate. We have not identified what this organism might be and whether or not it is fungal in nature. No research on selecting for resistance to "Aster Yellows" disease has been undertaken to the authors' knowledge. The report on the incidence of this disease was identified in larger production fields (for the essential oils) and linked to insect activity from local potato fields. Breeders and producers will have to be vigilant if this problem becomes more wide spread.

5. CONCLUSIONS - CHALLENGES FOR THE FUTURE

Many challenges remain for future breeding and advancement of *Monarda*. Breeders will have to be ever vigilant for disease resistance. There are many cultivars in the market today but few with the needed disease resistance package. Combining good disease resistance to a range of diseases with different flower colours and plant stature will present exciting challenges for many years.

Interspecific hybridization is one new road that requires further study. There are many species of *Monarda* and the ability to hybridize these should be explored more completely. Natural hybrids of *M. fistulosa* and *M. didyma* (*M. x media*) are known. Exciting new attributes might be found (flower colors, drought tolerance, disease resistance) but few have been systematically explored. Suggestions for future selection and cultivar development are listed in Table 28-7.

Table 28-7. Challenges for the future in *Monarda* breeding.

Reproductive Characteristics	Disease Resistance	Vegetative characteristics
Flower colour (true red, white, bi-colours)	Foliage diseases - Powdery mildew, rust and leaf spots	Plant stature (dwarf), pot plant production, stem strength
Flowering duration	Root pathogens - root rots	Ease of propagation
Inflorescence size	botrytis on flowers	Essential oils (flavors)

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Chapter 29

CLEMATIS

Clematis species

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Abstract: The genus *Clematis* contains ~300 species native to many regions of the globe. While cultivated since the 1500s, it wasn't until 1835 that the first hybrids, *C.* 'Eriostemon' and 'Henderson', were reported. Currently there are many interspecific hybrid cultivars and numerous mutations from numerous species on the market. While clematis are traditionally grown as vines, there are non-vining species which can be used to diversify the crop. Traits of interest to flower breeders for crop transformation include: plant architecture, flowering (color, size, timing), attractive seed heads, foliage (phyllotaxy, coloration, or other morphological traits), environmental adaptation to stressful conditions (cold tolerance, heat tolerance, drought stress, etc.)

Key words: Interspecific hybrids, mutations, Ranunculaceae, vines.

1. INTRODUCTION

Clematis could be thought of as a plant breeder's dream. With close to 300 species and several thousand clematis cultivars, the genetic diversity and variability available for clematis breeders is limitless. Although new selections of clematis are available each year, there is still a need in this very diverse genus for improved selections.

Native clematis species can be found throughout the world, from the Americas, Australia, Africa, India and Europe, to China, Japan, Siberia and Mongolia. They are truly an international plant. Clematis are botanically placed in the Ranunculaceae family, which includes columbine (*Aquilegia*), marsh marigold (*Caltha*), larkspur (*Delphinium*), buttercup (*Ranunculus*), and wind flower (*Anemone*) (Gleason and Cronquist, 1963).

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Gardening with clematis goes back several centuries, with the initial European use in the late 1500s (Evison, 1998). The first European interspecific hybrids are generally accepted as being *Clematis 'Eriostemon' and* '*Hendersonii*' in 1835 (Evison, 1998; Feltwell, 1999). In the 1800s, many new cultivars were being named and released. There have been numerous individuals responsible for release of the many hybrids. "The Gardener's Guide to Growing Clematis", by Raymond Evison (1998), provides an excellent summary on the plant breeders who have contributed to the number of new clematis hybrids and the introduction of species. The European plant breeders have been recognized as the primary contributors of clematis hybrids, but plant breeders from Japan should also be recognized for their longtime contributions. There are newer programs both in public and private sectors, which are actively involved with the selection and release of new clematis selections. Studying both the successes and failures of these past and present plant breeders can provide helpful background information for a clematis breeding project.

New selections of clematis may originate from individual species, hybridization between species, hybridization between species and cultivars, or hybridization between cultivars. Besides controlled crosses, new selections may come from plants that come from seed collected from open-pollinated plants. Open-pollinated seed may be the result of a plant being pollinated with its own pollen or with pollen from an unknown male parent. New cultivars can also be derived from the natural variation that occurs within a species, as well as from mutations. All of these methods have resulted in new cultivars of clematis.

Most breeding projects begin by determining which clematis species/selections have the desirable traits for the breeding objectives. Botanical gardens, nurseries selling clematis, and scientific/popular literature are sources of information on germplasm/breeding resources. Some of the best hybrids are obtained when one of the parents is adapted to local conditions (Beskaravainaya, 1992). The environment can have a significant influence on the expression of a clematis trait (Feltwell, 1999). Flower color, flower size and plant size will vary with the time of season, location, soil type, temperature, age of plant, elevation and moisture availability.

2. CLASSIFICATION

A basic understanding of the relationship between the various clematis species and hybrids is useful when selecting species to be used as parents in a breeding program. Clematis, like other genera of plants, does not have complete agreement between taxonomists on the number of species and the classification of species within the genus. Placement of species in various sections and subgenus will be left to the taxonomists. The discussion of breeding clematis in this chapter is based on the classification of clematis by Grey-Wilson (2000). Grey-Wilson (2000) has

classified clematis into nine subgenera, 22 sections, 28 subsections (not all sections are subdivided into subsections) and 297 species. A brief description of the nine subgenera as it relates to breeding and selection follows.

Subgenus Clematis includes 95 species from Australia/New Zealand, Asia, Europe and North Africa. These plants are primarily deciduous climbers, have flat-facing flowers that bloom on current year's growth. Flowers are perfect or unisexual depending on the section within the subgenus, sepal number is usually four but sometimes six, and leaves are usually compound but are sometimes simple. Sepals are usually yellow, white or greenish-white. The species *C. vitalba* is probably the most commonly recognized species in this subgenus. *Clematis marmoraria*, found in this subgenus, is considered the smallest species of clematis.

Subgenus Cheiropsis (D.C.) Peterm. includes 13 species from Asia and the Mediterranean region. These are climbers with compound leaves. They have solitary or clustered, light-colored (white, pink, cream) flowers. The species *C. Montana* of this subgenus is a parent of many cultivars.

Subgenus Flammula D.C. includes 54 species from Europe and Asia. They can be climbers, subshrubs or herbaceous types. Leaves are compound. Flowers are usually perfect with light-colored sepals. This subgenus contains some very important species used in hybridization including *C. flammula, C. recta, C. florida, C. viticella, C. lanuginosa* and *C. patens.* The latter two are the source of many of the large-flowered cultivars.

Subgenus Archiclematis (Tamura) contains just one (1) species, *C. alternata,* from Nepal and Tibet. This species is a climber, has alternate, simple leaves and urn-shaped flowers, red sepals with green anthers. It has not been used for hybridization, based on a current literature review.

Subgenus Campanella (Tamura) includes 77 species from Asia and Africa. These are mostly climbers with perfect flowers. Some species have very large seed heads. This subgenus contains many of the smaller-flowered species with yellow sepals. Species in the subsection Meclatis hybridize quite easily with each other.

Subgenus Atragene (L.) has 18 species from Europe, Asia and North America. Flowers are usually nodding, perfect, with 4 sepals produced on long pedicels and are usually early flowering. Flower color is usually blue, purple, cream or white. This subgenus has a set of staminodes outside the fertile stamens. The species *C. alpina* and *C.macropetala* are common parents of numerous cultivars. No hybrids are known between species in this subgenus with species in other subgenera (Evison, 2000).

Subgenus Tubulosae (Decne) includes eight (8) species from Asia. Plants are herbaceous or subshrubs and non-climbing. Flowers are terminal with four sepals, usually light blue, light purple or cream in color. Flowers are born on current year's growth. Two of the most important horticulture species in this group are *C. heracleifolia* and *C. stans*.

Subgenus Pseudoanemone (Prantl) includes eight (8) species from Africa. These are non-climbing, erect subshrubs or herbaceous plants. Flowers are terminal, usually cream or white in color. They are not cold hardy.

Subgenus Viorna (Tamura, non Reichb.) includes 23 species from North America, Asia and Europe. These are mainly subshrubs and/or herbaceous. They have opposite leaves. This subgenus includes three very important species for cultivar development, *C. crispa, C. integrifolia* and *C. texensis*. *Clematis integrifolia* has been used in many crosses to produce lower-growing border plants, *C. texensis* is important as a source of red in sepal color, and *C. crispa* blooms over a long period.

3. FLORAL ANATOMY

The typical clematis flower consists of the sepals, the stamens and the pistils (Figure 29-1). In most cases, they have no true petals. Sepals are the colorful part of the flower. The sepals are described as petaloid (petal-like) and are often called tepals (Great Plains Flora Association, 1986). Sepal number is normally four, but there may be up to ten or more per flower. Increasing sepal number leads to semi-double and double flower types. The stamens and pistils are numerous in clematis (Great Plains Flora Association, 1986). Most clematis have perfect or hermaphroditic flowers, which means they contain both the male and female sex organs (Toomey and Leeds, 2001). For clematis with perfect flowers, the pistils are in the middle of the flower, surrounded by the stamens. Imperfect clematis flowers can be monoecious or dioecious. *Clematis afoliata, C. paniculata, C. gentianoides, C. ligusticifolia* and *C. virginiana* are dioecious (Godley, 1976; Grey-Wilson, 2000). The stamen (male part of the flower) consists of two parts, the filament and anther. Stamens can add an attractive feature to a flower as their color may vary from white to yellow to crimson/red to dark purple, and stamens can vary in size and number per flower. Litvinenke and Beskaravainaya (1972) reported *C. fusca, C. integrifolia* and *C. viorna* had larger anthers with more pollen and the pollen had a higher germination rate compared to other species. Staminodes may be present or absent as well. Staminodes are sterile stamens or structures resembling stamens that produce no pollen. They can resemble small petals in some species and can be an attractive feature of a flower. The outer stamens of a flower may be modified as staminodes, or all stamens of a flower may be staminodia (sterile). Outside fertile stamens produce pollen first and as they mature, new stamens on the inside release their pollen. The female part of the clematis flower consists of numerous pistils. Each pistil is made up of the stigma, style and ovary. The stigma is at the top of the pistil. The style connects the stigma with the ovary. The ovary contains the egg, which when fertilized, produces the seed. The ovary on clematis is hypogynous, which

means that the stamens and sepals arise from beneath the ovary. Flowers may be produced on old stems or on the current season's stems.

The clematis fruit or seed is called an achene. The style is persistent, (remains attached to the seed), which aids in the dispersal of the seed, and it may be curled or moderately straight. Seed of *C. brachyura, C. crispa* and *C. viticella* are examples of species that do not retain the complete style but have a reduced style or tail. The seed head consists of numerous seeds. Mature seed may remain on the plants for only a few days or for as long as a year.

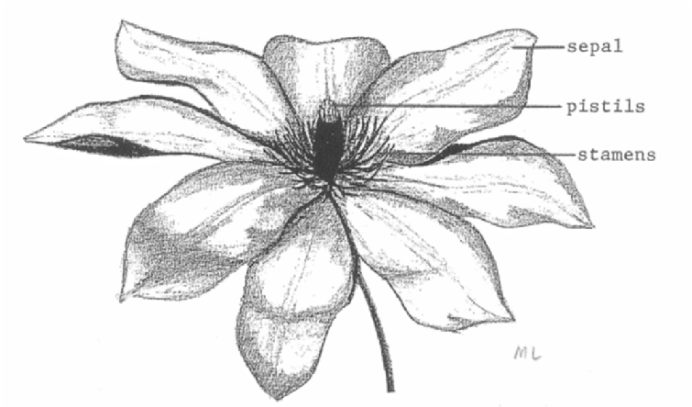

Figure 29-1. Floral anatomy of a *Clematis* flower, including the sepal, pistil (consisting of the stigma + style), and stamens (anthers + filaments).

4. TRAITS OF INTEREST

Breeding/hybridizing clematis can be conducted with specific objectives in mind or can be conducted for the enjoyment just to observe what the progeny may look like. Breeders usually visualize the needed improvement in a plant and make breeding decisions based on the ideotype or objectives of the project. There are many clematis traits that can be targeted for improvement. These include: 1) architectural forms and growth habits; 2) flowering traits; 3) seed heads; 4) foliage characteristics; 5) adaptive traits (cold hardiness, drought tolerance, disease resistance) and 6) medicinal uses (Southwell and Tucker, 1993; Johnson, 1997).

4.1 Plant Architecture

Plant architecture should be considered in any clematis breeding program. Clematis is most often thought of as a plant growing on a trellis, a clothesline post, or vining in a tree, but there are also woody subshrubs and herbaceous non-climbing types. Clematis plants can vary in height from 6 inches to over 25 feet. The genus

is unique in that the climbing species/cultivars of clematis cling by their twining leaf petioles (leaf stalks). Also, ease of pruning can be related to plant architecture in clematis.

Some clematis are considered herbaceous, such as *C. integrifolia* and some are considered subshrubs/semi-herbaceous such as *C. heracleifolia*. Developing small shrub-type clematis would be useful where a vining species/hybrid might be impractical such as for containers, small gardens, border gardens, rock gardens and ground covers. Good selections for growing in containers are *C*. 'Hagley Hybrid', *C*. 'Etiole de Paris', *C*. 'Edith', *C*. 'Mrs. George Jackman', *C*. 'The President' and many others (Evison, 2000; Fair and Fair, 1997; Feltwell, 1999). Some of the species and selections that merit attention as parents in developing smaller and non-vining plants include *C. integrifolia, C. hexapetala, C. heracleifolia, C. tangutica, C. marmoraria, C. gentianoides* and *C. recta* to name a few. *Clematis integrifolia* is one of the most useful plants for small gardens or for use as a border plant. Introductions of *C. integrifolia* include selections with blue, white, rose and pink-colored flowers. *C*. 'Blue Boy' and *C*. 'Olga' are two of the most popular blue-flowered *C. integrifolia* hybrids from this species. *Clematis Montana* and *C. tangutica* can be used as a ground cover (Evision, 2000; Fair and Fair, 1997) or for use in hanging baskets. Some clematis, such as *C. vitalba*, can be invasive as well (Bungard, Daly and McNeil, 1997; Ogle, et al., 2000).

4.2 Flowering Traits

Flowering traits will be one of the main features that plant breeders will concentrate on for improving. The major flower traits include flower color, flower size and flowering time. Other traits that also merit attention are flower scent, flower shape, flower texture, sepal margins and cut flower potential. Flowers may be solitary or clustered, and may be single, semi-double or fully double.

Flower shape/orientation in clematis can be grouped into several general classes. These include flat, open large flowers, bell-shaped, tulip-shaped, star-shaped, urn shaped, tubular, saucer, upright and nodding blossoms. As with many traits, flower shapes vary greatly and may well be classified as continuous rather than divided into definitive specific shapes. Flower size may range from less than an inch to over 8 inches in diameter.

In many of the ornamental plants, some gardeners feel that in efforts to improve flower size and quality, that scent has been lost. Scent is an important part of the garden/landscape environment. There are good examples of scented species and cultivars of clematis for use in a breeding project. These include: *C. recta, C. flammula, C. heracleifolia* var. *davidiana, C. heracleifolia* 'Wyevale', *C. Montana* 'Elizabeth', *C. integrifolia* 'Rosea' and *C. terniflora*. The cultivar *C. integrifolia* 'Olgae' is described as having 'vanilla'-scented flowers, and the species *C. forsteri* is described as having a lemon-verbena scent (Grey-Wilson, 2000). Numerous other

species/selections have notable fragrances for use in breeding programs (Fair and Fair, 1997). In general, smaller-flowered species have more scent than the larger-flowered species. Both flowers and/or foliage may be aromatic.

'True' yellow flower color is uncommon in the large-flowered clematis, but is quite common in the small-flowered species such as in *C. fruticosa, C. tangutica* and *C. tibetana*. Red is a color of interest. Many of the best reds in the trade have originated from *C. texensis*, a species from Texas, USA with scarlet, nodding flowers (Phillips and Barrell, 1993). True red-flowered selections could be used in the industry. Blue-colored clematis flowers often have a tint of purple, so pure blues are desirable. Petals with bi-colors can add a special palette to the inventory of new clematis. Color can vary within a flower as well. *C*. 'Miss Bateman', the result of a cross of *C*. 'Fortunei' x *C*. 'Standishii' is an example of a plant with attractive two-tone sepals (stripes in center of sepal) contrasting with red stamens.

Clematis can be successfully used as cut flowers. Breeding material for use in improving clematis for use as a cut flower include *C*. 'The President', *C.* 'Nelly Moser', *C*. 'Henryi', *C*. 'Polish Spirit' and cultivars of *C. heracleifolia* (Evison, 2000).

4.3 Seed Heads

Seed heads can be a very attractive feature of the clematis plant. A plant may flower for only a few weeks but can retain showy seed heads for months. Some species hold their seed heads for many months after maturing, while others drop their seed as soon as they reach maturity. Seed heads may vary in color from white to golden to pink or lavender. Seed tails (styles) may be smooth, fluffy, twisted or spiked. The seed tails are the showy portion of the head. *Clematis grandiflora* has one of the largest seed heads in the genus. Clematis with noted attractive seed heads include, but are not limited to, *C*. 'Nelly Moser', *C. flammula, C. integrifolia, C. tangutica,* and *C*. 'The President' (Evison, 2000). Along with the clematis seed head, seed size will vary greatly between species. The number of seed per pound can vary from 25,000 in *C. flammula*, to 192,000 in *C. virginiana* to 320,000 in *C. viticella* (Rudolf, 1974). The appearance of seed heads should not be overlooked as an ornamental trait for a clematis-breeding project.

4.4 Foliage

Foliage can vary extensively in clematis. Leaves are usually opposite, except for some species like *C. alternata*, which have alternate leaves. Some have leaves that are alternate as seedlings and develop into opposite leaves with maturity. The leaves can be simple or compound, including being trifoliate. Some species drop their leaves (deciduous) during the winter season while others are evergreen (*C. australis, C. cirrhosa, C. fasciculifora, C. gentianoides and C. microphylla*).

Leaf edges may be smooth or serrate. Foliage can change color with the species, cultivar or season, and the texture can be leathery to almost papery. Stems can be rough or smooth. *Clematis recta* 'Purpurea' has lavender stems and leaves. *Clematis heracleifolia* 'China Purple' also has purple stems. *Clematis tangutica* foliage will darken to lavender with the colder temperatures and shorter days of the fall.

4.5 Adaptation

Adaptation is a major issue when breeding clematis, especially if plants are expected to grow in stressful environments. Cold hardiness, heat tolerance, drought hardiness, site exposure, soil type, light intensity and humidity or lack of humidity will all effect adaptation. Although clematis can be found growing in many colder hardiness zones, there is considerable need to combine cold hardiness with many other characteristics such as larger flowers. Many of the beautiful cultivars that do well in the United Kingdom will not survive the winters of the northern USA and Canada without specific protection during the winter. However, there are species that are adapted to regions that typically get to –25° C. Tops may be killed, but the roots and crowns survive the winter. Beskaravainaya, et al. (1978), using cut shoots of 18 clematis selections, reported that *C. virginiana, C. ligusticifolia, C. serratifolia* and *C. glauca* withstood temperatures of minus 25 to 27° C without damage to shoot or bud tissue whereas the hybrids *C.* 'Sea Spray' and *C.* 'Phargesloides' only tolerated temperatures of minus 17 to 19° C, without damage to shoots and buds.

Clematis for north-facing sites may be quite different than selections for east, south or west-facing sites (Fair and Fair, 1997). Most clematis are described as needing moist soils, but exceptions do occur. For example, *C. fremontii*, from Nebraska and Kansas, USA, *C. tangutica* and others from the subsection Meclatis from China and surrounding areas, are examples considered to be more drought tolerant (Barr, 1983; Grey-Wilson, 2000). The ability to grow in drier sites is probably related to the type of root system because a more extensive root system can increase the plants ability to grow in drier sites. Clematis growth, in southern Crimen, was reported to be limited by root development and intolerance of high temperatures (Beskaravainaya, 1975; Falkova, et al., 1987; Donyushkina and Falkova, 1981). *Clematis recta* is reported to be more tolerant of alkaline soils. Other adaptive traits to consider include tolerance of long-term exposure to the sun and to the wind.

Susceptibility to pests (insects, disease, nematodes) are often not considered in a breeding project. Clematis wilt (*Phoma clematidina*) can be a serious problem. Mildew, damping off, grey mold, viruses, leaf spots, rust, slime flux and stem spots also infect clematis (Bellardi, et al., 1985; Howells, 1993; Kusakari, et al. 1997; Fair and Fair, 1997; Feltwell, 1999). The cultivars *C.* 'Aleksandrit', *C.* 'Nadezhda' and *C.* 'Fantaziya' were reported to be immune to powdery mildew as were all hybrids

with *C. heracleifolia* as a parent (Beskaravainaya and Mitrofanova, 1973). Problems, which might be confused with pathological diseases, include mineral deficiency, herbicide injury, drought and soil pH problems. Very few clematis projects have been designed with disease resistance/tolerance as a major objective. Insect pests include Japanese beetle, sawfly, black blister beetle, borers, aphids, mealy bugs, whitefly, earwigs and others. The importance of these pests will vary greatly with location. Other problems may include gophers, rabbits, deer and pets. In most cases, mammals may not have a specific preference for clematis species or cultivars, but it has been observed, for example, that *C. heracleifolia* has been especially susceptible to deer and gopher feeding in west central Nebraska (Lindgren, 2003, unpublished data). *Clematis socialis* seed is reported to be a favorite food of mice (Timmerman-Erskine and Boyd, 1999).

5. CLEMATIS BREEDING PROJECTS

One of the most prominent, earliest and still highly used clematis selections is *C.* 'Jackmanii'. *C.* 'Jackmanii', developed and released in 1863 by Jackman & Sons, was reported to be produced from *C. lanuginosa*, *C.* 'Hendersonii' and *C. viticella*. However, some question this parentage (Fretwell, 1997). It may well be the most recognized clematis cultivar with its large, deep bluish-purple flowers. It has served as one of the parents for numerous varieties including *C.* 'The President' (*C.* 'Jackmanii' x *C. patens*), *C.* 'Victoria' (*C. lanuginosa* x *C.* 'Jackmanii'), *C.* 'Ritenitis' (*C.* 'Victoria x *C.* 'Jackmanii'), *C.* 'Dacite' (*C.* 'Jackmanii x *C.* 'Victoria'), *C.* 'Iubileinyi-70' (*C.* 'Jackmanii x *C.* 'Blue Gem'), *C.* 'Nikolai Rubstov' (*C.* 'Jackmanii' x *C.* 'Nelly Moser'), plus others.

Lemoine of France produced many outstanding hybrids. Some of the parents he used in his crosses, included *C. lanuginosa, C. patens, C. viticella, C. texensis*, and *C. stans*. Brother Stefan Franczak of Poland has released many large-flowered climbing selections. Some of his selections, like *C.* 'Polish Spirit' are considered to be especially good for beginning growers. Maria Sharonvanbegor produced new clematis selections by taking known plants as the female parent and using a mixture of pollen (cocktail) for the pollen source (Green, 2000). This method has the advantage of increasing the chances of successful pollination and seed production but produce progeny of unknown male parentage. Dr. Frank Skinner of Canada was a successful hybrid clematis breeder, with emphasis on producing clematis that would tolerate the cold temperatures and stress of the climates of the northern USA and Canada. *C.* 'Blue Boy', a cross of *C. integrifolia* x *C. viticella,* is one of his results. Not only is *C.* 'Blue Boy' a good source of cold hardiness, but has attractive blue flowers on compact plants. Magnus Johnson released many varieties, and has to be known as the "father of clematis". The list of clematis breeders and their accomplishments can go on indefinitely, but these few examples illustrate some of

the breeders' many accomplishments. A list of a representative sample of reported interspecific crosses in clematis can be found in Table 29-1.

Table 29-1. Example *Clematis species* parents and their interspecific hybrids.

Female parent	Male parent	Interspecific hybrid cultivar
C. campaniflora	*C. viticella*	Lisboa
C. crispa	*C. pitcheri*	Anne Harvey
	C. viorna	Simsii
	C. vitcella	Betty Corning, Pendragon
C. fauriei	*C. sibirica*	Albina, Alpina Plena, Ametistina, Campanulina Plena, Pruinina
C. flammula	*C. integrifolia*	*C. x aromatica*
	C. viticella	Rubromarginata
C. forsteri	*C. paniculata*	Purity
C. heracleifolia	*C. recta*	Edward Prichard
	C. vitalba	Pracox, Jouiniana
C. integrifolia	*C. crispa*	Cylindrica
	C. lanuginosa	Pamiat Serdtsa
	C. viticella	Blue Boy, Heather Herschell, Olgae
C. koreana	*C. fauriei*	Brunette
C. lanuginosa	*C. patens*	Otto Froebel
	C. viticella	Perle di Azur
C. macropetala	*C. alpina*	Rosy O'Grady
	C. fauriei	Georg
	C. ochotensis	Floralia
	C. sibirica	White Swan, G. Steffner
C. marmoraria	*C. paniculata*	Joe
C. ochotensis	*C. alpina*	Tage Lundell
C. oreintalis	*C. intricata*	My Angel
C. patens	*C. lanuginosa*	Miss Crawshay, Reginae
C. potaninii	*C. vitalba*	Paul Farges™
C. recta	*C. ternifolia*	Pamela
C. serratifolia	*C. ligusticifolia*	Grace
C. tangutica	*C. tibetana*	Burford Variety, Corry
C. tibetana	*C. ladakhiana*	Tibetan Mix
C. tubulosa	*C. stans*	Crepuscule
C. virginiana	*C. ligusticifolia*	Western Virgin
C. viticella	*C. integrifolia*	Eriostemon
	C. lanuginosa	Prince Philip

6. MUTATIONS

Mutations (sports) occasionally occur in clematis. Some of the reported selections arising from sports include *C.* 'Pink Cameo' from *C.* 'Guiding Star', *C.*

'Petite Anna'™ from *C*. 'Anna Louise'™, *C*. 'Multi Blue' from *C*. 'The President', *C*. 'Königskind Rosa' from *C*. 'Königskind', *C*. 'Blue Light' from *C*. 'Mrs. Cholmondeley', *C*. 'Foxy' from *C*. 'Frankie', and *C*. 'Landsdowne Gem' from *C*. 'Freckles' (Green, 2000). *Clematis florida* 'Sieboldii' is considered unstable and sports frequently (Evison, 2000). Beskaravainaya, et al., (1981) and Beskaravainaya (1992) reported that treating clematis seed with the mutagens ethyleneimine, N-ethyl-nitrosourea or N-methyl-N-nitrosourea resulted in 76 forms of morphological variation.

7. HERITABILITY

There have been few studies reporting on the heritability of clematis traits. Because many of the cultivars that may be used as parents are made up themselves of an assortment of genetic combinations, heritability studies are challenging. Progeny from clematis parents that are closely related or have similar traits (foliage, flower color and growth habit) will likely have traits similar to the parents. If they differed in flower color, the progeny may be intermediate in color or may be closer to the color of one of the parents if one of the colors is dominant. Plants that vary in flower size and shape, such as *C. texensis* and *C*. 'Star of India' would be expected to have progeny that have flower size and shape that is intermediate, such as one of its progeny, *C*. 'Sir Trevor Lawrence' (Feltwell, 1999). The 2n = 2x = 16 chromosome number of clematis stays at a fairly constant number in the genus (Bhattacharjee and Sharma, 1980; Ozyurt, et al. 1997), but variation can occur such as with the reported *C. orientalis* as a amphidiploid with *C.mandschurica* as a tetraploid and with *C. paniculata*, as a hexaploid (Beskaravainaya, et al. 1979; Dennis, 1976). Phylogenetic studies (Hoot, et al. 1994; Miikeda et al. 2000; Schaal and Learn, 1988) have been conducted with clematis using chloroplast and nuclear ribosomal DNA which may lead to new hybrids based on using more current techniques of DNA transfer. Hybrids between *C. vitalba* and *C. heracleifolia* var. *davidiana* were reported to have disrupted meiosis with a high percentage of sterile pollen (Beskaravainaya and Dyakova, 1981). For clematis wilt, Steckelenburg (1972) found there were differences in clematis hybrids to wilt susceptibility, but no association with a particular group of hybrids was found. However, Van de Graff, et al. (2001) reported that in general, small-flowered clematis species were more resistant to stem rot and wilt caused by *Phoma clematidiona* than were the large-flowering hybrids.

8. CROSSING TECHNIQUES

The tools needed for making clematis crosses can include: pipe cleaners, small camel-hair brush, razorblade, small scissors, jewelers tags or similar tags, small paper bags, envelopes, pencil, and notebook. The objective of the crossing technique is to remove the pollen from one flower (male parent) and transfer it to the female part of another flower. This is the process of pollination. Begin by identifying the male and female parts of the flower, mainly the stamens and pistils. Flowers to be used as the female parent in a cross, should have all the anthers of the fertile stamens removed (emasculated) from that flower before the pollen in these stamens is released from the anthers. This is usually just before the fully developed bud opens. Stamens may be removed by using a forceps or tweezers to gently pull the stamens from the flower. However, pulling the stamens can result in damaging the pistils or accidentally pulling off the entire flower head. Some flowers, such as on *C. pitcheri*, have delicate heads, which may break off easily when removing stamens. The preferred method to removing stamens is to use a small sharp scissors or a sharp razor blade to cut the stamens away from the pistils. Caution should be used so as not to cut the pistils when using a razor blade to remove the stamens. In many cases, removing the sepals (especially of the urn-shaped flowers) will make it easier, if not necessary, to emasculate the flower. This emasculated flower (stamens removed) needs to be protected from pollination by unknown sources. A small paper or muslin bag can be placed over the emasculated flower to prevent accidental pollination.

Transferring pollen from the anthers of one flower to the pistils of another flower can be completed using several methods. Pollen can be collected by rubbing a small camel-hair brush or a pipe cleaner on the anthers to collect the pollen. Pipe cleaners have the advantage in that they are inexpensive and can be discarded after use to reduce the chance of pollen contamination. Pipe cleaners can also be reused for making multiple collections of the same pollen and can be stored for a few days in a refrigerator for later use. Camel-hair brushes need to be cleaned in alcohol after each use to ensure that pollen from different sources is not mixed or contaminated. In some cases, a whole flower with ripe anthers can be removed and then rubbed on the stigmas of another flower as a method of pollination. The fertile stamens on a clematis flower generally begin releasing the pollen from the outside group of stamens and ripen inward. The stigmas (tip of the pistil, female part of the flower) at this time, or several days later, become receptive for the pollen. Stigmas usually start to curve downward and become sticky as they become receptive to accept pollen. The pollen can be transferred to the stigma using pipe cleaners or camel-hair brush at this time. Once the controlled pollination is completed, the pollinated flower should be covered or protected for several weeks to prevent pollination from outside sources, by wind, insects, birds or even humans. This pollination procedure

can be repeated on the same flower in a few days to increase the chance of successful pollination.

After completion of successful pollination and fertilization, the seed (ovules) start to swell inside the ovary, and the sepals and stamens dry up and fall off. The seeds enlarge, turn green and then usually turn to a brown color as they mature. The mature seed (achene) can then be harvested as it turns to a darker brown color. Seed of some species will drop off immediately when mature, while seed on other species will be persistent and remain on the plant for months, if left undisturbed. The seed heads should be checked often for a color change and harvest as seeds change to a darker color to prevent the loss of mature seed. A muslin bag can be placed over the seed head to catch seeds of valuable crosses, if needed. Seed of some species will be ripe in several weeks, while others will take several months. The season may be too short in some climates for the seed to mature outdoors, so in this case, plants may have to be grown in containers and moved to a protected site as cold weather approaches. Seed heads should be dry when collected or should be allowed to gradually dry before storage. Seed should be stored in a cool, dry location. Although individual flowers have many pistils/carpels, rarely do all of the carpels result in a seed, especially from crosses. Seed from each clematis cross should be placed in a labeled envelope for storage.

The clematis cross should be labeled to designate the parentage of the cross. One popular method of doing this is on a jeweler's tag; list the female parent first, followed by an 'x', and then the name of the male parent and adding the date of pollination. Use a pencil or waterproof pen to label the tag. This is a standard method of designating crosses. The tag is then attached to the flower stem to ensure the parentage of the cross is correctly remembered for future reference. Records of both successful and unsuccessful crosses are extremely useful in making future breeding decisions and documenting previous crosses.

9. SEED GERMINATION

It is difficult to make specific recommendations on enhancing clematis seed germination because of the variation within this genera and because of the limited amount of published scientific studies on the requirements for clematis seed germination. Recommendations to enhance the germination of one selection or species may be detrimental to the germination of another species. Seed germination may occur in as short a time as 2 weeks and as long as 36 months (Fair and Fair, 1997; Malek, 1999). Reports on germinating clematis seed, would suggest that seed should be planted immediately after harvest or within a few weeks to a few months after harvest (Toomey and Leeds, 2001). However, for some species, seed can be stored up to 2 years with no refrigeration and still maintain viability (Rudolph, 1974). Lush et al. (1984) in studying the germination of *C. microphylla* seeds,

reported that seeds stored for 1 month after collection had 89% germination, and seeds stored for 6 months after collection had only 38% germination. Seed should be sown in clean, pasteurized growing media, several millimeters apart in clean containers and covered to a depth of their largest dimension (Malek, 1999). Do not crowd the seed. It is suggested by Grey-Wilson (2000) that it is not necessary to remove the tails (styles) of clematis seed. Keep the soil moist and check the containers often for germination. A fungicide can be applied, if desired, to the germination media to reduce the chance of disease (Toomey and Leeds, 2001). Temperature for germination will vary, but reports of 15-25^{o}C are suggested temperatures. Seedlings can be transplanted, when three to four weeks old, to other containers. When the roots of these seedlings are well established, the plants can be hardened off and transplanted outdoors. It is wise to keep the original germination media intact in case any remaining nongerminated seeds emerge at a later date. It is not unusual to have clematis seed germinate erratically. Record information on when the seed was planted, how much was planted, when it germinates, germination conditions and percent germination. Label the plants in the garden as well.

Reports on the value of stratifying seed are mixed (Fair and Fair, 1997; Stribling, 1986). The seed germination of *C. chinensis* was reported to increase when cold stratification was increased from 30 days to 131 days with temperatures of 20^{o} C (Sumi, et al., 1994). In addition, the seed dormancy was reduced in these studies when seed was treated with a 500 ppm gibberilic acid solution, and seed germinated best in the autumn regardless when the seed was sown (Sumi, et al. 1994). The low germination of *C. foetida* in dark treatments has been reported by Burrows (1996), and light, nitrogen and chilling increased germination of *C. vitalba* when these factors were combined rather than when supplied separately (Bungard, et al., 1997).

10. SEEDLING EVALUATION

Seedlings are usually evaluated for several years before a final decision is made concerning the value of these plants, even though some seedlings, especially the late season-flowering types, will flower the first year (Beskaravainaya, 1977). Many others will not flower until the second or third year. It may take several years to evaluate adaptation to a specific site. The progeny from the first generation of a cross may not display the combination of desired traits, so this first generation of plants may have to be self-pollination or crossed back to one of the parents in order to eventually obtain the desirable traits. The greater the number of progeny evaluated, the greater the chance of finding a choice new clematis plant. Good observations are essential for the selection of new clematis types.

The International Code of Nomenclature for Cultivated Plants (Trehane, et al. 1995) requests that all clematis cultivar names be registered with the International Clematis Registrar. Information on the procedure for registering a new cultivar can

be obtained by contacting the International Clematis Society. One of the easiest ways of finding out more about the International Clematis Society is to view their website (www.clematisinternational.com). Other clematis societies include the American Clematis Society, British Clematis Society, Estonian Clematis Society, Finnish Clematis Society, Pacific Northwest Clematis Society and the Swedish Clematis Society.

11. VEGETATIVE PROPAGATION

Once valuable seedlings have been identified, vegetative propagation can be used to multiply these plants for further evaluations. Division of plants can be used to propagate a few clematis selections, such as *C. recta, C. heracleifolia* and *C. integrifolia*, the clump-forming species. Division can take place before new growth starts in early spring by digging the plant, dividing it and resetting individual clumps. Outer edges of the plant, with crown buds and roots attached can also be cut away from the parent plant and transplanted to designed sites or containers.

Most of the vining forms of clematis can be propagated by layering, where a shoot is induced to root while still attached to the parent plant (Toomey and Leeds, 2001). Layering is accomplished by selecting a stem and laying this stem along the ground. Make cuts or slits on the underside of the stem about 0.5 in. below a node and partially cover with soil. Water as needed to keep moist. Once the stem has rooted down at the cuts, the stem can be divided into rooted sections, and the rooting sections either replanted or potted into containers. Clematis can be layered directly in a container of soil as well.

Cuttings, in general, are the best and most commonly used method of propagating clematis (Fair and Fair, 1997; Malek, 1999). Cuttings taken at a partially mature stage of the current season's growth normally are used. This is usually in late spring or early fall for outdoor plants. Cuttings should have at least one node on each stem cutting piece. The cutting can be placed in peat, perlite or a similar product and kept moist. A polyethylene bag can be placed over the cuttings to keep the environment humid if a mist chamber is not available. It may take anywhere from 3 weeks to 3 months for rooting to occur on a cutting but normally takes 25 to 35 days. Weyland (1978) found that cuttings from the older part of a clematis vine rooted 68% of the time, while cuttings from newer growth rooted 48% of the time. He demonstrated that the better the root system was on a cutting, the higher percentage there was of having a bud on the cutting develop into a shoot. Erwin et al. (1997) reported that peat and sand were good media for rooting clematis cuttings. They also suggested that the use of IBA to enhance rooting may be useful on difficult to root clematis cultivars/species like *C.* 'Jackmanii'. Weyland (1978) made the following recommendations when rooting clematis from cuttings: "1) protect the cuttings from drying out; 2) place the cuttings in intermittent mist when

rooting the cuttings; 3) harden off the rooted cuttings in the containers/media they were rooted in; 4) avoid root damage during transplanting; 5) avoid drying of the rooted cuttings before transplanting; 6) plant in moist soil and water immediately; 7) plant during humid weather; and 8) protect transplants from sun and wind until clearly established".

Successful somatic embryogenesis of *C. integrifolia x C. viticella* in tissue culture has been reported by Mandegaran and Sieber (2000), and culturing excised embryos of *C. viorna* using *C. orientalis* endosperm in the medium lead to the successful differentiation of shoots, roots and leaves from callus (Romanova, 1975). Many clematis were at one time grafted, but this method is seldom used except for selections that do not propagate well from division or cuttings (Fretwell, 1997).

12. GERMPLASM

Sources of clematis germplasm for breeding and evaluation can come from many sources. Seed is available through several commercial firms as well as through the seed exchange program of several plant societies, from many plant nurseries, from individual clematis collectors and from collection trips.

13. FUTURE BREEDING WORK

There is a bright future for clematis breeders. By finding new selections with unusual flower forms and colors, plants with unique plant habits, and selections adapted to special conditions, the value and demand for clematis will only increase. It can be a rewarding hobby or career.

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Chapter 30

CONEFLOWER

Echinacea species

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Abstract: *Echinacea*, commonly known as coneflower, is a North American native genus that includes eleven taxa. One species, *E. purpurea* or the purple coneflower, is a very popular garden plant, and has been subject to intensive breeding efforts. Several other species are also cultivated for ornamental purposes, including *E. angustifolia*, the blacksamsom, *E. pallida*, the pale purple coneflower, and *E. paradoxa*, the Bush's purple coneflower or yellow coneflower. Several of these taxa are also very popular in the medicinal plant trade. Habitat degradation and over collection from the wild threatens many of the taxa, most of which have been inadequately researched and sampled. There is evidence that most if not all of the taxa can be intercrossed, which potentially could lead to novel and superior forms for the horticultural trade. To date, this ability has not been exploited. Research is needed to determine the inheritance of such important traits as flower color, disease resistance, interspecific hybrid fertility, and pollen compatibility.

Key words: Blacksamson, coneflower, conservation, interspecific hybridization, propagation, seed germination, tissue culture

The genus *Echinacea* L. (Asteraceae), commonly known as coneflower, is composed of nine species indigenous to North America (McGregor, 1968). Three of these species, *E. angustifolia*, *E. pallida*, and *E. purpurea*, are of commercial interest for their reputed medicinal properties (Sari, Morales, and Simon, 1999). *Echinacea* is also recognized for its ornamental value (Armitage, 1997), most notably *E. purpurea*, the purple coneflower, which is widely grown both for cut flower production and as a garden plant. Plants remain in bloom for long periods of time, the flowers attract butterflies, and the seeds attract birds such as goldfinch. A number of cultivars have been developed from the purple coneflower. Several other species are also cultivated as ornamental plants, including *E. angustifolia*, *E. pallida*, *E. paradoxa*, and *E. tennesseensis*. However, these taxa have not been subject to the degree of breeding and selection that *E. purpurea* has received.

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Hybridization studies indicate that the different species of *Echinacea* can be crossed, and that many of the interspecific combinations form fertile F_1 hybrids (McGregor, 1968; Ault, unpublished data). Presumably, this could be utilized to develop plants with useful ornamental attributes. Currently, there does not appear to be any selected interspecific hybrids in commercial cultivation. Therefore *Echinacea* holds significant promise for further ornamental development. The breeding potential of this genus is the subject of this paper.

1.1 Taxonomic History

Echinacea purpurea was the first member of the genus described by botanists, in the 1700s. It was described more than once and so was given several different names, including *Rudbeckia purpurea* by Linnaeus. Moench first published the genus Echinacea in 1794. *Echinacea* was maintained as a valid genus until the late 1800s, when several authorities reassigned the taxa under it to the genus *Brauneria*. The validity of the two genera was in dispute until resolved at the ninth International Botanical Congress in 1959, when the taxa were reassigned back to *Echinacea* (McGregor 1968).

Currently, there are eleven recognized taxa in the genus *Echinacea* (McKeown, 1999a):

- *E. angustifolia* DC. var. *angustifolia* – blacksamson
- *E. angustifolia* DC. var. *strigosa* McGregor – strigose blacksamson
- *E. atrorubens* Nutt. – Topeka purple coneflower
- *E. laevigata* (Boynton & Beadle) Blake – smooth purple coneflower
- *E. pallida* (Nutt.) Nutt. – pale purple coneflower
- *E. paradoxa* (Norton) Britton var. *neglecta* McGregor – Bush's purple coneflower
- *E. paradoxa* (Norton) Britton var. *paradoxa* – Bush's purple coneflower, yellow coneflower
- *E. purpurea* (L.) Moench – eastern purple coneflower
- *E. sanguinea* Nutt. – sanguin purple coneflower
- *E. simulata* McGregor – wavyleaf purple coneflower
- *E. tennesseensis* (Beadle) Small – Tennessee purple coneflower

1.2 General Description

Echinacea are herbaceous perennial plants that form annually a basal rosette of petiolate leaves and may form one or more erect flowering stems that, depending on the taxon, can be from 1 to 15 dm tall. The foliage is coarsely pubescent for most of the taxa. A persistent underground rootstock can be vertical or horizontal, and is taprooted for all the species except *E. purpurea*, which has a fibrous root system. The flowering heads, which terminate the main stems and lateral branches, are

composed of many fertile disk flowers borne on a flattened to raised receptacle, and a single (rarely more) outer series of sterile ray flowers. The disk flowers are individually subtended by a single stiff, sharp palea, which gives the capitulum a bristly appearance. The corollas of the disk flowers are highly reduced and so are not showy. The pales can be a vibrant maroon or orange, notably in *E. purpurea*. The ray flowers, each with an elongated ligule, equal or exceed the width of the disk, and can be droopy (as in *E. pallida*), spreading (as in *E. purpurea*) or erect (as in *E. tennesseensis*). The ligules of the ray flowers are often, incorrectly so, referred to as petals of the flowering heads. Overall flowering head (disk plus ligules) diameter ranges from as little as 3.5 cm in *E. angustifolia* to nearly 18 cm in some cultivated forms of *E. purpurea*. One of the key ornamental attributes of the genus is the color of the ligules, which can be purple, rose, pink, yellow, or white. Many of the taxa are repeat blooming, such as *E. purpurea*, which can produce flowering heads from June until frost.

1.3 Distribution

The natural range of *Echinacea* is predominantly in the central portion of North America, from the Appalachian Mountains in the east to the Great Plains in the west, and from Texas to the south northward into Saskatchewan, Canada. *Echinacea* inhabits prairies, savannas, open woodlands, glades, slopes, bluffs, and barrens, typically on dry, shallow, or rocky, calcareous soils. The area of greatest diversity, and possibly the center of origin for the genus, is the Ozark Plateau of Missouri and Arkansas, and west into Kansas and Oklahoma. Nine of the eleven taxa occur in this region. The species richness of this area likely account for the hybrid swarms reported from Missouri and Oklahoma (McGregor, 1968; McKeown, 1999a).

Some taxa, such as *E. angustifolia* and *E. purpurea*, have very broad ranges. The distribution of *E. angustifolia* throughout the central plains may in part be due to historic dispersal and utilization as a medicinal plant by the Plains Indians (McKeown, 1999a). *Echinacea purpurea* occurs sporadically throughout the southeastern and Midwestern U.S., and has naturalized in the northeastern U.S. (McGregor, 1968), in northern Illinois (Swink and Wilhelm, 1994), and most likely elsewhere, due perhaps to the widespread use of this species in wildflower seed mixes and as a garden plant. Other taxa are more restricted, notably *E. tennesseensis*, which occurs only within a 14 mile radius within Tennessee, and *E. paradoxa* var. *neglecta*, which is endemic to the Arbuckle mountain region of Oklahoma (McKeown, 1999a).

1.4 Conservation

The greatest threats to natural populations of *Echinacea* are habitat loss and degradation, the practice of wild-harvesting seed, foliage, roots and plants for the

medicinal plant industry, and the potential for genetic pollution. Habitat loss and degradation likely threatens all the taxa. For example, the habitat for the rare taxon *E. laevigata* was thought to be prairie-like openings or post oak-blackjack oak savannas that were maintained by fires. Loss of these open habitats to fire suppression and to urbanization has resulted in the decline of the species (U.S. Fish and Wildlife Service, 1995). Similarly, *E. tennesseensis*, endemic to a small area of central Tennessee, is threatened predominantly by the urbanization (road construction, housing development, and industrial expansion) of its habitats, but potentially also by grazing, mowing, and the suppression of fire (U.S. Fish and Wildlife Service, 1995).

Three species, *E. angustifolia, E. pallida*, and *E. purpurea*, have long been of interest for medicinal utilization. Historically, *Echinacea* was the most widely used medicinal plant of the Plains Indians, who utilized coneflower leaves and roots to treat a variety of ailments such as snake bites, wounds, sores and aches, as a cough medicine, as a cold remedy, and for other uses (Foster, 1984, Kindscher, 1989). European settlers in the Midwest readily adapted its utilization in the 1800s, and exported the practice back to Europe. It was estimated that as many as two million coneflower roots were harvested in Kansas alone in 1902 (Kindscher, 1989).

Echinacea has undergone resurgence as an herbal supplement in the U.S. in recent decades, leading once again to the heavy harvesting of wild populations. One study estimated that 700,000 individual plants of *E. angustifolia* were removed from the wild in the northeastern region of Montana just in 1997 (McKeown, 1999b). The loss of wild stands led to legislation enacted in Missouri in 1987 making it illegal to harvest the three native species on state land (Kindscher, 1989). Since 1998, legislation was also enacted both in Montana and North Dakota for the regulation of wild harvesting in those states (McKeown, 1999b). Field cultivation of *Echinacea* has increased in the United States and Canada in recent years (Li, 1998; Sheldon, Balick, and Laird, 1997); hopefully, this will alleviate some of the collecting pressure on wild populations.

It has been demonstrated that the different species of *Echinacea* can readily interbreed, and that natural hybrid swarms exist (McGregor, 1968). Field and garden cultivation of *Echinacea* is increasing in the United States. *Echinacea purpurea* is thought to have become naturalized outside of its historic range (McKeown, 1999a). Therefore, as cultivated stands of *Echinacea* are grown in proximity to natural stands, or escape cultivation and become naturalized, the potential exists for genetic pollution of existent populations. A study examining gene flow between a wild type *E. purpurea* and the cultivar *E. purpurea* 'White Swan' indicated the resulting hybrid plants could successfully survive and reproduce under field conditions (Van Gaal, Galatowitsch, and Strefeler, 1998). Two taxa, *E. tennesseensis* and *E. laevigata*, are listed as nationally endangered in the United States (USDIFWS 1979, 1992). Hybrid populations, with presumably novel gene complexes, have been reported from Missouri and Oklahoma (McKeown, 1999a).

Hopefully, increased efforts will be made to preserve these rare and unique taxa *in situ* from the pressures of habitat degradation, wild collecting, and the potential for genetic pollution.

2. PRODUCTION

The economical production of *Echinacea* either for the medicinal plant industry or for the horticultural industry depends on understanding the propagation, cultivation, hardiness, disease susceptibility, and other parameters of this plant. These are detailed below.

2.1 Propagation

Echinacea may be propagated from seed, basal shoot cuttings, root cuttings, division of the crown, and through tissue culture. Propagation from shoot and root cuttings and by division of the crown is not practical for large-scale production but may be effective for small-scale production of selected genotypes. Mature, dormant plants can be dug and the crown divided into two to seven offsets each with a vegetative bud, which are immediately replanted (Foster, 1984). Root sections 10- to 12 cm in length can be planted for shoot regeneration (Li, 1998).

Seed germination protocols have been determined for several of the species. Protocols recommended for *E. angustifolia* include a 12 week stratification treatment (Baskin, Baskin, and Hoffman, 1992; USDA, NRCS. 2001); 2 weeks at 4°C combined with 1.0 mM ethephon and continuous light followed by 2 weeks at 25°C with 16 hours light per day, which resulted in over 95% germination (Feghahati and Reese, 1994); and a 10 minute treatment with 5.3 M KOH prior to germination at 14/10°C with 14 hours light per day (Gao, Zheng, and Gusta, 1998). Protocols for seed germination of *E. purpurea* include 4 weeks stratification at 4.5°C followed by germination at 20°C (Beattie and Berghage, 1997); 4 weeks of stratification at 5°C, which proved optimal from a range of temperatures tested for subsequent germination at 20°C (Brachter, Dole, and Cole, 1993); and priming seed for 6 or 9 days at 16°C with 50 mM K_2HPO_4 + KH_2PO_4 which significantly increased subsequent germination at 23°C in the dark (Samfield, Zajicek, and Cobb, 1990). This author routinely stratifies seed of various *Echinacea* taxa for 4 weeks at 5°C prior to sowing seed in the greenhouse, resulting in adequate germination.

Most *Echinacea* will not bloom until the second year of growth. Seed harvest can commence in the fall of the second year. Cleaned *E. angustifolia* seed (2.2 kg) contains about 128,000 seeds (USDA NRCS, 2001), and 2.2 kg of cleaned *E. purpurea* seed contains 96,000 seed (USDA NRCS, 2001). As the taxa appear for the main to be outcrossers with a high degree of interfertility (McGregor, 1968), it is recommended that stock blocks for seed production be well isolated.

Tissue culture propagation protocols have been reported for several *Echinacea* taxa. Field collected leaves of *E. angustifolia* were utilized to regenerate shoots on a medium with 14 μM Kinetin plus 0.5 μM NAA (Holden, Ellis, and Chen, 1978). Utilizing explants from *in vitro* grown *E. purpurea* seedlings, roots were induced from hypocotyl explants utilizing various concentrations of IAA and IBA (Choffee, Murch, and Saxena, 2000), and both somatic embryos and shoots were induced from petiole explants, with 2.5 μM BAP promoting the greatest number of regenerants per explant (Choffee, Victor, Murch, and Saxena, 2000). Both the somatic embryos and shoots rooted readily on basal medium, and were successfully acclimatized to greenhouse conditions. Another study utilizing hypocotyl explants from *in vitro* grown *E. purpurea* seedlings reported optimal shoot regeneration from treatment with 4.6 μM Kinetin in combination with 5.4 or 10.8 μM NAA; the regenerated shoots also rooted on these media (Coker and Camper, 2000). In a series of experiments on the micropropagation of *E. angustifolia*, *E. pallida*, and *E. purpurea* (Harbage, 2001), adventitious shoots were initiated for all three taxa by treatment with 4.44 μM BA of both zygotic embryo and stem segment explants. Separate experiments indicated that the number of shoots formed per explant varied with the taxa and BA concentration, and that rooting percentages also varied with the taxa. In summary, additional research is needed to refine micropropagation protocols, but the technique appears to offer potential as a method of propagating the economically important *Echinacea* taxa. This may prove most valuable for the large-scale propagation of selected clones, as the alternative methods of propagation by root cuttings and division yields a low number of propagules. The technology is now being utilized for a few horticultural selections; for example, *Echinacea purpurea* 'Kim's Knee High' (PPAF), a compact selection introduced in 1999 by Niche Gardens (Chapel Hill, N. Carolina, U.S.) is being propagated through tissue culture.

2.2 Cultivation

Seed can either be field sown or started in the greenhouse. Fall sowing is preferred for direct field seeding; in one study, both *E. pallida* and *E. purpurea* germinated better with a fall sowing (Albrecht and Smith-Jochum, 1990). Fall field planting of seed is also recommended for *E. angustifolia* (USDA NRCS, 2001). Greenhouse production of seedlings followed by field transplanting enhanced establishment for three *Echinacea* species in comparison with direct seeding (Smith-Jochum and Albrecht, 1988). Seed can be treated for germination as outlined above, then the seedlings can be grown on for containers production, or for either a spring or fall field planting. The tap rooted taxa can become pot bound, and so should be planted out while still small or grown on in larger containers. Plants of *E. purpurea* may bloom the first year, if started early enough, but the other taxa tend to not bloom until the second year in the field. For field production, a spacing between plants from 30 cm (Li, 1998) to 46 cm (Foster, 1984) between plants have been

recommended. In field cultivation, *Echinacea* are not competitive with other plants, so appropriate weed control is required. Plants being field grown for medicinal utilization are typically harvested in the fall of the third or fourth year from seed (USDA, NRCS, 2001). The fresh root yield from field grown *E. angustifolia* can be 2,500 kg/ha (Hobbs, 1989).

With appropriate germplasm selection, *Echinacea* can be winter hardy to -40°C, and can be heat tolerant to 38°C. All *Echinacea* prefer to be grown in full sun to light shade. A deep, well-drained soil is recommended. The taxa prefer a neutral to alkaline soil, tolerating a soil pH as high as 8.0. Plants are not tolerant of a poorly drained soil, especially in the winter. Plants are drought tolerant, especially the taprooted taxa. Under optimal conditions, individual plants can be long lived.

2.3 Pest Problems

Echinacea for the most part seem to be little bothered by insect or animal herbivores. However, there are several diseases of concern. A survey of field and greenhouse growers in Alberta, Canada, identified six major diseases of *Echinacea*: aster yellows, Sclerotinia stem rot, damping-off, Fusarium crown and root rot, botrytis blight, and Alternaria leaf spot (Chang, Howard, Hwang, and Blade, 1999). Aster yellows may pose the greatest problem for field grown plants. The disease is caused by a phytoplasma, which is primarily vectored by leafhoppers. Symptoms include yellowing of leaves, especially the new growth in spring, leaf reddening, plant stunting, proliferation of axillary shoots, virescence and phyllody of the floral organs, and the formation of smaller florets arising from the disks of the primary flower heads; the foliage symptoms can appear on first year plants, and the floral symptoms on second year plants (Chang, et al., 1999; Chang, Howard, Blade, and Hwang, 2000a). Diseased plants will not recover and need to be promptly removed to prevent the spread of infection. Other measures include removal of susceptible weed plants and controlling the insect vector. *Echinacea purpurea* appears to be the most susceptible of the taxa to aster yellows, and *E. angustifolia* the least susceptible (Chang, et al., 1999; Sari, et al., 1999). McKeown (1999a) notes that selection for greater leaf pubescence in the former species may confer greater resistance by deterring the insect vector. Research needs to be directed towards the genetics and breeding of resistance to both the vector and to the disease.

The other diseases listed for *Echinacea* can also be problematic; for example, a survey of greenhouse grown *E. angustifolia* seedlings in Alberta, Canada, indicated that the incidence of damping-off and root rot was as high as 35% (Chang, Howard, Blade and Hwang, 2000b). *Fusarium* species, which were the most commonly isolated causal agents, can be controlled by avoiding injury to the roots and by avoiding overwatering of the growing medium. Sclerotinia stem and root rot can be prevalent in field grown plants under moist soil conditions, leading to rapid wilt of plants, leaf disintegration, and root rot; *E. purpurea* is more resistant than *E.*

angustifolia (Chang, et al., 1999). Symptoms and control measures for the aforementioned diseases are detailed in the latter reference.

Other potential problems in *Echinacea* may include several other fungal diseases and viruses (Li, 1998). Plants do not seem to be bothered by herbivorous insects or by deer.

3. BREEDING

Echinacea breeding needs to progress in two directions, one for the ornamental plant industry, and the other for the medicinal plant industry. Both areas have some common goals – selection for disease and insect resistance, and improved hardiness for example – but selection for other useful traits will vary depending on the ultimate use of the crop. This discussion will focus largely on selection for the ornamental plant industry, as it appears that all of the breeding and selection work to date has been for that use, and not for the medicinal industry (McKeown, 1999b). Certainly the latter could benefit from the selection of uniform, vigorous selections with known chemical composition.

3.1 Crop Ideotype

The ideal *Echinacea* for ornamental utilization would have the following characteristics: bloom the first year from seed; reproduce as a homozygous seed line (i.e. overcome the self-incompatibility barrier); produce sweetly fragrant flower heads throughout the season on compact, sturdy stems; produce ray flowers in colors of white, yellow, pink, magenta, orange, or red; produce ray flowers with different orientation (i.e. drooping, horizontal, erect); selection of doubled forms, including an increase in the number of ray flowers, and petalody of the disk flowers; resistance to leaf hoppers, aster yellows, and Fusarium and Sclerotinia rots; greater tolerance to wet soils and to shade; and the combined heat and cold tolerances of the different taxa. The ideal plant for medicinal utilization would have the same characteristics for seed reproduction, disease resistance, and soil moisture, shade, and temperature tolerance, as well as; higher biomass production of both foliage and roots; shorter production time; increased and uniform accumulation of the medicinally active compounds; and control over which active compounds are accumulated. As will be discussed below, most of these goals may be possible to achieve.

3.2 Reproductive Barriers

Successful breeding and selection of *Echinacea* is dependent on understanding the reproductive barriers of this crop. Previously, the genus was described as being completely self-incompatible (McGregor, 1968). *E. angustifolia* plants from a

Minnesota population proved to be completely self-incompatible in a controlled pollination study (Wagenius, 2000). The author of this paper has seen little evidence of successful self-pollination in controlled crosses involving four species and their interspecific hybrid combinations. Conversely, a different report states that every species of *Echinacea* is self-compatible to some degree (McKeown, 1999b), while another study of *E. angustifolia* reported successful self-pollinations as high as 9% for wild populations from South Dakota (Leuszler, Tepedino, and Alston, 1996).

Additional research is needed to more adequately quantify the degree and type of self-incompatibility both within and between the taxa of *Echinacea*, but the high degree of self-incompatibility that appears to exist in this crop suggests some breeding strategies. In cross-pollinated crops like *Echinacea*, both the individual plants of the breeding population and their progeny tend to be heterozygous, as they are all the products of cross-pollination with other heterozygous plants. Therefore, to maintain a reasonably uniform seed line the breeding focus needs to be on selecting desirable traits at the population level rather than at the individual plant level (unless individual plants are being selected for clonal propagation). Phenotypic recurrent selection is a straightforward selection model that should be applicable for the development and maintenance of *Echinacea* seed lines. Plants are selected from the source population based on desirable visible traits – for ornamental *Echinacea*, this could include plant height, capitulum size, and ray flower color – then the progeny from these plants are grown out and intercrossed to form a new source population. The cycle can be repeated to maintain and improve the population based on the desired, selected traits.

Most of the *E. purpurea* cultivars in the market place today are seed propagated, such as the large-flowered, rose-colored selection ‘Magnus’ (Beattie and Berghage, 1997) and the white flowered selection ‘White Swan’ (Armitage, 1997). These and the other seed selections are frequently quite variable in appearance, at times to such an extreme as to not be recognizable against their descriptions. This variability is most likely due to some of the production nurseries not understanding the crop traits and the appropriate breeding responses. The nurseries producing these selections need to be aware of the self-incompatibility of this genus, and to therefore grow out populations of plants and reselect seed stock on a routine basis. The ease with which interspecific hybrids can form also necessitates isolating seed stock blocks from one another to ensure adequate seed quality control.

Seed stock blocks can be isolated either spatially or temporally. Temporal isolation can be difficult as the taxa may have extended and overlapping bloom periods, which precludes utilizing differential bloom times for isolation. Spatial isolation is the preferred method, which can be accomplished either by barriers or sufficient distance between plant populations. *A variety of insects, including honeybees (Apis), bumblebees (Bombus), and numerous butterflies can pollinate Echinacea*. The foraging distances for these insects can be extensive; for example,

the median foraging distance for honey bees (*Apis mellifera*) was 6.1 km in one study (Beekman and Ratnieks, 2000). There does not seem to be a recommended isolation distance for *Echinacea*, but it may be possible to adapt recommended distances for *Helianthus* (sunflowers), which is also a cross-pollinating taxon from the Asteraceae. For field grown sunflower crops, the recommended distances for isolation range from 0.8 km (Kwon and Kim, 2001) to 6.4 km (Smith, 1978). These distances can likely be modified to take into account restrictive barriers such as alternate crops and windbreaks between *Echinacea* populations, and the likelihood of growing smaller populations than would be typically grown for *Helianthus*.

Echinacea can also be field grown in pollination cages with the appropriate pollinator introduced inside the barrier. The United States Department of Agriculture (USDA) has successfully utilized this approach to reproduce the accessions of *Echinacea* and other crops being maintained within the U.S. National Plant Germplasm System (Widrlechner, Abel, and Wilson, 1996). Production nurseries and plant breeders could readily adopt this technique.

3.3 Reproductive Biology

The previous section briefly discussed insect-mediated pollination for mass propagation. For more selected breeding, it is essential to utilize techniques that will better control pollination. Previously, this author has not emasculated *Echinacea* flowers as it was thought the taxon was self-incompatible; however the reports cited above of some level of self-compatibility existing in the genus suggests that emasculation may be prudent. Manual emasculation with forceps would likely be too laborious, as each capitulum is an aggregate of many, tightly grouped, pollen-producing florets; for example, wild *E. angustifolia* produced 156 florets per capitulum in one study (Wagenius, 2000). One procedure that may be effective is a modification of the clip and wash method adopted for lettuce, in which the elongating corollas of the disk flowers were first cut, then the pollen removed at anthesis via a stream of water from a spray bottle (Nagata, 1992). The corollas of *Echinacea* disk flowers are very short, and so do not need clipping; however, the subtending pales can extend beyond the height of the receptive stigmas, and so interfere with pollination efficiency. This author routinely trims the pales; this is best accomplished by selecting an immature capitulum that has produced the pales, but has yet to reach floral anthesis. The receptacle at this stage is generally flattened, making removal of the pales easier. The pales can be readily cut off to just above the immature disk flowers with a one-sided razor blade. Be sure to sterilize the blade or utilize a new one between plants to prevent the possible spread of diseases. Removal of the pales in this manner more readily exposes the anthers and stigmas at floret maturity, making pollen collection and pollination easier.

Once a capitulum has had the pales removed, it may be possible to wash the self-produced pollen off prior to outcrossing; however, this will be a laborious

procedure. The disk flowers of *Echinacea* open in concentric rows over a number of days, proceeding from the outer portion of the receptacle to the center. Self-produced pollen would then have to be washed off on a daily basis, immediately followed by outcrossing, then the process repeated until final floral anthesis is reached.

The disk flowers of *Echinacea* are protandrous, with the anthers of all the disk flowers located in one row dehiscing and shedding their pollen one-day, followed by the styles emerging from the same florets the next day. The anthers of the adjacent, inner row of florets in turn dehisce as the stigmas of the outer row become receptive. This pattern is repeated until all of the disk florets have bloomed. Depending on the taxon, the florets of a single capitulum can take up to 14 days to all bloom. The individual styles can remain receptive for several days each; for example, the styles were receptive on average 2.4 to 2.7 days for one population of *E. angustifolia* examined for a three year period (Wagenius, 2000). This extended period of stigma receptivity can optimize pollination effectiveness, as an individual stigma can then be readily pollinated several times while still receptive.

One recently reported trait of *E. angustifolia* may be useful for the breeding of all *Echinacea* taxa. Wagenius (2000) observed that the stigmas of *E. angustifolia* would persist for several days if not pollinated with compatible pollen. When the stigmas were pollinated with compatible pollen, they rapidly shriveled, whereas when stigmas were either self-pollinated or pollinated with incompatible pollen, they did not shrivel immediately after pollination. Subsequent seed set for the compatible pollinations verified this physical response of the stigmas, and the lack of seed set for the incompatible pollinations. If this trait is true for other *Echinacea* taxa, then it may prove to be a valuable predictor of self-compatibility, cross-compatibility and of subsequent seed production.

To exclude pollinators from the flowers of *Echinacea*, either caging the entire plants (Widrlechner, et al., 1996) or covering individual capitula with mesh bags (Wagenius, 2000) is effective. This author routinely utilizes the latter with no apparent negative effect on fertilization or seed maturation. Caution must be taken in opening and closing the individual bags to ensure no openings remain for possible insect pollinators to enter through; the uncovered capitulum must also be closely watched as bees will readily land on it even as the breeder is pollinating the florets. The stem bearing the bagged capitulum should be supported with a stake to ensure it is not broken off, as the bag over the capitulum can act like a sail in the wind. Keeping the bag over the capitulum through seed maturation and collecting is effective in deterring insect and bird predation of the ripening or mature seed.

Pollen can be collected either by gently bending a capitulum bearing florets with dehisced anthers sideways and tapping it over a petri dish, or by gathering up clumps of pollen with fine, curved-tip forceps. The pollen is “sticky” and tends to persist in a clump until disturbed by a pollinator or strong wind, which makes it easy to gather from the individual florets. There does not appear to be any published

data on the long term storage of *Echinacea* pollen, but from the author's experience, pollen can be stored in gelatin capsules in a sealed jar without desiccation and under refrigeration (5°C) for up to several days and still be viable. However, with the extended bloom period of so many of the *Echinacea* taxa, it may not be necessary to store pollen for breeding purposes as the parent plants will likely bloom at the same time.

3.4 Species Traits and Selected Forms

Before a discussion of the interspecific breeding of *Echinacea*, it is beneficial to detail the potentially useful ornamental traits of the different taxa, both of the undomesticated species and of the selected, cultivated forms. The current commercial availability in the U.S. of these taxa will also be mentioned.

Echinacea angustifolia var. angustifolia. The taxon that was widely utilized for medicinal purposes by the Plains Indians, it is now being grown in increasing acreage for medicinal utilization. While perhaps not as showy as some of the other *Echinacea* due to its short ray flowers, this taxon still has its ornamental usage, notably for native plant gardens, prairie restorations and creations, and in habitats otherwise too severe for the other coneflowers. This taxon has the northernmost (into Canada) as well as the westernmost (eastern Montana) distribution in the genus. Populations at the extreme ranges should be well adapted for cold hardiness and drought tolerance, respectively, traits which would be useful in a selection program. The plants are compact, generally less than 0.5 meters tall. *Echinacea angustifolia* reputedly has good resistance to aster yellows. The ray flowers are broad relative to their length, which could be useful in developing types with broader or overlapping ligules. McGregor (1968) notes that this is the only taxon of the genus with sclereid cells in the stems, which presumably confers stem strength to this species, and the hybrids from it. The drawbacks to this taxon include the short rays, the few flower heads produced, the short duration of bloom relative to the repeat blooming taxa, and poor adaptability to wet clay soils. The taxon is available on a limited basis for ornamental use, both as seed and as plants. There is one selected form, 'Mecklenberg Select', a seed cultivar with reputedly darker rose ray flowers. It persisted for several seasons for the author, which is more than can be said of the other plants of the taxon that were trialed.

Echinacea angustifolia var. strigosa. The taxon occurs in a narrow band from Kansas to northeastern Texas. It branches more than the previous variety, and has broader flower heads. The ligules are a darker red color. Its resistance to aster yellows has not been characterized. Presumably, it may not be as cold hardy as the previous variety. The taxon may be a natural hybrid of *E. atrorubens* and *E. angustifolia* var. *angustifolia* (McGregor, 1968); presumably, there exists unique and useful genotypes among the natural populations. Tetraploid forms of the plant have been noted (McGregor, 1968), which could be valuable in breeding with the other tetraploid species, *E. pallida*. *Echinacea angustifolia* var. *strigosa* does not appear to be in cultivation.

Echinacea atrorubens. Another narrow endemic taxon, found only in Texas, Oklahoma, and Kansas. It has tall, sturdy stems to 0.9 meters in height. The ligules are a deep reddish purple or magenta color, among the darkest in the genus, which

would be useful to breed into other taxa. However, the ligules are also rather short, less than 3.3 cm, and recurve down and inwards so strongly as to often touch the stem. Hardiness may also be problematic due to the taxon's more southern U.S. nativity. The taxon is available on a limited basis as seed.

Echinacea laevigata. One of the two federally endangered species, it has not been widely cultivated. Found at the easternmost end of the distribution for the genus, it is native to the Appalachian Mountains and the piedmont from Georgia north to Virginia. Presumably, from its nativity, the taxon has useful heat and humidity tolerances. Plants produce handsome flowers on tall, upright stems that can reach 1.5 meters in height. The narrow, gracefully drooping ray flowers are among the largest for the genus, up to 8 cm long, and can be white to light pink to deep magenta. The taxon has great ornamental potential, but its federal endangered status places restrictions on the collecting and the interstate commerce of the plant. Seed or plants should not be collected from the wild. It is occasionally available from nurseries within its native states. Several nurseries also sell plants labeled as *E. laevigata* hybrids, presumably with *E. purpurea*. The hybrid, which has been hardy for the author, is intermediate to the parent species. Robust, vigorous plants produce a profusion of flower heads, each with very prominent, slightly drooping, narrow ray flowers tending towards light pink. The plants have been fertile, and will hybridize with other genera. The hybrids have been very susceptible to aster yellows, indicating *E. laevigata* may also be susceptible.

Echinacea pallida. The species can be found in dry prairies, glades, and other habitats from Wisconsin to Texas. It can grow to over a meter tall, producing a few flowering heads that are individually borne on top of the typically unbranched stems. The ray flowers are long, to 9 cm, narrow, and rather droopy. The plant makes a good addition to the naturalistic garden and the less former border, where the flowers on their tall stems can rise above the surrounding vegetation. With such a north to south range, there are likely differences in heat and cold tolerances that could be utilized in breeding. The ray flowers tend to be a darker purple pink from the northern end of its range. A seed selection with uniformly dark pink ligules would be an attractive addition to the trade. This species is a tetraploid, which may limit its breeding potential in crosses with the other taxa. It is available both as plants and as seed. There do not appear to be any developed selections available.

Echinacea paradoxa var. neglecta. This variety has a very narrow range, being restricted to south central Oklahoma. The flowers are similar to those of *E. pallida*, with long (to 7 cm), narrow, drooping ray flowers that can be white to pink to lavender. Plants can grow to 0.8 meters tall. The taxon has not been widely garden tested, and so its usefulness in a breeding program is unknown. Hardiness may be an issue due to its more southern provenance. It can be mistaken for other taxa, and is under pressure from collectors. It does not appear to be commercially available.

Echinacea paradoxa var. paradoxa. The species epithet is very apt as it is truly a paradox why this taxon has yellow ray flowers, the only such member of the genus. Native to the Ozark Mountains of Kansas, Arkansas, Missouri, and Texas, the attractive flowering heads are terminally borne on upright stems approaching 1 meter in height. The drooping, narrow, yellow rays can be up to 7 cm long. In the north, this taxon is one of the earliest of the genus to bloom, and tends to produce a single flush of flowers. It has proven hardy well north of its natural range. Invaluable for its unique ray flower color alone, artificial hybrids with it can produce

unique ray flower colors (see below). The species forms hybrids with several taxa in the wild, some of which have significant ornamental potential; McGregor (1968) described hybrids with orange-red ligules, as well as yellow ligules with reddish bases. None of the natural hybrids appear to be in cultivation. The species has become more readily available in recent years, both as seed and as plants. There do not appear to be any developed selections available.

Echinacea purpurea. It is safe to say that there have been more selections made from this taxon than from all the other *Echinacea* taxa combined. This is a very popular garden plant, grown for its showy flowers that are produced over a long bloom season, for its ease of cultivation, its hardiness and drought tolerance, and for its attraction of butterflies and goldfinches. The ray flowers range from white, pink, magenta, to deep red purple, can be held stiffly horizontal or slightly drooping, are broad to the point of overlapping in many selections, and can be up to 7 cm in length. Some selections have sweetly fragrant flowers. The prominent orange to red pales on the raised cones is also quite attractive and provides an interesting contrast to the ray flowers. Plants can range from 0.3 to 1.2 meters tall. The flowering stems are upright, and branch throughout the growing season, producing new flowering heads. Unique among the *Echinacea*, this species has a fibrous root system, which possibly makes it easier to transplant. New selections continue to appear on the marketplace, notably in recent years clonal selections that are being propagated though tissue culture. This species has and will continue to take a central position in the development of the genus, contributing its branching habit, long bloom season, showy flowers, and garden adaptability.

Perhaps the one greatest drawback to the species is its susceptibility to aster yellows. Selections with greater leaf pubescence may confer some resistance to the leaf hopper vector of the disease (McKeown, 1999a), as may crossing this species with the more resistant species such as *E. angustifolia*.

Although the species has long been subject to breeding and selection, there may still be useful genotypes in the wild that should be evaluated. McKeown (1999a) mentions that there are populations in Louisiana and Missouri with striking, deep lavender ligules; these populations likely also have genes for heat and humidity tolerances as well. Plants from the western end of its natural distribution tend to have greater pubescence, which may be useful in developing improved aster yellow resistance. McKeown (1999a) also reports that this species prefers a semishaded habitat in nature; as the genus is typically thought of as plants for cultivation in full sun, there may be an opportunity to utilise appropriate genotypes of this species for the development of more shade tolerant selections.

While not meant to be exhaustive, here are some of the *E. purpurea* selections currently in the trade:

- ‘Alba' – a seed grown cultivar with white ray flowers and a greenish brown disk, it seems to also be loosely applied by many nurseries and gardeners to any white flowered plant of the species. Likely misapplied to other white-flowered selections. The white flower color is recessive to the more common pink or magenta color, so care must be exercised in isolating seed stock from the non-white flowering forms. The white-flowered selections often tend to not be as

vigorous, as tall, or as large flowered as the non-white flowering forms. The disks on the white flowered selections tend to be greenish gold.

- 'Bravado' – a seed propagated selection with 10-12 cm wide flower heads. Grows to 72 cm tall. The ray flowers are rose red, are broader than most other selections, and are held horizontally.
- 'Bright Star' – see 'Leuchstern'.
- 'Crimson Star' – One of the most deeply pigmented selections available, the ray flowers are a deep crimson red, and are quite horizontal. Plants can become 60-76 cm tall. A clonal selection that needs to be vegetatively propagated to be true to type.
- 'Cygnet White' – a white flowered selection, the rays are held horizontally. A compact selection growing to 50 cm tall. Similar to 'White Swan' except more compact.
- 'Kim's Knee High' – a compact, clonal selection growing only 40 cm tall, with smaller flower heads bearing drooping clear pink purple rays. Vegetatively propagated.
- 'Kim's Mop Head' – a compact, clonal selection growing to 40 cm tall, with drooping white ray flowers that have fringed tips, and a gold tinged green cone. Resulted as a mutation from 'Kim's Knee High'.
- 'Leuchstern' – plants grow from 90 to 120 cm tall, producing 10 cm wide flower heads with flat to slightly drooping, bright rose to maroon rays. A seed selection, thus plants are variable. Also called 'Bright Star', 'Star Bright' and 'Starlight'. Very floriferous.
- 'Magnus' – propagated mainly from seed, and perhaps occasionally by vegetative means, 'Magnus' can grow to 120 tall and produces 10-12 cm wide flower heads with deep rose magenta ray flowers that are horizontal to slightly drooping. There can be extra ray flowers produced, giving the impression of a fuller flower. The center disk is an attractive dark red with rusty orange pales. The original 'Magnus' was reputedly a clonal selection with huge, 17 cm wide, flower heads and intensely dark ray flowers on compact, 76 cm tall plants; unfortunately, the original clone may be lost due to the advent of seed propagation of this selection.
- 'Robert Bloom' – a vigorous selection growing to 90 cm tall, producing large, 12 cm wide and flower heads with flattened to slightly upturned reddish purple rays. Selected from the German strain 'Abendsonne'.
- 'Ruby Giant' – a clonal selection derived from the seed strain 'Rubinstern'. Produces 12-18 cm wide flowers with bright pink rays, each with an up curved tip. The flowers are also fragrant. Grows to 90 cm tall.
- 'Ruby Star' – see 'Rubinstern'.
- 'Rubinstern' – produces large, intense carmine red flowers with horizontal rays and a dark orange brown cone. A robust selection with stems to 100 cm tall.

Like 'Magnus', this was reputedly a clonal selection that has become muddled through the practice of seed propagation. Also known as 'Ruby Star'.

- 'Springbrook Crimson Star' – a selected form derived from 'Crimson Star', it has sturdy, compact stems and flowers with flat, crimson-red rays. The flowers do not fade in summer heat. Vegetatively propagated.
- 'White Luster' – gracefully drooping white ray flowers on 60 cm tall plants. The disks are orange brown. Both seed and vegetatively propagated.
- 'White Swan' – 10 cm wide flower heads with white ray flowers that are often green tinged. Grows to 90 cm tall. Seed propagated. A vigorous selection.

Echinacea sanguinea. Native to open, acidic, sandy pine barrens of the south central U.S., from Louisiana into Texas and north into Arkansas and Oklahoma. This taxon is similar in appearance to *E. pallida*, with mostly unbranched stems up to 0.9 meters tall, and flower heads with 4 – 7 cm long, narrow, pendulous ray flowers that can be a very pale pink to rose red. The disk corolla can be a blood red color, which could be a useful horticultural trait. Plants from Texas and Louisiana did not prove hardy for McGregor (1968) in Kansas in each of five years tested. The taxon does not appear to be available. Its lack of hardiness may limit its horticultural usefulness.

Echinacea simulata. This taxon is native to the southeastern U.S., from south central Missouri though Tennessee into northern Georgia. It is very similar to *E. pallida* in appearance, with mostly unbranched stems to 1.0 meter tall, and flower heads bearing drooping, narrow, 4-9 cm long ray flowers that range from a pale pink to an attractive deep magenta. McKeown (1999a) notes that plants are "remarkably fragrant", which, coupled with the darker colored ray flowers, could make this species a useful plant. It may be available on a very limited basis from a few native plant nurseries.

Echinacea tennesseensis. Listed as an endangered species within the U.S., this taxon is found only in central Tennessee, where its cedar glade habitat is threatened by woody plant invasion and development. It is an easy plant to cultivate, and the species may turn out to be one of the most horticulturally significant of the genus. Plants are compact; growing to 0.4 meters in height, long lived, and produce many simple to branched flowering stems in cultivation. The flower heads are smaller than most *Echinacea*, up to 7 cm in diameter, but are produced in great profusion from midsummer into fall. The ray flowers are an attractive pink violet to purplish pink, and, unique among all *Echinacea*, are upturned. The taxon appears to have good resistance to aster yellows, perhaps due to the dense pubescence of the foliage and stems. Some authorities consider this taxon as closely related to *E. angustifolia* (McGregor, 1968), to which it has some morphological similarities. Federal regulations preclude the interstate commerce of this species without a permit; however, many nurseries have circumvented this by offering plants for sale that are unselected hybrids with other *Echinacea*, most likely *E. purpurea*. These are for the main part inferior to the species. There are no selected forms of *E. tennesseensis*

offered for sale as of yet. The species (or its hybrids) are becoming more available each year, a testimony to its ease in cultivation and to its attractiveness.

Both McGregor (1968) and McKeown (1999a,b) reported on the considerable morphological variability between and within populations of the different taxa, as well as on the presence of hybrid plants where species ranges overlap. It is very likely that potentially useful and unique combinations of traits could be derived from these populations and natural hybrids. Unfortunately, the multiple threats of habitat degradation and destruction, and the largely uncontrolled practice of wild harvesting plants for the medicinal trade, may well obliterate these populations before they can be adequately surveyed, described, and sampled.

3.5 Interspecific Hybridization

The greatest unrealized potential for development of *Echinacea* as an ornamental crop is through the interspecific hybridization of the species and their varieties. Only a few interspecific hybrids appear to be commercially available, and these are as yet in limited supply. Hybrids of *E. purpurea* and *E. laevigata*, and of *E. purpurea* and *E. tennesseensis* have been marketed; however, they appear to be unselected populations that are quite variable in appearance and in general inferior to other *Echinacea* selections. This is unfortunate, considering how many years ago McGregor (1968) reported that F_1 hybrids could be produced from all possible species combinations (with the exception of *E. tennesseensis*, which was not included in the study due to its rarity); he also observed that "the best possibility for obtaining a new valued cultivar is in the hybrids between *Echinacea purpurea* L. and *E. angustifolia* DC. var. *angustifolia*." While McGregor was able to obtain seed from all species combinations he tested, the degree of success varied, from the combination of *E. simulata* and *E. sanguinea* yielding about 10% viable seed, to the combination of *E. angustifolia* var. *angustifolia* and *E. purpurea* yielding over 90% viable seed. He also reported that backcrosses were relatively successful, with the progeny segregating "much as would be expected". Several of the taxa also form natural hybrids in the wild, such as between *E. pallida* and *E. angustifolia* var. *angustifolia* as well as with *E. simulata*; *E. paradoxa* var. *paradoxa* with *E. pallida* and *E. simulata*; and a complex of hybrid swarms involving putatively *E. paradoxa* var. *neglecta*, both varieties of *E. angustifolia*, and *E. atrorubens* (McKeown, 1999a).

Ault (unpublished data) has also attempted a number of interspecific *Echinacea* crosses, which are summarized below (Table 30-1). Crosses were conducted by collecting pollen from donor capitula into disposable petri dishes, then pollinating the receptive stigmas on receptor capitula utilizing small paintbrushes. Both the donor and receptor capitula were enclosed in mesh bags to prevent pollen contamination by insects and other visitors. Pollen was generally utilized the day it was collected. Brushes were thoroughly cleaned in 70% ethanol then allowed to air

dry overnight in a laboratory between uses. Receptive stigma were pollinated two to four times each over a two-day period to ensure a sufficient pollen load for fertilization.

Table 30-1. Fertility of interspecific *Echinacea* hybrids (Ault, unpublished data).

Taxa Crossed[1]	Viable Seed Set[2]	Progeny Fertile[3]	Comments
ang × tenn	+++	++	Recip the same
para × purp	+++	+++	Recip the same
para × tenn	+	0	Recip the same
purp × tenn	+++	+++	Recip the same
[ang × tenn] × purp	+++	+++	
[para × tenn] × para	0		
[para × tenn] × purp	0		
Purp × [para × tenn]	+++	+++	
[purp × laev] × purp	+++	++	
[purp × laev] × tenn	+		Recip the same
[purp × tenn] × purp	+++	+++	Recip the same
tenn × [para × tenn]	0		
[ang × tenn] × [purp × para]	+		
[ang × tenn] × [tenn × purp]	+, +++		
[para × tenn] × [purp × para]	0		
[[purp × tenn] × purp] × tenn	+++	+++	
[purp × [purp × tenn]] × purp	+++	+++	
[[purp × laev] × purp] × para	+		
[purp × para] × [purp × [[para × tenn]]	+		Recip the same
[purp × [purp × laev]]× [purp × tenn]	+++		Recip the same
[tenn. × [purp. × tenn.]]× [ang. × tenn.]	+++		
[tenn × [purp × laev]] × [[purp × laev] × purp]	+		
[[purp × laev] × purp] × [tenn × [purp × laev]]	+++		

[1]Abbreviations: *ang = E. angustifolia; laev = E. laevigata; para = E. paradoxa; purp = E. purpurea; tenn = E. tennesseensis*; recip = reciprocal cross.
[2]Progeny fertility tested by cross-pollinating the sib plants and germinating the seed. Blank scores indicate not tested.
[3]0, +, ++, +++ = none, low, moderate, and high, respectively.

Many of these hybrid combinations have yielded attractive and unique plants. Among the more promising interspecific hybrids made to date are: both the magenta and white flowered forms of *E. purpurea* crossed with *E. tennesseensis*, which has yielded bushy, floriferous plants with broad, horizontal to slightly upturned ray flowers in colors of white, pink, magenta, and dark violet. The hybrids are quite fertile. The cross of *E. angustifolia* and *E. tennesseensis* has yielded attractive plants that have been more vigorous than either parent species, yet still compact,

bushy and floriferous. The plants have not shown symptoms of aster yellows, whereas adjacent plants of *E. purpurea* have exhibited a high incidence of symptoms. Robust plants with flower heads to 18 cm across have been observed from the *E. purpurea – E. laevigata* hybrids. Unique ligule colors of gold and orange and flame red have segregated in advanced generations from crosses of *E. paradoxa* with white-flowered forms of *E. purpurea*. Undoubtedly other species combinations will in turn yield horticulturally promising hybrid plants.

While not all pair-wise hybrid combinations of *Echinacea* species have been attempted, from this research and the reports by McGregor (1968) and McKeown (1999a) it appears highly likely that most if not all *Echinacea* taxa can be intercrossed. Some hybrid combinations, such as *E. purpurea* and *E. tennesseensis*, have been highly fertile though three generations of sibbing and in backcrosses to both species. For species combinations that yield low percentages of viable seed or progeny with low fertility, it may be possible to bridge genes from the two species through another taxa. For example, crossing *E. paradoxa* and *E. tennesseensis* yielded few viable seed, and sibbing the plants from this seed failed to produce viable seed. Conversely, crosses of either of these taxa individually with *E. purpurea* readily produced viable seed and interfertile progeny. While intercrossing these latter hybrid combinations has to date yielded only a few offspring, judicious selection of the parent plants may eventually yield more fertile progeny. Fertile, three-species hybrids have been produced, and more complex crosses are planned. Thus it may eventually prove possible to combine traits from almost any pairing of *Echinacea* taxa.

One factor to consider in the interspecific breeding of *Echinacea* are the reported differences in ploidy levels. Most of the *Echinacea* taxa are diploid ($2n = 2x = 11$), except *E. pallida* and some populations of *E. angustifolia* var. *strigosa,* which are tetraploid ($2n = 4x = 22$)(McGregor, 1968). Where hybrid populations occur in the wild involving these taxa and diploid taxa, sterile triploid plants have been observed (McKeown, 1999a). Therefore the utilization of these two taxa in a more comprehensive breeding program would be limited, unless polyploidy can be induced in the other taxa, or diploid forms induced in these two taxa through such technology as the *in vitro* generation of plants from pollen mother cells. Currently, there are no reports either on polyploid induction or reduction for *Echinacea*.

3.6 Identified Traits

Unfortunately, as McKeown (1999b) observed, there is an almost complete lack of knowledge on the genetics, reproductive biology, and physiology of *Echinacea*. This poses difficulties for hybridization research in this genus, as there are no reports on the genetic control of such important traits as ray flower color and morphology, plant height, hardiness, and pest resistance. The aforementioned reports on interspecific hybrid fertility are also incomplete, as not all species

combinations have been attempted, and, at least for this author's results, are based on crosses made between only a few plants for each taxon tested. The fertility results could vary tremendously if other genotypes for each taxon were tested. This author has also grown out too few plants from most of the crosses to be able to statistically verify trait inheritance. But with these caveats in mind, a few generalized observations can be presented here. Flower color inheritance may be under control of only a few genes. Selections of *E. purpurea* exist with either lavender ray flowers, which is typical of the species, or with white ray flowers. Crosses of the selections 'Magnus' and 'Bravado', both of which have magenta ray flowers, with 'White Swan' and 'White Luster', both of which have white flowers, yielded progeny with flowers in varying shades of light magenta. Sibbing these populations segregated plants with dark magenta, light magenta, and white ray flowers. Sibbing the plants with white ray flowers yielded 100% plants with white flowers. Thus, at least in this taxon, white ray flowers is recessive to magenta ray flowers, and segregates in proportions that suggests ligule color may be controlled by a single recessive allele.

A similar inheritance may be possible for some of the interspecific hybrids. White ray flowers have not been observed in *E. tennesseensis*. A cross of *E. purpurea* 'White Swan' with *E. tennesseensis* yielded plants only with rose pink ray flowers. Sibbing these plants in turn segregated plants with dark magenta, to pink, to white ray flowers. Sibbing only the plants with white ray flowers yielded 100% plants with white ray flowers. The similar pattern of inheritance of flower color in both *E. purpurea* and the hybrids of *E. purpurea* and *E. tennesseensis* suggests that flower color in these two taxa may be controlled by the same gene(s). It may therefore be possible to utilize the white flowered forms of *E. purpurea*, and through a series of interspecific crosses, backcrosses, and sibbings, to yield plants that appear similar to the various species but with white ray flowers.

Flower color inheritance is more complicated when *E. paradoxa* is used in interspecific breeding. The cross of *E. paradoxa* with either magenta colored forms of *E. purpurea* or *E. tennesseensis* yielded progeny with ray flowers in shades of magenta. Sibbing these populations (when possible) in turn yielded progeny with dark magenta to light magenta ray flowers, often with a muddy appearing oranges or tan coloration. None of these has been especially noteworthy. In turn, the cross of *E. paradoxa* with a white flowered form of *E. purpurea* yielded plants with pink ray flowers; when the latter were sibbed, plants were produced with varying colors of white to pink to yellow to gold to orange to orange-red ray flowers. A similar result has been obtained with the white flowered hybrids produced from white flowered *E. purpurea* with *E. tennesseensis* and then crossed with *E. paradoxa*. Some of these plants have been quite showy, and indicate a good direction for future breeding. Research is needed to elucidate the inheritance of ray flower color in these more complex hybrids involving *E. paradoxa*.

Some other ornamentally useful traits have been observed. Occasional plants with attractive, dark red stems have arisen in both *E. purpurea* and hybrids of *E. purpurea* and *E. tennesseensis*. Crosses have been made to attempt to enhance this coloration. Selection for forms that exhibit the stem coloration during periods of high temperatures would be useful. Plants of the same taxa have also been observed with very dark magenta ray flowers. Sibbing such plants tends to yield plants with dark ray flowers, indicating the trait is under genetic control and can be selected. Again the same taxa have also yielded plants with longitudinally fused ray flowers; this trait has spontaneously appeared in several independent breeding lines, and in one line, has proven to be heritable; the original plant expressing the character was crossed with another plant without the trait. The first generation plant did not express the trait, but upon sibbing the population, the trait reappeared in the next generation. Sibbing two plants with the trait resulted in a population that all exhibited fused ray flowers, indicating at least for this population, that the trait may be under control by a single recessive allele. Plants with more than one whorl of ray flowers have also arisen in several independent lines, but to date the trait has not been duplicated in subsequent generations.

One ornamental aspect of *Echinacea* that is rarely mentioned is the potential for fragrant flowers. McKeown (1999a) mentions that *E. simulata* is "remarkably fragrant." A recently introduced cultivar, *E. purpurea* 'Ruby Giant', is promoted in part for its fragrance. This author has occasionally observed hybrid plants between *E. purpurea* and *E. paradoxa* that were noticeably, sweetly fragrant. The species of *Echinacea* that can contribute genes for fragrance need to be identified; presumably, this is a trait that can be selected both for intensity and perhaps qualitative differences.

Under the author's growing conditions, both *E. paradoxa* and *E. angustifolia* tend to produce a single flush of flower heads, whereas the flowering stems of *E. purpurea* and *E. tennesseensis* branch and continue to produce new flowering heads throughout the growing season. The F1 hybrids between *E. paradoxa* and *E. tennesseensis*, and between *E. angustifolia* and *E. tennesseensis*, also produced only a single flush of flowers, whereas the F1 hybrids between *E. purpurea* and *E. tennesseensis* were repeat blooming. Advanced generations of both the latter two hybrids have also been repeat blooming, indicating this trait may be selectable. For ornamental purposes, a plant that has an extended bloom season is of obvious greater value than one that does not repeat bloom.

Tremendous variability has been observed in quantitative traits such as plant height, bushiness, ray flower length, etc. These are likely all traits under complex genetic control, and are beyond the scope of this author's studies to elucidate. Again, research is needed to understand the genetics of these and all the other potentially useful traits in *Echinacea*.

3.7 Future Breeding Directions

Echinacea has a long history of being cultivated as an ornamental crop. While numerous ornamental selections have been made from *E. purpurea*, the other taxa have barely been developed. The ease with which interspecific hybrids involving two, three, and perhaps more taxa opens up the possibility of making myriad selections combining the most useful traits from the different taxa. Of greatest priority should be breeding and selection for greater disease resistance, especially to aster yellows, but it should prove feasible to also make selections based on ray flower color, orientation; fusion; and proliferation; flower fragrance; hardiness; drought tolerance; heat and humidity tolerance; compact, bushy plants; repeat blooming; and other useful traits. Overcoming the self-incompatibility of this genus would improve the uniformity of seed lines. As propagation techniques are refined, additional clonal selections should reach the marketplace. These breeding directives may prove difficult if greater attention is not given to protecting the indigenous populations from habitat degradation and over-harvesting for the medicinal plant market. Research is needed to better understand the underlying genetics of all the ornamentally useful traits.

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