James V. Anderson *Editor*

Advances in Plant Dormancy

Advances in Plant Dormancy

James V. Anderson
Editor

Advances in Plant Dormancy

Editor
James V. Anderson
Sunflower and Plant Biology Research
USDA-Agricultural Research Service
Fargo, North Dakota
USA

ISBN 978-3-319-14450-4 ISBN 978-3-319-14451-1 (eBook)
DOI 10.1007/978-3-319-14451-1

Library of Congress Control Number: 2015936526

Springer Cham Heidelberg New York Dordrecht London
© Springer International Publishing Switzerland 2015
This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.
The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.
The publisher, the authors and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, express or implied, with respect to the material contained herein or for any errors or omissions that may have been made.

Printed on acid-free paper

Springer is the brand of Springer International Publishing.
Springer is part of Springer Science+Business Media (www.springer.com)

Preface

As the earth makes its yearly elliptical orbit around the sun, the angle of its axis causes the northern and southern hemispheres to experience changes in photoperiod and temperature that result in the changing seasons. As a consequence of a plant's specific geographic location, these seasonal changes often induce cold-, heat-, and/or dehydration-related stress during an annual life cycle. Therefore, plants have evolved specific adaptive mechanisms for surviving periods of seasonally-induced stress. From a life cycle perspective, deciduous and annual plant species often sacrifice tissues as an adaptive response to environmentally-induced stress, whereas evergreens do not. However, in all cases, these diverse plant species respond to seasonal environmental cues to induce well-defined phases of dormancy within reproductive structures such as seeds and vegetative buds that can initiate a new life cycle once seasonal conditions are conducive for growth. Understanding how upstream plant receptors perceive these seasonal changes in photoperiod, temperature, and moisture to orchestrate the timing of downstream cellular, molecular, and physiological networks regulating dormancy induction, maintenance, and release are critical for global agricultural production; particularly in the context of global climate change.

Historically, a small group of experts and stakeholders interested in various aspects of plant dormancy has gathered, on average, every 4–5 years at an International Plant Dormancy Symposium (IPDS) to exchange information, develop collaborations, and share ideas on plant dormancy mechanisms. Presentations at these meetings generally cover updated research from experimental plant systems ranging from agronomic, horticultural, and tree crops to model plants and weeds. Previous presentations from IPDS meetings have resulted in a series of published documents. Presentations from the 1st IPDS held in Corvallis, Oregon, USA, in 1995 and the 2nd IPDS held in Angers, France, in 1999 were published as book chapters by CAB International in 1996 and 2000, respectively; whereas presentations from the 4th IPDS held in Fargo, North Dakota, USA, 2009 were published as peer-reviewed papers in a special issue of Plant Molecular Biology (Vol. 73, No. 1–2, 2010). This book includes proceedings from the 5th IPDS held on November 4–7, 2014 in Auckland, New Zealand, as well as other invited chapters. Based on several chapters covering comparisons between dormancy mechanisms common to buds

and seeds, it seems logical that the IPDS is often sponsored by or held in conjunction with the International Seed Science Society.

Collectively, the chapters in this book constitute reviews, research, and perspectives among scientists interested in disseminating advances in our understanding of cellular, genetic, molecular, and physiological mechanisms involved in plant dormancy processes in both buds and seeds. It was recognized that many previously published IPDS articles inadequately discussed the impact that global climate change may have on these plant dormancy processes. Consequently, numerous chapters contained in this book fill this gap by highlighting the potential for global climate change to impact not only dormancy, but also flowering processes. Because dormancy and flowering appear to share overlapping pathways, it will be particularly important to understand how global climate change will impact specific factors (e.g., chilling requirements and alternative chemicals) needed to break dormancy and uniformly induce flowering in horticultural crops and tree species. Although global climate change is not likely to affect seasonal changes in photoperiod, it is likely to impact seasonal changes in temperature. Thus, in crops where dormancy and flowering are known to be controlled by temperature alone (such as apple and pear), global climate change has the potential to change the geographic landscape for some of these horticultural species. Therefore, understanding how environmental and biochemical factors impact processes regulating the complex nature of dormancy in diverse plant systems and its overlap with flowering, covered by numerous chapters in this book, will be essential for ensuring crop production and food security for future generations.

I hope that the scientific community benefit from the information reported in this book and it raises a new level of interest in understanding plant dormancy. Finally, I want to give special thanks to all of the contributors and reviewers who helped to make this book a reality.

James V. Anderson

Contents

Part I Seed Dormancy, Reviews, Research, and Perspectives

1 **Molecular and Hormonal Regulation of Thermoinhibition of Seed Germination** ... 3
Heqiang Huo and Kent J. Bradford

2 **Regulation of Seed Dormancy Cycling in Seasonal Field Environments** ... 35
W.E. Finch-Savage and S. Footitt

Part II Bud Dormancy, Reviews, Research, and Perspectives

3 **Role of the Circadian Clock in Cold Acclimation and Winter Dormancy in Perennial Plants** ... 51
Mikael Johansson, José M. Ramos-Sánchez, Daniel Conde, Cristian Ibáñez, Naoki Takata, Isabel Allona and Maria E. Eriksson

4 **Dormancy Behaviors and Underlying Regulatory Mechanisms: From Perspective of Pathways to Epigenetic Regulation** ... 75
Zongrang Liu, Hong Zhu and Albert Abbott

5 **Recent Advances in Genetics and Molecular Control of Bud Dormancy in Pipfruits** ... 107
Vítor da Silveira Falavigna, Diogo Denardi Porto, Carolina Pereira Silveira and Luís Fernando Revers

6 **Flower Bud Dormancy in Prunus Species** ... 123
Erica Fadón, Maria Herrero and Javier Rodrigo

7 **Dormancy-Associated *MADS-BOX* Genes: A Review** ... 137
David P. Horvath

8 Functional Characterization of Japanese Apricot (*Prunus mume*) *DORMANCY-ASSOCIATED MADS-box1* (*PmDAM1*), a Paralog of *PmDAM6*, Using *Populus* Transformants 147
Hisayo Yamane and Ryutaro Tao

9 The Genetic Components Involved in Sensing Chilling Requirements in Apricot 159
Taly Trainin, Irit Bar-Ya'akov and Doron Holland

10 Prolonged Exposure of Grapevine Buds to Low Temperatures Increases Dormancy, Cold Hardiness, and the Expression of *Vvα-AMYs* Genes 169
Francisco J. Pérez and Sebastián Rubio

Part III Dormancy Mechanisms in Buds of Underground Structures

11 Bridging Dormancy Release and Apical Dominance in Potato Tuber 187
Dani Eshel

12 Meta-Analysis Identifies Potential Molecular Markers for Endodormancy in Crown Buds of Leafy Spurge; a Herbaceous Perennial 197
Münevver Doğramacı, David P. Horvath and James V. Anderson

Part IV Comparison of Seed and Bud Dormancy Mechanisms

13 A Comparison of Transcriptomes Between Germinating Seeds and Growing Axillary Buds of Arabidopsis 223
Dawei Yan, Kiyoshi Tatematsu, Kazumi Nakabayashi, Akira Endo, Masanori Okamoto and Eiji Nambara

14 Dormancy Induction and Release in Buds and Seeds 235
Wun S. Chao, Münevver Doğramacı, David P. Horvath, Michael E. Foley and James V. Anderson

15 Comparing Genetic Mechanisms of Bud Chilling Fulfillment and Seed Cold Stratification: A Role for Peach (*Prunus persica*)? 257
Douglas G. Bielenberg

Part V Chemical Manipulation of Bud Dormancy

16 Chemical Release of Endodormancy in Potato Involves Multiple Mechanisms 269
Michael Campbell

17 Exploring Alternative Bud Break Enhancing Product in 'Zesy003' (Gold9) by Painting Application 279
Gustavo Hernández and Rob Craig

18 Assessment of Alternative Bud Break Enhancers for Commercial Kiwifruit Production of 'Zesy002' (Gold3) 289
Gustavo Hernández, Rob Craig and David Tanner

19 Effect of BLUPRINS® Application on Bud Release from Dormancy in Kiwifruit, Cherry, and Table Grape 301
V. Ziosi, A. Di Nardo, A. Fontana, F. Vitali and G. Costa

Index 309

Contributors

Albert Abbott Forest Health Research and Education Center, University of Kentucky, Lexington, KY, USA

Isabel Allona Biotechnology and Plant Biology, Centre for Plant Biotechnology and Genomics (CBGP/UPM-INIA), Polytechnic University of Madrid, Pozuelo de Alarcón, Madrid, Spain

James V. Anderson USDA-Agricultural Research Service, Biosciences Research Lab, Sunflower and Plant Biology Research Unit, Fargo, ND, USA

Irit Bar-Ya'akov Department of Fruit Tree Sciences, Newe Ya'ar Research Center, Agricultural Research Organization, Ramat Yishay, Israel

Douglas G. Bielenberg Department of Biological Sciences, Clemson University, Clemson, SC, USA

Kent J. Bradford Seed Biotechnology Center, Department of Plant Sciences, University of California, Davis, CA, USA

Michael Campbell School of Science, Penn State Erie, Erie, PA, USA

Wun S. Chao USDA-Agricultural Research Service, Biosciences Research Lab, Sunflower and Plant Biology Research Unit, Fargo, ND, USA

Daniel Conde Biotechnology and Plant Biology, Centre for Plant Biotechnology and Genomics (CBGP/UPM-INIA), Polytechnic University of Madrid, Pozuelo de Alarcón, Madrid, Spain

G. Costa Dipartimento di Colture Arboree, Università di Bologna, Bologna, Italy

Rob Craig Punchbowl Ltd., Pukekohe, New Zealand

A. Di Nardo Biolchim S.p.A., Medicina, Bologna, Italy

Münevver Doğramaci USDA-Agricultural Research Service, Biosciences Research Lab, Sunflower and Plant Biology Research Unit, Fargo, ND, USA

Akira Endo Department of Cell & Systems Biology, University of Toronto, Toronto, Canada

Present address; Crop Breeding Research Division, National Agriculture and Food Research Organization (NARO), Naro, Japan

Maria E. Eriksson Plant Physiology, Umeå University, Umeå, Sweden

Dani Eshel Department of Postharvest Science of Fresh Produce, The Volcani Center, ARO, Bet-Dagan, Israel

Erica Fadón Estación Experimental Aula Dei, CSIC, Zaragoza, Spain

Centro de Investigación y Tecnología Agroalimentaria (CITA), Zaragoza, Spain

Vítor da Silveira Falavigna Graduate Program in Cell and Molecular Biology, Centro de Biotecnologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

W.E. Finch-Savage School of Life Sciences, Wellesbourne Campus, University of Warwick, Warwickshire, UK

Michael E. Foley USDA-Agricultural Research Service, Biosciences Research Lab, Sunflower and Plant Biology Research Unit, Fargo, ND, USA

A. Fontana Biolchim S.p.A., Medicina, Bologna, Italy

S. Footitt School of Life Sciences, Wellesbourne Campus, University of Warwick, Warwickshire, UK

Gustavo Hernández Punchbowl Ltd., Pukekohe, New Zealand

Maria Herrero Estación Experimental Aula Dei, CSIC, Zaragoza, Spain

Doron Holland Department of Fruit Tree Sciences, Newe Ya'ar Research Center, Agricultural Research Organization, Ramat Yishay, Israel

David P. Horvath USDA-Agricultural Research Service, Biosciences Research Lab, Sunflower and Plant Biology Research Unit, Fargo, ND, USA

Heqiang Huo Seed Biotechnology Center, Department of Plant Sciences, University of California, Davis, CA, USA

Cristian Ibáñez Departamento de Biología, Universidad de La Serena, La Serena, Coquimbo, Chile

Mikael Johansson Molecular Cell Physiology, Bielefeld University, Bielefeld, Germany

Zongrang Liu USDA-ARS, Appalachian Fruit Research Station, Kearneysville, WV, USA

Kazumi Nakabayashi Department of Plant Breeding and Genetics, Max Planck Institute for Plant Breeding Research, Köln, Germany

Eiji Nambara Department of Cell & Systems Biology, University of Toronto, Toronto, Canada

King Abdulaziz University, Jeddah, Saudi Arabia

Masanori Okamoto Arid Land Research Center, Tottori University, Tottori, Japan

Francisco J. Pérez Facultad de Ciencias, Laboratorio de Bioquímica Vegetal, Universidad de Chile, Santiago, Chile

Diogo Denardi Porto Centro de Pesquisa Agropecuária Trópico Semiárido, Empresa Brasileira de Pesquisa Agropecuária, Petrolina, PE, Brazil

José M. Ramos-Sánchez Biotechnology and Plant Biology, Centre for Plant Biotechnology and Genomics (CBGP/UPM–INIA), Polytechnic University of Madrid, Pozuelo de Alarcón, Madrid, Spain

Luís Fernando Revers Laboratory of Plant Molecular Genetics, Centro Nacional de Pesquisa de Uva e Vinho, Empresa Brasileira de Pesquisa Agropecuária, Bento Gonçalves, RS, Brazil

Javier Rodrigo Centro de Investigación y Tecnología Agroalimentaria (CITA), Zaragoza, Spain

Sebastián Rubio Facultad de Ciencias, Laboratorio de Bioquímica Vegetal, Universidad de Chile, Santiago, Chile

Carolina Pereira Silveira Laboratory of Plant Molecular Genetics, Centro Nacional de Pesquisa de Uva e Vinho, Empresa Brasileira de Pesquisa Agropecuária, Bento Gonçalves, RS, Brazil

Naoki Takata Forest Bio-Research Center, Forestry and Forest Products Research Institute, Hitachi, Ibaraki, Japan

David Tanner Zespri International Limited, Mt Maunganui, New Zealand

Ryutaro Tao Laboratory of Pomology, Graduate School of Agriculture, Kyoto University, Kyoto, Japan

Kiyoshi Tatematsu Laboratory of Plant Organ Development, National Institute for Basic Biology, Okazaki, Japan

Taly Trainin Department of Fruit Tree Sciences, Newe Ya'ar Research Center, Agricultural Research Organization, Ramat Yishay, Israel

F. Vitali Biolchim S.p.A., Medicina, Bologna, Italy

Hisayo Yamane Laboratory of Pomology, Graduate School of Agriculture, Kyoto University, Kyoto, Japan

Dawei Yan Department of Cell & Systems Biology, University of Toronto, Toronto, Canada

Hong Zhu Chinese Academy of Sciences, South China Botanical Garden, Tianhe District, Guangzhou, China

V. Ziosi Biolchim S.p.A., Medicina, Bologna, Italy

Part I
Seed Dormancy, Reviews, Research, and Perspectives

Chapter 1
Molecular and Hormonal Regulation of Thermoinhibition of Seed Germination

Heqiang Huo and Kent J. Bradford

Introduction

The life cycles of most flowering plants begin as a seed. However, rather than germinating immediately, most seeds exhibit some level of primary dormancy to prevent precocious germination during development and to ensure that the environmental conditions are favorable for seedling growth. In addition to primary dormancy, seeds can also enter secondary dormancy in response to unfavorable environmental conditions such as temperature or water stresses, enabling them to persist over multiple seasons or years until conditions are appropriate for germination (Hilhorst 2007). Temperature is a primary factor regulating seasonal changes in seed dormancy status (Bewley et al. 2013; Footitt et al. 2013). With the prospect of global warming, we can anticipate that plants will be exposed to wider fluctuations in temperature and may be subjected to higher temperatures in the future, which may significantly impact crops and ecosystems worldwide (Franks et al. 2014; Long and Ort 2010). In particular, it is of interest to anticipate how the germination and dormancy responses of seeds to temperature might be affected under climate change scenarios (Donohue et al. 2010; Kimball et al. 2011; Saatkamp et al. 2011).

Temperature influences seed germination in multiple ways. For nondormant seeds, germination rates (inverse of the time to germination following imbibition) generally increase above a minimum or base temperature (T_b) to an optimum temperature (T_o) and then decrease above T_o until germination ceases at a maximum or ceiling temperature (T_c) (Bewley et al. 2013; Covell et al. 1986). The increase in germination rates as temperature increases is generally in accordance with the thermodynamics of temperature effects on plant growth. The decrease in germination rates above T_o appears to be due to an increase in the threshold water potential required for completion of germination (Ψ_b) (Alvarado and Bradford 2002); however,

K. J. Bradford (✉) · H. Huo
Seed Biotechnology Center, Department of Plant Sciences, University of California, Davis, CA 95616, USA
e-mail: kjbradford@ucdavis.edu

© Springer International Publishing Switzerland 2015

J. V. Anderson (ed.), *Advances in Plant Dormancy,* DOI 10.1007/978-3-319-14451-1_1

the molecular mechanisms underlying this effect are essentially unknown. In addition to these broad germination responses of nondormant seeds to temperature, upper temperature limits for germination are associated with primary dormancy and after-ripening. In some seeds, primary dormancy is expressed as restricted temperature ranges allowing germination and these ranges widen as dormancy is alleviated by after-ripening or other factors (Allen et al. 2007; Probert 1992). On the other hand, some seeds will enter thermodormancy and/or thermoinhibition when imbibed at warm temperatures. This is distinct from the inhibition of germination described above, as it generally occurs at temperatures considerably lower than T_c. It may also occur over a small range of temperatures, such that germination can go from 100 to 0% when the upper temperature limit is exceeded by only a few degrees. This germination behavior is often associated with a winter annual life cycle common in Mediterranean-type climates with wet winters and dry summers, as seeds that are shed in early summer and have subsequently after-ripened nonetheless may not germinate when hydrated at warm temperatures, waiting instead until the fall for cooler temperatures and more certain rainfall before germinating. While advantageous in the wild, this can result in economic losses due to poor stand establishment in crops such as lettuce or carrot during warm seasons (Lafta and Mou 2013; Nascimento et al. 2013).

The terms "thermoinhibition" and "thermodormancy" are used to describe this type of germination behavior at warm temperatures, although there are subtle distinctions between the two terms. Seed thermoinhibition is the situation when germination of seeds that have been imbibed at warm temperature is prevented, but the seeds will subsequently germinate rapidly when the temperature is reduced (Argyris et al. 2008; Huo et al. 2013). It is therefore a temporary inhibition of germination that can be alleviated simply by lowering the temperature. In contrast, thermodormancy generally refers to a type of secondary dormancy that is induced by extended exposure of hydrated seeds to warm temperatures that prevent germination. Thermodormant seeds will not germinate immediately when the temperature is reduced, indicating that a more persistent type of inhibition or dormancy has been induced (Corbineau et al. 1988; Kepczynski and Bihun 2002; Leymarie et al. 2008; Toh et al. 2012). Such seeds may require a dormancy-breaking condition such as chilling to overcome this induced dormancy. Thermoinhibition could be likened to light requirements for germination, as the seed is not deeply dormant, but rather is not germinating due to responses to its current situation, for example warm temperature or lack of light, that selection over evolutionary history has indicated are not conducive to seedling survival. The molecular mechanisms underlying these different levels of regulation of germination for different life cycle strategies are now being revealed (Donohue 2014; Footitt et al. 2013).

Despite the wealth of empirical information about the role of temperature in regulating seed dormancy and germination (Baskin and Baskin 1998; Bewley et al. 2013), relatively little is known about the mechanism(s) by which seeds can sense temperature and transduce that signal into gene expression and developmental events controlling germination (Penfield and MacGregor 2014; Wigge 2013). Seed dormancy and germination in general have been reviewed in many recent reports

(Arc et al. 2013; Graeber et al. 2012; Linkies and Leubner-Metzger 2012; Nambara et al. 2010; Rajjou et al. 2012; Weitbrecht et al. 2011). Here, we will focus on the hormonal and molecular regulation of seed thermoinhibition and principally on the initial responses to imbibition at warm temperatures that prevent the developmental progression to germination, that is, rupture of any tissues enclosing the embryo and growth of the embryonic seedling.

Abscisic Acid (ABA) Is a Major Regulator of Seed Thermoinhibition

The plant hormone ABA plays a leading role in regulating seed dormancy and germination (Graeber et al. 2012; Nambara et al. 2010). ABA biosynthesis is required for the induction of primary dormancy during seed development and in general is an inhibitor of germination (Bewley et al. 2013). Biochemical, genetic and genomics analyses have revealed the molecular basis of ABA metabolism and action (Fig. 1.1) (Cutler et al. 2010; Nambara and Marion-Poll 2005; Schwartz and Zeevaart 2010).

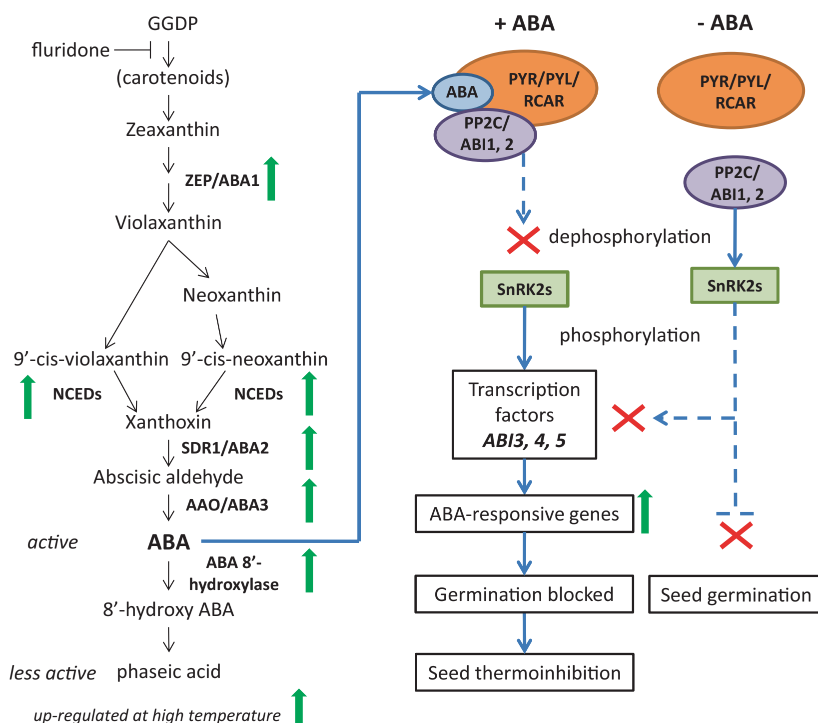


Fig. 1.1 Pathways of abscisic acid (ABA) biosynthesis and signaling. (Modified from Bewley et al. 2013; Cutler et al. 2010; Yamaguchi et al. 2007). Gene/enzyme names are in Table 1.1

Genetic studies in particular have demonstrated that alterations in the ABA biosynthetic pathway can greatly influence seed dormancy and germination (Holdsworth et al. 2008; Martinez-Andujar et al. 2011; Nambara et al. 2010). Several key genes/enzymes have been characterized for their functional roles in ABA biosynthesis and catabolism and in regulating seed germination and dormancy (Table 1.1) (reviewed in Nambara et al. 2010). Loss of function in ABA biosynthetic genes, including *ZEP*/*ABA1* (Koornneef and Jorna 1982), *NCED*s (Frey et al. 2012; Lefebvre et al. 2006), *ABA2*/*GIN1*/*SDR1* (Gonzalez-Guzman et al. 2002; Leon-Kloosterziel et al. 1996a), or *ABA3/LOS5* (Leon-Kloosterziel et al. 1996a; Xiong et al. 2001), results in reduced dormancy, whereas overexpression of ABA biosynthetic enzymes enhances dormancy (Lin et al. 2007; Martinez-Andujar et al. 2011). Disruption of ABA catabolism leads to higher levels of ABA and seed germination inhibition. For example, mutation in the *CYP707A2* gene encoding ABA-8′hydroxylase, a P450 enzyme that catalyzes conversion of ABA to inactive 8′-hydroxyl-ABA, resulted in a strong seed dormancy phenotype (Kushiro et al. 2004; Okamoto et al. 2006).

When nondormant seeds are imbibed under favorable conditions such as optimum temperature and light requirements, the endogenous ABA present in dry seeds rapidly declines; in contrast, in thermoinhibited seeds the endogenous ABA contents generally decline initially followed by accumulation and maintenance of elevated levels (Argyris et al. 2008; Huo et al. 2013; Toh et al. 2008). This indicates that *de novo* ABA biosynthesis is induced in thermoinhibited seeds. It is also known that application of ABA biosynthesis inhibitors (e.g., fluridone) can relieve the inhibition of seed germination by high temperature (Argyris et al. 2008; Gonai et al. 2004; Toh et al. 2008; Yoshioka et al. 1998), implying that ABA-deficient mutants might be resistant to inhibition by high temperature. However, not all ABA-deficient Arabidopsis mutants display both reduced seed dormancy and thermoinhibition, suggesting that the mechanisms for regulating seed thermoinhibition are not entirely the same as the ones for regulating primary dormancy (Table 1.1). NCEDs (9-*cis*-epoxycarotenoid dioxygenases) catalyze oxidative cleavage of 9-*cis*-violaxanthin or 9-*cis*-neoxanthin to generate xanthoxin (Fig. 1.1) (Sawada et al. 2008; Schwartz et al. 1997; Schwartz and Zeevaart 2010). There are five *NCED* gene family members that exhibit enzyme activity in Arabidopsis (*NCED2*, *NCED3*, *NCED5*, *NCED6*, and *NCED9*) (Auldridge et al. 2006). All five *NCED*s might regulate seed dormancy and/or thermoinhibition by contributing to ABA synthesis in developing or imbibed seeds (Table 1.1) (Auldridge et al. 2006; Lefebvre et al. 2006; Toh et al. 2008). Among them, *NCED6* and *NCED9* are the two major *NCED* family members that regulate seed dormancy in Arabidopsis. Loss of function in these two genes leads to significant reduction of endogenous seed ABA content (Lefebvre et al. 2006). While dormancy was normally established during seed development in *nced6* and *nced9* mutants, and freshly harvested seeds of these two single mutants displayed similar germination kinetics as Columbia wild-type seeds, both single mutants were more resistant to paclobutrazol (PAC), a gibberellin biosynthesis inhibitor, typical of the phenotype seen in other ABA-deficient mutants such as *aba2* and *aba3* (Lefebvre et al. 2006; Leon-Kloosterziel et al. 1996a). However, only the *nced9* mutant is resistant to high temperature during seed germination, while ger-

Table 1.1 Some genes known to affect seed thermoinhibition. The Arabidopsis gene names are used; homologous genes are known to have similar function in other species in some cases. *LOF* loss of function, *ND* not determined

Gene	Biological function	Dormancy phenotype of LOF mutant	Thermoinhibition phenotype of LOF mutant	References
ABA biosynthesis				
ABA1/ZEP	ABA biosynthesis	Reduced dormancy	Resistance to thermoinhibition	Koornneef and Jorna (1982) Tamura et al. (2006)
ABA2	ABA biosynthesis and sugar responsive	Reduced dormancy	Reduced inhibition	Leon-Kloosterziel et al. (1996a) (Toh et al. 2008) Toh et al. (2012)
ABA3	ABA biosynthesis	Reduced dormancy	ND	Leon-Kloosterziel et al. (1996a)
NCED2	ABA biosynthesis	Normal dormancy	Improved tolerance to thermoinhibition combined with *nced9* mutant	Toh et al. (2008) Frey et al. (2012)
NCED3 *LsNCED2* *LsNCED3*	ABA biosynthesis	Normal dormancy	ND	Qin and Zeevaart (2002) Tan et al. (2003) Huo et al. (2013)
NCED5	ABA biosynthesis	Normal dormancy	Improved tolerance to thermoinhibition combined with *nced9* mutant	Toh et al. (2008) Frey et al. (2012)
NCED6	ABA biosynthesis	Normal dormancy but more resistant to paclobutrazol	Sensitive to high temperature as wild type	Lefebvre et al. (2006) Toh et al. (2008)
NCED9 *LsNCED4* *HvNCED1* *McNCED*	ABA biosynthesis	Normal dormancy but more resistant to paclobutrazol	Resistance to thermoinhibition	Leymarie et al. (2008) Toh et al. (2008) Huo et al. (2013) Tuan and Park (2013)
ABA signaling				
ABI1	ABA signaling	*abi1-2*, *abi1-3*, hypersensitivity to ABA at seed germination; *abi1-1*, insensitivity to ABA response at seed germination	*abi1-1*, resistance to thermoinhibition	Koornneef et al. (1984) Finkelstein and Somerville (1990) Leung et al. (1997) Tamura et al. (2006)

Table 1.1 (continued)

Gene	Biological function	Dormancy phenotype of LOF mutant	Thermoinhibition phenotype of LOF mutant	References
ABI2	ABA signaling	*abi2-1*, insensitivity to ABA response at seed germination	*abi2-1* resistance to thermoinhibition	Koornneef et al. (1984) Finkelstein and Somerville (1990) Leung et al. (1997) Tamura et al. (2006)
HAB1	ABA signaling	*hab1-1*, hypersensitivity to ABA at seed germination	ND	Saez et al. (2004) Saez et al. (2006)
ABI3	Seed maturation and ABA signaling	Reduced dormancy	Resistance to thermoinhibition	Koornneef et al. (1984) Tamura et al. (2006) Lim et al. (2013)
ABI4	ABA and sugar signaling	Normal dormancy	Sensitive to high temperature as wild type	Finkelstein (1994) Tamura et al. (2006)
ABI5	ABA signaling	Reduced sensitivity to ABA at germination	Overexpression of *ABI5* causes hypersensitivity to high temperature	Finkelstein (1994) Tamura et al. (2006) Lim et al. (2013)
Gibberellin signaling				
RGL2	GA signaling negative regulator	More resistant to paclobutrazol	Resistance to thermoinhibition	Lee et al. (2002) Tyler et al. (2004) Toh et al. (2008)
SPINDLY	GA signaling negative regulator	More resistant to paclobutrazol	Resistance to thermoinhibition	Jacobsen and Olszewski (1993) Toh et al. (2008)
DELLA	GA signaling negative regulator	More resistant to paclobutrazol	Resistance to thermoinhibition	Kim et al. (2008)
Ethylene biosynthesis and signaling				
CTR1	Ethylene signaling	Reduced ABA and glucose sensitivity during seed germination	Resistance to thermoinhibition	Beaudoin et al. (2000) Wang et al. (2007) Cheng et al. (2009) Huo et al., unpublished data

Table 1.1 (continued)

Gene	Biological function	Dormancy phenotype of LOF mutant	Thermoinhibition phenotype of LOF mutant	References
ETO1	Negative regulator of ethylene synthesis	Faster germination rate	Partial resistance to thermoinhibition	Cheng et al. (2009) Huo et al., unpublished data
Dormancy maintenance				
FUS3	Seed maturation	Reduced dormancy	Resistance to thermoinhibition	Holdsworth et al. (2008) Chiu et al. (2012)
RDO1	Unknown	Reduced dormancy	Resistance to thermoinhibition	Leon-Kloosterziel et al. (1996b) Tamura et al. (2006)
RDO2	Transcription elongation factor SII	Reduced dormancy	Resistance to thermoinhibition	Tamura et al. (2006) Liu et al. (2011) Leon-Kloosterziel et al. (1996b)
RDO3	Unknown	Reduced dormancy	Resistance to thermoinhibition	Tamura et al. (2006) Leon-Kloosterziel et al. (1996b)
RDO4/ HUB1	Histone H2B monoubiquitination	Reduced dormancy	Resistance to thermoinhibition	Leon-Kloosterziel et al. (1996b) Tamura et al. (2006) Liu et al. (2007)
DOG1	Unknown	Reduced dormancy	Resistance to thermoinhibition	Bentsink et al. (2006) Huo et al. unpublished data
Jasmonic acid biosynthesis				
OPR3	Jasmonic acid synthesis	No obvious phenotype in germination	More sensitive to thermoinhibition	Dave et al. (2011)
Strigolactone synthesis and signaling				
MAX1	Strigolactone synthesis	Reduced germination	More sensitive to thermoinhibition	Toh et al. (2012)
MAX2	Strigolactone signaling	Reduced germination	More sensitive to thermoinhibition	Toh et al. (2012)
Other				
SOM	CCCH-type zinc finger protein	Enhanced germination in the dark	Resistance to thermoinhibition	Kim et al. (2008) Lim et al. (2013)

Table 1.1 (continued)

Gene	Biological function	Dormancy phenotype of LOF mutant	Thermoinhibition phenotype of LOF mutant	References
DAG1	DOF AFFECTING GERMINATION1	Enhanced germination in the dark	Resistance to thermoinhibition	Papi et al. (2002) Gabriele et al. (2010) Rizza et al. (2011)
TT7	Required for flavonoid-3′-hydroxylase activity	Faster germination rate	Resistance to thermoinhibition	Debeaujon et al. (2000) Salaita et al. (2005) Tamura et al. (2006)
CAP2	DREB2A-like gene, responsive to salt, dehydration	ND	Overexpression of CAP2 causes thermotolerance of tobacco seeds at germination	Shukla et al. (2009)
HSP26	Small heat shock protein, responsive to heat stress	ND	Overexpression of wheat sHSP26 caused thermotolerance of Arabidopsis seeds at germination	Chauhan et al. (2012)
CODA	Choline oxidase that converts choline to glycinebetaine	ND	Overexpression of *CODA* gene caused higher germination under heat shock stress	Alia et al. (1998) Li et al. (2011)

mination of *nced6* mutant seeds was still sensitive to thermoinhibition (Toh et al. 2008). When their mRNA levels were investigated in seeds imbibed at 34 °C, transcripts increased for *NCED9* but not *NCED6*, while mRNA amounts for both genes decreased dramatically in seeds imbibed at 22 °C. This indicates specialization of the *NCED* gene family in response to environmental signals.

Similar results were also observed in other species (Argyris et al. 2008; Huo et al. 2013; Leymarie et al. 2008). There are four *NCED* members (*LsNCED1-4*) in lettuce (*Lactuca sativa*) (Sawada et al. 2008). Phylogenetic analysis revealed that *LsNCED2* and *LsNCED3* have high similarity to *AtNCED3*, whereas *LsNCED4* is more homologous to *AtNCED6* and *AtNCED9* (Huo et al. 2013; Sawada et al. 2008). Genetic quantitative trait locus (QTL) mapping pointed to *LsNCED4* as being responsible for the capacity for germination at high temperature exhibited by a *Lactuca serriola* accession (UC96US23) (Argyris et al. 2011). Functional analysis demonstrated that *LsNCED4* mRNA decreased in lettuce seeds imbibed at 20 °C, associated with a decline in endogenous ABA content (Argyris et al. 2008). When

lettuce seeds were imbibed at 35 °C, *LsNCED4* expression in the thermoresistant accession (UC96US23) was still down-regulated as in seeds imbibed at 20 °C, whereas *LsNCED4* mRNA level in a thermosensitive line (Salinas) increased after an initial decline. *LsNCED4* can rescue the thermoresistant phenotype of Arabidopsis *nced6-1 nced9-1* double mutants that was mainly caused by the loss of function in *NCED9* (Huo et al. 2013; Toh et al. 2008). Silencing of *LsNCED4* using RNA interference (RNAi) in the thermosensitive cultivar Salinas overcame the inhibitory effect by high temperature during seed germination, while expression of Salinas *LsNCED4* under its own promoter in the thermoresistant accession resulted in complete inhibition of germination at 35 °C (Huo et al. 2013). This indicated that the thermoresistance of UC96US23 seeds was primarily due to lack of response of the promoter of its *LsNCED4* allele to high-temperature signals. In barley (*Hordeum vulgare*), high imbibition temperature (> 30 °C) also inhibited germination of primary dormant seeds and caused secondary thermodormancy (Leymarie et al. 2008). *HvNCED1* was up-regulated by high temperature during barley seed imbibition at 30 °C (Leymarie et al. 2008), and sequence analysis showed that *HvNCED1* is more homologous to Arabidopsis *NCED9* than to *NCED6* (Chono et al. 2006).

Arabidopsis *NCED2* and *NCED5* genes have also been demonstrated to be regulated by high temperature during seed germination, although seeds of both single mutants did not show significant thermoresistant phenotypes comparable to *nced9-1* mutant seeds (Toh et al. 2008). However, triple mutants of *nced9-1 nced5-1 nced2-1* displayed stronger thermotolerance during seed germination than did the *nced9-1* single mutant, suggesting that *NCED5* and *NCED2* play minor roles in regulation of seed thermoinhibition as they do in primary seed dormancy (Frey et al. 2012; Toh et al. 2008). In bitter melon (*Momordica charantia*), *McNCED*, a homolog of Arabidopsis *NCED9* and *NCED3*, was responsive to high temperature during seed germination (Tuan and Park 2013). The results from Arabidopsis, lettuce, barley and bitter melon suggest that *NCED9*-like genes are specifically regulated by high temperature and are essential for induction of thermoinhibition. By contrast, *NCED3*-like genes were mainly responsive to water stress (Huo et al. 2013; Iuchi et al. 2001; Qin and Zeevaart 2002; Tan et al. 2003). This multiplication of genes encoding ABA-biosynthetic enzymes and diversification of their regulation by environmental cues (e.g., temperature and water deficit) explains how plant hormones can play such diverse developmental and physiological roles.

In Arabidopsis, *NCED* family members are not the only genes encoding ABA biosynthetic enzymes that are induced by high temperature. *ABA1*/*ZEP* was also up-regulated by high temperature during seed imbibition (Argyris et al. 2008; Chiu et al. 2012; Huo et al. 2013; Toh et al. 2008). The *ABA1*/*ZEP* (zeaxanthin epoxidase) gene initiates the biosynthetic pathway leading to ABA by converting zeaxanthin to violaxanthin in a two-step epoxidation (Fig. 1.1; Marin et al. 1996; Nambara and Marion-Poll 2005). Although *ABA1/ZEP* expression does not increase as greatly as *NCED9* during imbibition at high temperature, the *aba1-1* mutant displays a significant thermotolerance phenotype (Tamura et al. 2006). The *ABA2* gene encodes a short-chain alcohol dehydrogenase that catalyzes the conversion of xanthoxin to abscisic aldehyde which is then oxidized to ABA by an abscisic aldehyde oxidase

(AAO3). The activity of AAO3 enzyme requires a sulfurated molybdenum cofactor, which is activated by a Mo-cosulfurase (ABA3) (Fig. 1.1; Nambara and Marion-Poll 2005). Similar to *aba1-1*, *aba2-2* mutant seeds are also resistant to thermoinhibition, although *ABA2* expression was unchanged during seed imbibition at high temperature (Toh et al. 2008; Toh et al. 2012). This could be due to its critical role in ABA biosynthesis. Unlike the *NCED*s, *ABA1* and *ABA2* do not have other homologous genes in the Arabidopsis genome, and mutations of these two genes could cause more severe defects in ABA biosynthesis than mutation of individual *NCED* members (Nambara and Marion-Poll 2005).

The inhibitory effect of ABA on seed germination is transduced through an ABA signaling pathway. In the PYR/RCAR-PP2C-SnRK2 signal transduction model, protein phosphatase 2Cs (PP2Cs such as *ABI1*, *ABI2* and *HAB1*) dephosphorylate SNF1-related protein kinase 2s (SnRK2s) in the absence of ABA to repress their activity and downstream activation of ABA response factors (ABFs) by SnRK2s (Fig. 1.1; Cutler et al. 2010). ABA can interact with the PYR/RCAR receptors and PP2Cs to form a complex and release the suppression of PP2Cs on SnRK2s; activated SnRK2s phosphorylate numerous downstream target proteins involved in ABA responses, including transcription factors ABI3 (ABA-INSENSITIVE3, a B3-domain transcription factor) and ABI5 (ABA-INSENSITIVE5, a bZIP transcription factor) (Fig. 1.1; Cutler et al. 2010; Umezawa et al. 2010).

Both ABI1 and ABI2 are negative regulators in the ABA signaling pathway (Fig. 1.1). Recessive loss-of-function alleles of *ABI1* and *HAB1* (*abi1-2*, *abi1-3*, and *hab1-1*) displayed hypersensitivity to ABA at seed germination (Saez et al. 2004; Saez et al. 2006). By contrast, mutations *abi1-1* and *abi2-1* prevent their interaction with the ABA receptors in the presence of ABA (Ma et al. 2009; Park et al. 2009). However, *abi1-1* and *abi2-1* mutant seeds efficiently dephosphorylate and inactivate SnRK2, resulting in dominant insensitivity to ABA response at seed germination (Finkelstein and Somerville 1990; Fujii et al. 2009; Koornneef et al. 1984; Leung et al. 1997; Umezawa et al. 2009). Seeds of *abi1-1* and *abi2-1* mutants showed resistance to thermoinhibition at 32 °C; however, only *abi1-1* seeds could germinate well at 34 °C (Tamura et al. 2006). In addition, Arabidopsis *abi3-1* (L*er* background) and *abi3-8* (Col background) mutants showed intermediate levels of thermoinhibition at 34 °C (Tamura et al. 2006). A recent report also demonstrated that seeds with two additional *abi3* mutant alleles (*abi3-sk11* and *abi3-sk22*) displayed strong resistance to thermoinhibition (Lim et al. 2013). On the other hand, germination of ABA-insensitive *abi4-3* and *abi5-7* seeds were inhibited as severely as the wild-type seeds at 32 °C (Tamura et al. 2006). ABI4 and ABI5 are involved in repressing Arabidopsis seed germination and mutant seeds display resistance to ABA during germination (Finkelstein 1994; Finkelstein and Lynch 2000; Lopez-Molina et al. 2002; Lopez-Molina et al. 2001). However, unlike seeds with *abi1-1*, *abi2-1* and *abi3* mutations that have reduced dormancy (Koornneef et al. 1984), seeds with *abi4* and *abi5* mutations have normal dormancy, consistent with the lesser effects of these mutations on thermoinhibition (Table 1.1; Finkelstein 1994; Tamura et al. 2006).

Expression of both *LsABI3* and *LsABI5* was up-regulated in thermosensitive Salinas lettuce seeds imbibed at 35 °C, whereas this up-regulation was not observed in the thermoresistant accession UC96US23 (Argyris et al. 2008; Huo et al. 2013). Similarly, *LsABI5* expression was suppressed in Salinas seeds in which the *LsNCED4* gene was completely silenced, consistent with the up-regulation of *ABI3* and *ABI5* expression by ABA (Lopez-Molina et al. 2002; Lopez-Molina et al. 2001). The endogenous ABA content is much lower in UC96US23 and RNAi-*LsNCED4* Salinas seeds imbibed at high temperature than in wild-type Salinas seeds (Huo et al. 2013), suggesting that the up-regulation of *LsABI5* in Salinas seeds at high temperature may be due to the high level of *de novo* ABA biosynthesis rather than a direct response to high temperature. Although *abi5* mutant Arabidopsis seeds are as sensitive to high temperature as the wild-type seeds, overexpression of *ABI5* resulted in hypersensitivity to high temperature at seed germination (Lim et al. 2013). These results suggest that the lack of resistance of *abi5* single mutants to high temperature at germination could be due to the redundant function of different bZIP transcription factors.

GA Counteracts ABA to Improve Seed Germination at High Temperature

Gibberellin (GA) is generally required for seed germination (Debeaujon and Koornneef 2000; Ogawa et al. 2003; Sun 2008). Mutations of GA biosynthetic genes such as *ent-COPALYL DIPHOSPHATE SYNTHASE* (*CPS*/*GA1*), *ent-KAURENE SYNTHASE* (*KS/GA2*), *ent-KAURENE OXIDASE* (*KO/GA3*) and *GIBBERELLIN-3-OXIDASE*s (*GA3ox1* and *GA3ox2*) lead to failure of germination in the light and dark (Fig. 1.2; Koornneef and Vanderveen 1980; Mitchum et al. 2006; Yamauchi et al. 2004). Imbibition at lower temperatures results in induction of GA biosynthetic genes, such as *GA3ox1* and *GIBBERELLIN-20-OXIDASE1* (*GA20ox1*) in Arabidopsis seeds (Yamauchi et al. 2004), while high temperature suppresses the expression of *GA3ox* and *GA20ox* genes in Arabidopsis and lettuce (Argyris et al. 2008; Gonai et al. 2004; Huo et al. 2013; Toh et al. 2008). We can ask whether the GA biosynthetic genes are suppressed directly by high temperature or by the elevated endogenous ABA content derived from the *de novo* ABA biosynthesis. The expression of *GA3ox* and *GA20ox* genes was not suppressed in thermoresistant lettuce seeds imbibed at high temperature, and the suppression of these genes in a thermosensitive line was reversed by silencing the *LsNCED4* gene (Argyris et al. 2008; Huo et al. 2013). Similar results were observed in Arabidopsis *aba2-1* mutant seeds, which displayed thermotolerance of germination at 33 °C and elevated expression of *GA3ox1, GA3ox2* and *GA20ox3*, whereas all three genes were strongly suppressed in wild-type seeds in which the ABA content was increased by up-regulation of *NCED9*, *NCED5* and *NCED2* (Toh et al. 2008). There was no difference in expression of genes encoding GA catabolism enzymes such as *GIBBERELLIN-2-OXIDASE* (*GA2ox*) between germinating seeds and thermoinhibited seeds in

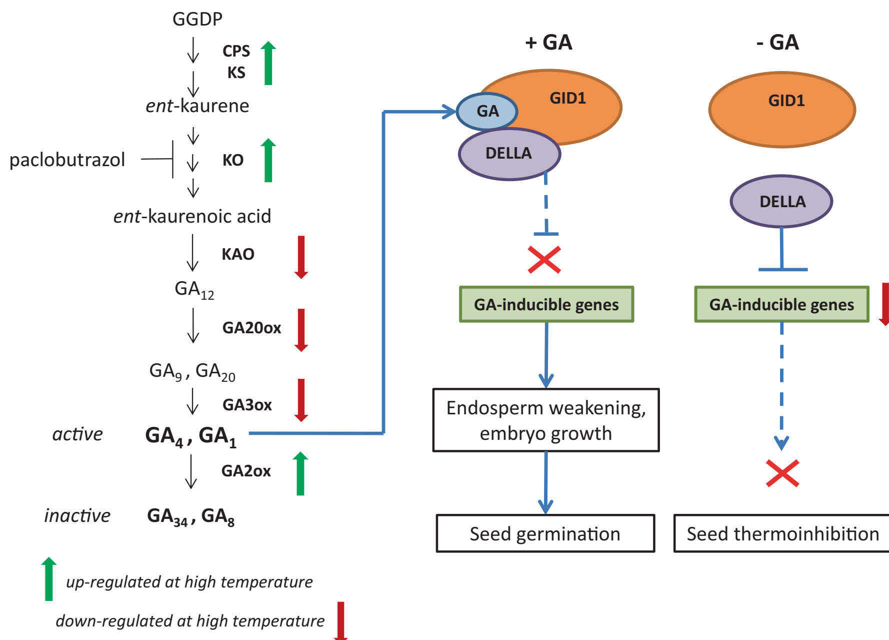


Fig. 1.2 Pathways of gibberellin biosynthesis and signaling. (Modified from Bewley et al. 2013; Yamaguchi et al. 2007). Gene/enzyme names are in Table 1.1

Arabidopsis (Toh et al. 2008), but the expression of *LsGA2ox1* in thermoinhibited lettuce seeds was higher than in thermoresistant seeds (Argyris et al. 2008). This up-regulation by high temperature did not occur in Salinas seeds when *LsNCED4* was silenced, thereby lowering ABA content (Huo et al. 2013), indicating that in some but not all seeds, the regulation of GA catabolism during seed germination at high temperature is ABA dependent. Some genes involved with early steps in the GA biosynthetic pathway were up-regulated by high temperature (*CPS*, *KS*, *KO*) in lettuce (Fig. 1.1); this is likely due to feedback inhibition of these genes by GA, resulting in their up-regulation when synthesis of active GAs is inhibited (Argyris et al. 2008).

Although application of GA alone can partially alleviate thermoinhibition in lettuce, Arabidopsis, tomato (*Solanum lycopersicum*) and pepper (*Capsicum annuum*) seeds, combined application of GA and fluridone was more effective in promoting seed germination (Argyris et al. 2008; Carter and Stevens 1998; Gonai et al. 2004; Kepczynska et al. 2006; Toh et al. 2008). In addition, germination of the thermoinhibition-resistant Arabidopsis *aba2-1* and lettuce UC96US26 seeds was suppressed by a GA biosynthesis inhibitor (PAC) (Huo et al. 2013; Toh et al. 2008). This suggests that thermotolerance might be achieved by maintaining ABA content below an inhibitory threshold, but GA is still required for seed germination to proceed.

GA acts on seed germination through several key signaling components (Fig. 1.2), including GA receptors (GIDs) (Nakajima et al. 2006), negative regula-

tors (e.g., SPINDLY [SPY] and DELLA-domain proteins such as RGL2) (Jacobsen and Olszewski 1993; Lee et al. 2002; Piskurewicz et al. 2008) and positive regulators (e.g., F-box protein SLEEPY1 [SLY1]) (McGinnis et al. 2003). As expected from the promotive effect of GA on germination, seeds of *gid* and *sly1* mutants fail to germinate (Griffiths et al. 2006; McGinnis et al. 2003; Steber et al. 1998; Willige et al. 2007). By contrast, *rgl2* and *spy* mutant seeds are insensitive to PAC during germination (Jacobsen and Olszewski 1993; Lee et al. 2002; Tyler et al. 2004). Consistent with this, Toh et al. (2008) found that seeds of Arabidopsis *rgl2* and *spy-4* mutants are resistant to thermoinhibition. Seeds of pentuple *DELLA* mutants also exhibited germination thermotolerance (Kim et al. 2008). The tolerance of *DELLA* mutants could be due to the lower ABA contents, as Piskurewicz et al. (2008) found that *RGL2* inhibits Arabidopsis seed germination by stimulating ABA synthesis and *ABI5* expression. In Arabidopsis, *SPY* transcripts decreased to low levels at 22 °C, but were induced and maintained at relatively high levels at 34 °C; this elevated *SPY* expression was suppressed in *aba2-2* seeds imbibed at 33 °C (Toh et al. 2008). While this suggests that suppression of *SPY* may result from the lower ABA content in the *aba2-2* mutant seeds, in other studies *SPY* expression was not responsive to ABA treatment (Qin et al. 2011).

Red light can increase the upper temperature limits for germination and overcome the thermoinhibition of lettuce seeds incubated in the dark (Argyris et al. 2008; Cantliffe et al. 2000; Saini et al. 1989). Light promotes GA accumulation in lettuce seeds and decreases ABA content by promoting or inhibiting expression of their respective biosynthetic genes (Sawada et al. 2008; Toyomasu et al. 1993; Toyomasu et al. 1994). In Arabidopsis, red light, acting through phytochrome and degradation of PHYTOCHROME-INTERACTING FACTOR3-LIKE5 (PIL5), induces the expression of genes involved in GA biosynthesis and influences responsiveness to GA while also acting to reduce ABA levels (Oh et al. 2007; Yamaguchi et al. 1998). Thus, it is likely that light increases upper temperature limits for germination through these effects on the relative balance between ABA and GA action.

Ethylene Promotes Seed Germination at High Temperature

Ethylene promotes seed germination of diverse species (reviewed in Arc et al. 2013; Bogatek and Gniazdowska 2012; Kepczynski and Kepczynska 1997; Matilla and Matilla-Vazquez 2008). Thermoinhibition of lettuce, chickpea (*Cicer arietinum*), sunflower (*Helianthus annuus*) and tomato seeds could be alleviated by application of ethylene or its biosynthetic precursor 1-aminocyclopropane-1-carboxylic acid (ACC) (Corbineau et al. 1988; Dutta and Bradford 1994; Gallardo et al. 1991; Gallardo et al. 1994; Kepczynska et al. 2006; Nascimento et al. 2000; Saini et al. 1989), suggesting that ethylene biosynthesis is involved in regulation of thermoinhibition across different species. For carrot (*Daucus carota*) seeds, there was a positive correlation among genotypes between their ethylene production and their capacity for germination at high temperatures (Nascimento et al. 2008). Thermoinhibition in

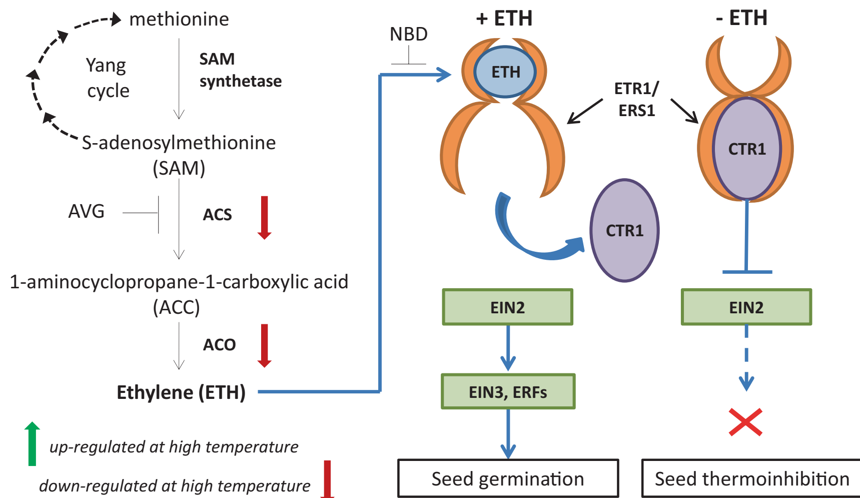


Fig. 1.3 Pathways of ethylene biosynthesis and signaling. (Modified from Bewley et al. 2013). Gene/enzyme names are in Table 1.1

marigold (*Tagetes minuta*) seeds was alleviated by a combination of treatments that reduced ABA levels and increased ethylene levels, although ethylene alone was less effective (Taylor et al. 2005).

In higher plants, ethylene is derived from the amino acid methionine which is converted to *S*-adenosyl-L-methionine (*S*-AdoMet) by *S*-AdoMet synthetase (SAM synthetase) (Fig. 1.3; Yang and Hoffman 1984). ACC synthase (ACS) catalyzes the conversion of *S*-AdoMet to ACC, which is further converted to ethylene by ACC oxidase (ACO) (Bleecker and Kende 2000). Up-regulation of ACS and/or ACO and enhanced ethylene evolution are associated with seed germination in several species (Chiwocha et al. 2005; Foley et al. 2013; Hermann et al. 2007; Iglesias-Fernandez and Matilla 2010; Leubner-Metzger et al. 1998; Linkies et al. 2009; Machabee and Saini 1991; Petruzzelli et al. 2000). The promotive effect of red light on releasing thermoinhibition in the dark could be blocked by 2-aminoethoxyvinyl glycine (AVG, an ethylene biosynthesis inhibitor) and 2,5-norbornadiene (NBD, an ethylene action inhibitor) (Saini et al. 1989), suggesting that endogenous ethylene action was essential for the light-induced alleviation of lettuce seed thermoinhibition in the dark. Higher ethylene evolution was also detected from thermoresistant than from thermosensitive genotypes of lettuce seeds imbibed at 35 °C (Nascimento et al. 2000). Exogenous ACC increased seed germination of thermosensitive lettuce seeds at 35 °C and simultaneously increased endo-β-mannanase activity (involved in endosperm cell wall degradation), whereas AVG and silver thiosulfate (STS, an inhibitor of ethylene signal transduction) resulted in decreased or no activity of endo-β-mannanase. This result indicated that ethylene may overcome the inhibitory effect of high temperature in thermosensitive lettuce seeds by increasing endo-β-mannanase expression, leading to weakening of the endosperm tissue enclosing the radicle (Nascimento et al. 2000, 2001, 2004).

Seed priming (controlled hydration followed by drying) alleviates thermoinhibition by increasing the maximum germination temperature (Guedes and Cantliffe 1980; Valdes and Bradford 1987). The ACC level in primed lettuce seeds peaked before germination at 35 °C and AVG reduced the ACC level and inhibited germination at 35 °C (Huang and Khan 1992). ACC application in association with seed priming further enhanced seed germination performance at 35 °C (Korkmaz 2006). The expression of genes encoding enzymes in the GA and ethylene biosynthetic pathways (*LsGA3ox1* and *LsACS1*, respectively) was suppressed by imbibition of untreated lettuce seeds at elevated temperatures but enhanced in primed seeds (Schwember and Bradford 2010). At the same time, transcript levels of *LsNCED4* decreased in the primed seeds compared to untreated control seeds imbibed at high temperature. There is an "escape time" from thermoinhibition, that is, transfer to higher temperatures after initial imbibition for 6–12 h at low temperature (in the case of lettuce seeds) does not result in inhibition of germination (e.g., Argyris et al. 2008). Similarly, increased expression of *LsGA3ox1* and *LsACS1* and repression of *LsNCED4* during priming treatment is not reversed by subsequent imbibition of primed (and dried) seeds at high temperature (Schwember and Bradford 2010). Thus, the developmental regulation of *LsNCED4*, which is expressed during late seed maturation (Huo et al. 2013) and is only inducible by elevated temperature for a limited period of time after imbibition, seems to be associated with either preventing or allowing the increases in expression of GA and ethylene biosynthetic genes that promote germination.

The temperature at which lettuce seeds mature influences the upper temperature limit inducing thermoinhibition (Contreras et al. 2009; Harrington and Thompson 1952; Hayashi et al. 2008; Sung et al. 1998). Seeds that mature at higher temperature (HTM seeds) germinate to higher percentages at warm temperatures than do seeds matured at lower temperature (LTM seeds). The effect of seed maturation environment on thermoinhibition may act in part via ethylene. HTM seeds produced more ethylene during germination than LTM seeds, regardless of imbibition conditions, and application of exogenous ACC also increased the germination of both HTM and LTM seeds (Kozarewa et al. 2006). This was not due to differences in ethylene perception between HTM and LTM germinating seeds, indicating that ethylene biosynthesis rather than ethylene perception in both types of seeds led to the difference in thermoinhibition. Expression of *LsACS* and *LsACO* was strongly repressed in seeds of a thermosensitive lettuce genotype at 35 °C in the light, whereas their expression in a thermoresistant genotype was only delayed but still expressed as well as at 20 °C (Argyris et al. 2008). As for GA biosynthetic genes, the repression of *LsACS* and *LsACO* at high temperature could be due to the elevated endogenous ABA, because silencing of *LsNCED4* (reducing ABA content) resulted in increased expression of these genes in association with germination at 35 °C (Huo et al. 2013).

Because elevated ethylene synthesis is associated with thermoresistance in some species, mutations that result in ethylene overproduction, such as in *ETHYLENE OVERPRODUCER 1* (*ETO1*), might be expected to display resistance to high temperature during seed germination. ETO1 negatively regulates ACS5 activity by recognizing and directly interacting with the C-terminal region of the protein.

This interaction allows CUL3 (a component of ubiquitin E3 ligase) to bind ACS5, leading to its degradation via the ubiquitin-26S-proteasome pathway (Wang et al. 2004). The *eto1-4* mutant overproduces ethylene and has a rapid seed germination phenotype (Cheng et al. 2009), whereas inhibition of ACO and ACS or loss of function of ACO resulted in less ethylene production and delayed seed germination (Gallardo et al. 1994; Iglesias-Fernandez and Matilla 2010; Kepczynski et al. 2006; Linkies et al. 2009). Consistent with this, our preliminary data showed that the *eto1-4* mutant can increase the upper temperature limit for seed germination (Table 1.1).

Although numerous reports have demonstrated that ethylene can promote seed germination and release seed thermoinhibition, the molecular mechanism behind ethylene action is still unclear. Ethylene signaling components comprise ethylene receptors (ETR1/ETS1), the negative regulator CTR1 (CONSTITUTIVE TRIPLE RESPONSE1), an essential ER-membrane-localized EIN2 protein, and downstream nuclear-localized transcription factors such as EIN3, EIL1 and EREBPs/ERFs (Fig. 1.3; Merchante et al. 2013). Ethylene might act on seed germination through altering the sensitivity to endogenous ABA, as the *ctr1* and *ein2* mutants were isolated as enhancer and suppressor, respectively, of germination of *abi1-1* mutant seeds that have an ABA-resistant germination phenotype (Beaudoin et al. 2000; Wang et al. 2007). However, in other reports, the *ein2* and *etr1-1* mutants displayed enhanced germination sensitivity to ABA due to over-accumulation of endogenous ABA (Cheng et al. 2009; Ghassemian et al. 2000). Interestingly, these ABA-sensitive *ein2* and *etr1-1* mutants had higher ethylene production than the wild-type seeds whereas *ctr1* mutant seeds had reduced ethylene production (Cheng et al. 2009). A reduction in endogenous ABA, as observed in the *ein2 aba2* double mutant, considerably promoted seed germination in a manner that resembled the effect of the *ctr1-1* mutant (Cheng et al. 2009), suggesting that ethylene promotes seed germination through alteration of endogenous ABA content and/or ABA sensitivity. Linkies et al. (2009) also found that ethylene promotes seed germination in *Lepidium sativum* and Arabidopsis by counteracting the effects of ABA and promoting endosperm rupture. On the other hand, ERF1 was implicated in transducing GA and/or mechanical signals between the embryo and endosperm in tomato seeds (Martínez-Andújar et al. 2012). In addition, other genes involved in the ethylene signaling pathway like *RAN1* and *XRN4* may also regulate seed germination/thermoinhibition (Bogatek and Gniazdowska 2012).

Jasmonates and Strigolactones May Be Involved in Regulating Seed Thermoinhibition

Jasmonates such as jasmonic acid (JA) and methyl jasmonate (MeJA) have been found to inhibit seed germination of various plant species (reviewed in Linkies and Leubner-Metzger 2012). Jasmonic acid and its biosynthetic precursor 12-oxo-phytodienoic acid (OPDA) and associated metabolites (MeJA and jasmonoyl-L-isoleucine [JA-Ile]) are signaling compounds in plant stress responses, physiologi-

cal reactions and developmental processes (Wasternack 2007). In the pathway for jasmonate biosynthesis, OPDA is first produced in chloroplasts from α-linolenic acid by 13-lipoxygenase (LOX), allene oxide synthase (AOS) and allene oxide cyclase (AOC) (Fonseca et al. 2009). The ATP-binding cassette (ABC) transporter COMATOSE (CTS) is responsible for transporting OPDA to the peroxisome for β-oxidation in Arabidopsis (Wasternack 2007). In the peroxisome, OPDA is catalyzed by OPDA reductase (OPR3 in Arabidopsis) to OPC-8:0 that is eventually converted to JA (Wasternack 2007). Genetic analysis revealed that *cis*-OPDA rather than JA or JA-Ile has strong inhibitory effects on seed germination in Arabidopsis (Dave et al. 2011). *cis*-OPDA treatment is more effective than JA in inhibiting wild-type Arabidopsis seed germination; in addition, OPDA has also been shown to affect seed germination at high temperature. Although seeds of both *opr3-1* and *aos* mutants germinate similarly at 20 °C, thermoinhibition at 31 °C was observed only in *opr3-1* mutant seeds which had elevated levels of *cis*-OPDA. The inhibition of seed germination by *cis*-OPDA is likely not due to decreased GA levels since exogenous GA and after-ripening treatment could not rescue *cts* mutants having high *cis*-OPDA and strong dormancy (Pinfield-Wells et al. 2005; Russell et al. 2000); moreover, the endogenous GA level in *cts-2* mutant seeds is even higher than in wild-type seeds (Dave et al. 2011).

This type of thermoinhibition also is not caused by elevated endogenous ABA levels, because endogenous ABA contents in dry and imbibed seeds of the *cts-2* mutant are similar to those in wild-type seeds. Instead, the inhibitory effect of *cis*-OPDA is synergistic with ABA; combined ABA and OPDA treatment inhibited seed germination more efficiently than either treatment alone (Dave et al. 2011). The two hormonal signals may converge at ABI5, which can inhibit germination. In seeds of the mutant *ped3*, which is allelic to *cts-2*, *ABI5* transcript levels remain elevated relative to the wild type upon seed imbibition (Kanai et al. 2010); in addition, exogenous OPDA treatment induced ABI5 protein accumulation during seed imbibition and exogenous ABA treatment could enhance this induction (Dave et al. 2011). These results suggest that the seed germination inhibition by OPDA at high temperature could be mediated by this increase in ABI5, as its overexpression enhanced germination sensitivity to high temperature (Lim et al. 2013). Whether OPDA biosynthesis is responsive to temperature remains to be investigated. It should be noted that in contrast to the results for Arabidopsis and other species, MeJA reduced dormancy in wheat (*Triticum aestivum*) grains and this was associated with lower expression of *TaNCED1* and lower ABA content (Jacobsen et al. 2013).

Strigolactones are terpenoid lactones derived from carotenoids that are known for their ability to stimulate germination of seeds of some parasitic weeds, such as *Striga lutea* (witchweed) (Ruyter-Spira et al. 2013). A synthetic strigolactone (GR24) alleviated Arabidopsis seed thermoinhibition at 32 °C (Toh et al. 2012). Germination of seeds of a strigolactone biosynthetic mutant (*max1-1*) and a signaling mutant (*max2-1*) were 3 °C more sensitive to elevated temperature than were wild-type seeds. Exogenous GR24 can rescue seeds of *max1-1* but not of *max2-1*, indicating that strigolactone action has a promotive effect on seed germination at high temperature (Toh et al. 2012). Alleviation of thermoinhibition by GR24 was

blocked by the GA inhibitor PAC, suggesting that GA is required for the action of strigolactones on germination; in addition, GR24 can increase the endogenous GA_4 content of seeds. GR24 also suppressed *NCED9* expression, resulting in a lower level of endogenous ABA in seeds imbibed at 32 °C (Toh et al. 2012). These results indicate that strigolactone signaling is also involved in regulating seed thermoinhibition.

Cytokinins might also be involved in the regulation of seed germination by strigolactone, because hormonal profiling results revealed that the cytokinin levels increased significantly in GR24-treated *Striga hermonthica* seeds that are particularly sensitive to the GR24 signal for germination (Toh et al. 2012). Cytokinin also, at least partially, alleviates seed thermoinhibition in lettuce (Khan and Huang 1987; Saini et al. 1986; Small et al. 1993). Cytokinins have been shown to promote Arabidopsis seed germination through antagonizing ABA action by suppression of *ABI5* expression (Wang et al. 2011). However, the promotive effect of cytokinins on seed germination might also be due to their enhancement of ethylene production (Chiwocha et al. 2005; Khan and Prusinski 1989; Siriwitayawan et al. 2003).

Other Genetic Factors Influencing Thermoinhibition

FUSCA3 (FUS3), ABI3 and LEAFY COTYLEDON (LEC1 and LEC2) are four key regulators that play prominent roles in embryogenesis and seed maturation (Holdsworth et al. 2008). Mutations in all four genes cause reduced seed dormancy (Holdsworth et al. 2008). FUS3 is also involved in Arabidopsis seed thermoinhibition (Chiu et al. 2012). At high temperature, FUS3 was greatly induced and accumulated in imbibed Arabidopsis seeds. A *FUS3* overexpression line had a slower germination rate and displayed hypersensitivity to high temperature during seed germination, whereas seeds of a loss of function *fus3* mutant were strongly resistant to thermoinhibition at 32 °C (Chiu et al. 2012). The thermoinhibition of the *FUS3*-overexpression line could be partly rescued by application of an ABA biosynthesis inhibitor, suggesting that *de novo* ABA synthesis is required for the hypersensitivity to high temperature. ChiP-chip and microarray assays revealed that FUS3 can directly regulate *NCED5*, *NCED9* and several GA biosynthetic genes (Wang and Perry 2013; Yamamoto et al. 2010), suggesting that the thermotolerance of the *fus3* mutant seeds could be due to a decrease in *NCED5* and *NCED9* expression. Our microarray data (unpublished results) also showed that *LsFUS3* transcripts decreased in lettuce seeds imbibed at 20 °C, but remained at a high level when thermosensitive seeds were imbibed at 35 °C for 24 h, raising the possibility of *LsFUS3* as a candidate transcription factor for regulating *LsNCED4*. The increased abundance of *FUS3* transcript in thermoinhibited seeds could also be part of a syndrome of maintaining expression of maturation-related genes in dormant seeds and prevention of the developmental phase change from embryogenesis to germination (Cadman et al. 2006; Chiu et al. 2012).

Testa structure, color and permeability can influence germination and dormancy (Chen et al. 2012; Debeaujon and Koornneef 2000; Debeaujon et al. 2000; Downie et al. 2003; Leubner-Metzger 2002; Salaita et al. 2005). Germination of some *transparent testa* (*tt*) mutant seeds, which generally exhibit reduced dormancy, is temperature dependent (Salaita et al. 2005; Tamura et al. 2006). Seeds of Arabidopsis *tt7-4* and *tt7-1* mutants displayed resistance to thermoinhibition and also germinated faster at lower temperature than did wild-type seeds (Tamura et al. 2006). The partial loss of thermoinhibition in *tt7* mutants could be caused by reduced seed dormancy and/or reduced seed coat strength as a barrier to radicle emergence (Debeaujon et al. 2000).

Several reduced-dormancy mutants (e.g., *rdo1*, *rdo2*, *rdo3* and *rdo4*) have also exhibited strong resistance to thermoinhibition of germination although they are not deficient in ABA (Leon-Kloosterziel et al. 1996b; Tamura et al. 2006). Among these four reduced-dormancy mutants, *rdo2* and *rdo4* have been well characterized (Liu et al. 2011; Liu et al. 2007; Mortensen et al. 2011). *RDO4* encodes HISTONE MONOUBIQUITINATION1 (HUB1), a C3HC4 RING finger protein while *RDO2* encodes a TFIIS transcription elongation factor that facilitates transcript elongation by assisting RNA polymerase II (Grasser et al. 2009; Liu et al. 2011; Liu et al. 2007; Mortensen et al. 2011). These proteins may also interact with *DELAY OF GERMINATION* (*DOG*) genes identified from the Cape Verde Islands (Cvi) accession of Arabidopsis that require extended after-ripening to alleviate dormancy (Alonso-Blanco et al. 2003; Bentsink et al. 2006). Genetic analysis showed that *HUB1-2* is epistatic to *DOG3* because seeds containing *hub1-2* and *Cvi-DOG3* were non-dormant, while the near-isogenic line with wild-type *HUB1* is very dormant (Liu et al. 2007). The expression of *NCED9* was dramatically reduced in freshly harvested *hub1-2* mutant seeds compared to the wild-type seeds, possibly contributing to its resistance to thermoinhibition (Liu et al. 2007). In *rdo2-1* and *hub1-2* mutants, expression of the *DOG1* gene also was significantly decreased, suggesting that *DOG1* might also be involved in thermoinhibition (Liu et al. 2007; Mortensen et al. 2011). This is supported by recent results from our lab, where silencing of *LsDOG1* in a thermosensitive lettuce genotype resulted in loss of germination thermoinhibition at 35 °C (our unpublished data). Graeber et al. (2014) also reported that overexpression of *DOG1* in Arabidopsis and *Lepidium sativum* lowered the maximum germination temperature. DOG1 may therefore be involved in the shifts in germination temperature sensitivity in response to the ambient temperature during seed development or imbibition (Chiang et al. 2011; Kendall et al. 2011).

SOMNUS (SOM), a CCCH-type zinc finger protein, has been reported recently to regulate seed germination at high temperature (Lim et al. 2013). Seeds of *som* mutants germinate better than Arabidopsis wild-type seeds at high temperature in the dark. Further analysis showed that *SOM* expression is induced by high temperature, and this induction by high temperature requires ABI3 and DELLA proteins, which bind to the *SOM* promoter, although their enrichment on the promoter of *SOM* was not affected by high temperature. Overexpression of *ABI5* also caused higher expression of *SOM* in the seeds imbibed at higher temperature compared to those imbibed at lower temperature. However, ABA synthesis and ABA content

were also lower in seeds of *som* mutants than in wild-type seeds, which could also contribute to the thermotolerance of germination, typical of the thermoresistance in other ABA-deficient mutants such as *aba1, aba2* and *nced9* (Kim et al. 2008; Lim et al. 2013; Tamura et al. 2006; Toh et al. 2008).

DOF AFFECTING GERMINATION 1 (*DAG1*) has been reported to be a negative regulator of Arabidopsis seed germination (Papi et al. 2002). Mutation of *DAG1* improved seed germination at high temperature, particularly under salinity stress (Rizza et al. 2011). Loss of function in *dag1* mutants caused increased expression of *GA3ox1* and down-regulation of *ABA1/ZEP*, *NCED6* and *NCED9* (Gabriele et al. 2010). In addition, *dag1* mutant seeds have a more permeable testa than wild-type seeds, a characteristic typical of *tt* mutants that have reduced dormancy (Debeaujon et al. 2000; Papi et al. 2002).

Using a recombinant inbred line population derived from two cultivars of winter wheat, Lei et al. (2013) mapped temperature sensitivity of germination to the short arm of chromosome 3. This QTL (*QTsg.osu-3A*) is also reported to regulate seed germination and dormancy in spring wheat (Nakamura et al. 2011). *TaMFT-A1* (a homolog of *MOTHER OF FT*) and *TFL1* (*TERMINAL FLOWER 1-LIKE*) genes co-localized with *QPhs.ocs-3A*. Although *TaMFT-A1* expression in seeds was responsive to imbibition at high temperature, its effect on seed germination at high temperature was not significant in the RIL population (Lei et al. 2013).

Thermoinhibition can be considered to be a specific case of more general responses of plants to heat and/or dehydration stresses. Dehydration-responsive element binding (DREB)/C-repeat-binding factors regulate the expression of many stress-inducible genes and play critical roles in improving plant abiotic stress tolerance (Lata and Prasad 2011). CAP2 is a single AP2 domain-containing transcription activator from chickpea (*Cicer arietinum*) that is similar to DREB2A in Arabidopsis; its overexpression in tobacco (*Nicotiana tabaccum*) improved seed germination at high temperature (Shukla et al. 2009). Further analysis revealed that *CAP2* expression could be transiently induced by heat stress and could improve thermotolerance in yeast, suggesting that the thermotolerance caused by overexpression of *CAP2* could involve activation of heat shock proteins such as HSP70 (Shukla et al. 2009).

Overexpression of a *CODA* gene encoding a choline oxidase that converts choline to glycinebetaine led to accumulation of glycinebetaine in tomato and Arabidopsis seeds, which increased germination under heat shock stress (Alia et al. 1998; Li et al. 2011). Further analysis showed that increased germination following heat shock could be due to the activation of HSP70 expression. However, all tomato seeds were treated first with high temperature then transferred to room temperature for the germination assay, and the germination percentage was not significantly improved under continuous high temperature (34 °C), so the role of heat shock proteins in thermoinhibition of germination remains unclear. However, another group found that a nuclear-encoded chloroplast small heat shock protein from wheat (sHSP26) can regulate seed germination at high temperature (Chauhan et al. 2012). *TaHSP26* was highly induced by heat stress and overexpression of *TaHSP26* in Arabidopsis resulted in better germination and faster seedling growth at continuous

35 °C. Moreover, *TaHSP26*-overexpressing plants that were grown under constant 35 °C produced viable seeds but the wild-type Arabidopsis plants could not. These results suggest that heat stress-responsive genes such as DREBs and HSPs might be involved in protecting seeds from the heat stress associated with thermoinhibition.

Perspective

ABA, especially *de novo* synthesized ABA, seems to be essential for thermoinhibition of seed germination. In particular, *NCED9*-like genes in Arabidopsis and their homologs in other species play a pivotal role in *de novo* ABA biosynthesis during thermoinhibition. However, how these genes are regulated by temperature is not fully understood. Further studies are needed on the cellular mechanisms and molecular components that perceive temperature during seed imbibition and mediate the expression of *NCED9*-like genes (Franklin and Wigge 2014; Penfield 2008; Wigge 2013). Other hormones, particularly GA and ethylene, can at least partially alleviate thermoinhibition, but direct connections among ABA, temperature and these pathways are not fully characterized. Expression of GA- and ethylene-related genes is in general oppositely regulated in comparison with ABA-related genes during seed imbibition, but whether this is in response to changes in ABA levels or action or to independent temperature sensitivity mechanisms remains to be definitively determined. Similarly, whether additional signaling compounds such as jasmonates and strigolactones act through these same pathways or distinct ones is of interest. Elucidating this is not a trivial task, as expression of many genes increases when germination is triggered, but not all of these will be directly involved in regulating the initiation of germination *per se*. It is particularly difficult, but critical, to distinguish correlation from causality in such studies, as any gene whose expression increases soon after germination *sensu stricto* is completed will show a high correlation with germination percentages in bulk samples of seed populations, but such genes would not be associated with the causal mechanisms preventing or enabling the germination process to proceed. Precise attention to developmental timing and tissue localization in sample collection, such as for transcriptomic analyses, is essential to distinguish early regulatory events controlling germination from subsequent events associated with endosperm weakening and embryo growth. Mutants and manipulation of expression (overexpression or silencing) have also been extremely useful in identifying and characterizing such regulatory relationships.

Another topic for future research would be the relationships among primary dormancy, secondary dormancy and thermoinhibition. The gene expression and signaling networks involved in regulating whether germination occurs are shared among these types of dormancy (Bewley et al. 2013; Footitt et al. 2013; Graeber et al. 2012; Huo et al. 2013; Toh et al. 2008), but specifically how they are distinguished as separate phenomena is unclear. In Arabidopsis, ABA-related and *DOG1* genes are required for establishment of primary dormancy, and genetic analysis showed that the lack of either one results in reduced dormancy (Nakabayashi et al. 2012).

Loss of primary dormancy and down-regulation of *DOG1* in *rdo2* and *rdo4* mutants are associated with seed thermoresistance, indicating a correspondence between primary dormancy and thermoinhibition. This is further supported by the effects of the environment during seed development on subsequent temperature sensitivity of germination (Chiang et al. 2011; Hayashi et al. 2008; Kendall et al. 2011; Kozarewa et al. 2006; Sung et al. 1998), which could implicate epigenetic mechanisms in the regulation of thermoinhibition (Nonogaki 2014). The ecological consequences of various types of dormancy are the same: seeds do not germinate at a particular time or environmental condition, yet remain capable of doing so at a later time or when environmental conditions change. Adaptation to an array of climates and ecological niches has resulted in a broad diversity of seed germination environmental sensitivities and behaviors (Baskin and Baskin 1998), and we must expect that a similarly wide array of genetic and physiological mechanisms are present, although they may converge on a common core of regulators controlling the developmental phase transition from dormancy to germination (Chiu et al. 2012; Donohue et al. 2010; Huang et al. 2013; Nonogaki 2010).

References

Alia, Hayashi H, Sakamoto A, Murata N (1998) Enhancement of the tolerance of Arabidopsis to high temperatures by genetic engineering of the synthesis of glycinebetaine. Plant J 16:155–161

Allen PS, Benech-Arnold RL, Batlla D, Bradford KJ (2007) Modeling of seed dormancy. In: Bradford KJ, Nonogaki H (eds) Seed development, dormancy and germination. Blackwell Publishing, Oxford, pp 72–112

Alonso-Blanco C, Bentsink L, Hanhart CJ, Blankestijn-de Vries H, Koornneef M (2003) Analysis of natural allelic variation at seed dormancy loci of *Arabidopsis thaliana*. Genetics 164:711–729

Alvarado V, Bradford KJ (2002) A hydrothermal time model explains the cardinal temperatures for seed germination. Plant Cell Environ 25:1061–1069

Arc E, Sechet J, Corbineau F, Rajjou L, Marion-Poll A (2013) ABA crosstalk with ethylene and nitric oxide in seed dormancy and germination. Front Plant Sci 4:63

Argyris J, Dahal P, Hayashi E, Still DW, Bradford KJ (2008) Genetic variation for lettuce seed thermoinhibition is associated with temperature-sensitive expression of abscisic acid, gibberellin, and ethylene biosynthesis, metabolism, and response genes. Plant Physiol 148:926–947

Argyris J, Truco MJ, Ochoa O, McHale L, Dahal P, Van Deynze A et al (2011) A gene encoding an abscisic acid biosynthetic enzyme (*LsNCED4*) collocates with the high temperature germination locus *Htg6.1* in lettuce (*Lactuca* sp.). Theor Appl Genet 122:95–108

Auldridge ME, McCarty DR, Klee HJ (2006) Plant carotenoid cleavage oxygenases and their apocarotenoid products. Curr Opin Plant Biol 9:315–321

Baskin CC, Baskin JM (1998) Seeds: ecology, biogeography, and evolution of dormancy and germination. Academic Press, New York

Beaudoin N, Serizet C, Gosti F, Giraudat J (2000) Interactions between abscisic acid and ethylene signaling cascades. Plant Cell 12:1103–1115

Bentsink L, Jowett J, Hanhart CJ, Koornneef M (2006) Cloning of *DOG1*, a quantitative trait locus controlling seed dormancy in Arabidopsis. Proc Natl Acad Sci U S A 103:17042–17047

Bewley JD, Bradford KJ, Hilhorst HWM, Nonogaki H (2013) Seeds: physiology of development, germination and dormancy, 3rd edn. Springer, New York

Bleecker AB, Kende H (2000) Ethylene: a gaseous signal molecule in plants. Annu Rev Cell Dev Biol 16:1–18

Bogatek R, Gniazdowska A (2012) Ethylene in seed development, dormancy and germination. In: McManus MT (ed) Annual plant reviews 44. Blackwell Publishing Ltd., Oxford, pp 189–218

Cadman CSC, Toorop PE, Hilhorst HWM, Finch-Savage WE (2006) Gene expression profiles of Arabidopsis Cvi seeds during dormancy cycling indicate a common underlying dormancy control mechanism. Plant J 46:805–822

Cantliffe DJ, Sung Y, Nascimento WM (2000) Lettuce seed germination. Hortic Rev 224:229–275

Carter AK, Stevens R (1998) Using ethephon and GA_3 to overcome thermoinhibition in 'Jalapeño M' pepper seed. HortScience 33:1026–1027

Chauhan H, Khurana N, Nijhavan A, Khurana JP, Khurana P (2012) The wheat chloroplastic small heat shock protein (sHSP26) is involved in seed maturation and germination and imparts tolerance to heat stress. Plant Cell Environ 35:1912–1931

Chen MX, Wang Z, Zhu YN, Li ZL, Hussain N, Xuan LJ et al (2012) The effect of *TRANSPARENT TESTA2* on seed fatty acid biosynthesis and tolerance to environmental stresses during young seedling establishment in Arabidopsis. Plant Physiol 160:1023–1036

Cheng WH, Chiang MH, Hwang SG, Lin PC (2009) Antagonism between abscisic acid and ethylene in Arabidopsis acts in parallel with the reciprocal regulation of their metabolism and signaling pathways. Plant Mol Biol 71:61–80

Chiang GCK, Bartsch M, Barua D, Nakabayashi K, Debieu M, Kronholm I et al (2011) *DOG1* expression is predicted by the seed-maturation environment and contributes to geographical variation in germination in *Arabidopsis thaliana*. Mol Ecol 20:3336–3349

Chiu RS, Nahal H, Provart NJ, Gazzarrini S (2012) The role of the Arabidopsis FUSCA3 transcription factor during inhibition of seed germination at high temperature. BMC Plant Biol 12:15

Chiwocha SDS, Cutler AJ, Abrams SR, Ambrose SJ, Yang J, Ross ARS et al (2005) The *etr1–2* mutation in *Arabidopsis thaliana* affects the abscisic acid, auxin, cytokinin and gibberellin metabolic pathways during maintenance of seed dormancy, moist-chilling and germination. Plant J 42:35–48

Chono M, Honda I, Shinoda S, Kushiro T, Kamiya Y, Nambara E et al (2006) Field studies on the regulation of abscisic acid content and germinability during grain development of barley: molecular and chemical analysis of pre-harvest sprouting. J Exp Bot 57:2421–2434

Contreras S, Bennett MA, Tay D (2009) Temperature during seed development affects weight, germinability and storability of lettuce seeds. Seed Sci Technol 37:398–412

Corbineau F, Rudnicki R, Come D (1988) Induction of secondary dormancy in sunflower seeds by high temperature. Possible involvement of ethylene biosynthesis. Physiol Plant 73:368–373

Covell S, Ellis RH, Roberts EH, Summerfield RJ (1986) The influence of temperature on seed germination rate in grain legumes. 1. A comparison of chickpea, lentil, soybean and cowpea at constant temperatures. J Exp Bot 37:705–715

Cutler SR, Rodriguez PL, Finkelstein RR, Abrams SR (2010) Abscisic acid: emergence of a core signaling network. Annu Rev Plant Biol 61:651–679

Dave A, Hernandez ML, He Z, Andriotis VM, Vaistij FE, Larson TR et al (2011) 12-oxo-phytodienoic acid accumulation during seed development represses seed germination in Arabidopsis. Plant Cell 23:583–599

Debeaujon I, Koornneef M (2000) Gibberellin requirement for Arabidopsis seed germination is determined both by testa characteristics and embryonic abscisic acid. Plant Physiol 122:415–424

Debeaujon I, Leon-Kloosterziel KM, Koornneef M (2000) Influence of the testa on seed dormancy, germination, and longevity in Arabidopsis. Plant Physiol 122:403–413

Donohue K (2014) Why ontogeny matters during adaptation: developmental niche construction and pleiotropy across the life cycle in *Arabidopsis thaliana*. Evolution 68:32–47

Donohue K, Rubio de Casas R, Burghardt L, Kovach K, Willis CG (2010) Germination, postgermination adaptation, and species ecological ranges. Annu Rev Ecol Evol Syst 41:293–319

Downie AB, Zhang D, Dirk LM, Thacker RR, Pfeiffer JA, Drake JL et al (2003) Communication between the maternal testa and the embryo and/or endosperm affect testa attributes in tomato. Plant Physiol 133:145–160

Dutta S, Bradford KJ (1994) Water relations of lettuce seed thermoinhibition. II. Ethylene and endosperm effects on base water potential. Seed Sci Res 4:11–18
Finkelstein RR (1994) Mutations at two new Arabidopsis ABA response loci are similar to the *abi3* mutations. Plant J 5:765–771
Finkelstein RR, Lynch TJ (2000) The Arabidopsis abscisic acid response gene *ABI5* encodes a basic leucine zipper transcription factor. Plant Cell 12:599–609
Finkelstein RR, Somerville CR (1990) Three classes of abscisic acid (ABA)-insensitive mutations of Arabidopsis define genes that control overlapping subsets of ABA responses. Plant Physiol 94:1172–1179
Foley ME, Chao WS, Horvath DP, Dogramaci M, Anderson JV (2013) The transcriptomes of dormant leafy spurge seeds under alternating temperature are differentially affected by a germination-enhancing pretreatment. J Plant Physiol 170:539–547
Fonseca S, Chico JM, Solano R (2009) The jasmonate pathway: the ligand, the receptor and the core signalling module. Curr Opin Plant Biol 12:539–547
Footitt S, Huang Z, Clay HA, Mead A, Finch-Savage WE (2013) Temperature, light and nitrate sensing coordinate Arabidopsis seed dormancy cycling, resulting in winter and summer annual phenotypes. Plant J 74:1003–1015
Franklin KA, Wigge PA (eds) (2014) Temperature and plant development. Wiley, Ames
Franks SJ, Weber JJ, Aitken SN (2014) Evolutionary and plastic responses to climate change in terrestrial plant populations. Evol Appl 7:123–139
Frey A, Effroy D, Lefebvre V, Seo M, Perreau F, Berger A et al (2012) Epoxycarotenoid cleavage by NCED5 fine-tunes ABA accumulation and affects seed dormancy and drought tolerance with other NCED family members. Plant J 70:501–512
Fujii H, Chinnusamy V, Rodrigues A, Rubio S, Antoni R, Park SY et al (2009) In vitro reconstitution of an abscisic acid signalling pathway. Nature 462:660–U138
Gabriele S, Rizza A, Martone J, Circelli P, Costantino P, Vittorioso P (2010) The Dof protein DAG1 mediates PIL5 activity on seed germination by negatively regulating GA biosynthetic gene *AtGA3ox1*. Plant J 61:312–323
Gallardo M, Delgado MD, Sanchezcalle IM, Matilla AJ (1991) Ethylene production and 1-aminocyclopropane-1-carboxylic acid conjugation in thermoinhibited *Cicer arietinum* L. seeds. Plant Physiol 97:122–127
Gallardo M, Derueda PM, Matilla A, Sanchezcalle IM (1994) The relationships between ethylene production and germination of *Cicer arietinum* Seeds. Biologia Plantarum 36:201–207
Ghassemian M, Nambara E, Cutler S, Kawaide H, Kamiya Y, McCourt P (2000) Regulation of abscisic acid signaling by the ethylene response pathway in Arabidopsis. Plant Cell 12:1117–1126
Gonai T, Kawahara S, Tougou M, Satoh S, Hashiba T, Hirai N et al (2004) Abscisic acid in the thermoinhibition of lettuce seed germination and enhancement of its catabolism by gibberellin. J Exp Bot 55:111–118
Gonzalez-Guzman M, Apostolova N, Belles JM, Barrero JM, Piqueras P, Ponce MR et al (2002) The short-chain alcohol dehydrogenase ABA2 catalyzes the conversion of xanthoxin to abscisic aldehyde. Plant Cell 14:1833–1846
Graeber K, Nakabayashi K, Miatton E, Leubner-Metzger G, Soppe WJ (2012) Molecular mechanisms of seed dormancy. Plant Cell Environ 35:1769–1786
Graeber K, Linkies A, Steinbrecher T, Mummenhoff K, Tarkowská D, Turečková V et al (2014) *DELAY OF GERMINATION 1* mediates a conserved coat-dormancy mechanism for the temperature- and gibberellin-dependent control of seed germination. Proc Natl Acad Sci U S A 10:1073/pnas.1403851111
Grasser M, Kane CM, Merkle T, Melzer M, Emmersen J, Grasser KD (2009) Transcript elongation factor TFIIS is involved in Arabidopsis seed dormancy. J Mol Biol 386:598–611
Griffiths J, Murase K, Rieu I, Zentella R, Zhang ZL, Powers SJ et al (2006) Genetic characterization and functional analysis of the *GID1* gibberellin receptors in Arabidopsis. Plant Cell 18:3399–3414
Guedes AC, Cantliffe DJ (1980) Germination of lettuce seeds at high temperature after seed priming. J Am Soc Hortic Sci 105:777–781

Harrington JD, Thompson RC (1952) Effect of variety and area of production on subsequent germination of lettuce seed at high temperatures. Proc Am Soc Hortic Sci 59:445–450

Hayashi E, Aoyama N, Still DW (2008) Quantitative trait loci associated with lettuce seed germination under different temperature and light environments. Genome 51:928–947

Hermann K, Meinhard J, Dobrev P, Linkies A, Pesek B, Hess B et al (2007) 1-Aminocyclopropane-1-carboxylic acid and abscisic acid during the germination of sugar beet (*Beta vulgaris* L.): a comparative study of fruits and seeds. J Exp Bot 58:3047–3060

Hilhorst HWM (2007) Definitions and hypotheses of seed dormancy. In: Bradford KJ, Nonogaki H (eds) Seed development, dormancy and germination. Blackwell Publishing, Oxford, pp 50–71

Holdsworth MJ, Bentsink L, Soppe WJJ (2008) Molecular networks regulating Arabidopsis seed maturation, after-ripening, dormancy and germination. New Phytol 179:33–54

Huang XL, Khan AA (1992) Alleviation of thermoinhibition in preconditioned lettuce seeds involves ethylene, not polyamine biosynthesis. J Am Soc Hortic Sci 117:841–845

Huang D, Koh C, Feurtado JA, Tsang EW, Cutler AJ (2013) MicroRNAs and their putative targets in *Brassica napus* seed maturation. BMC Genomics 14:140

Huo H, Dahal P, Kunusoth K, McCallum CM, Bradford KJ (2013) Expression of *9-cis-EPOXYCAROTENOID DIOXYGENASE4* is essential for thermoinhibition of lettuce seed germination but not for seed development or stress tolerance. Plant Cell 25:884–900

Iglesias-Fernandez R, Matilla AJ (2010) Genes involved in ethylene and gibberellins metabolism are required for endosperm-limited germination of *Sisymbrium officinale* L. seeds. Planta 231:653–664

Iuchi S, Kobayashi M, Taji T, Naramoto M, Seki M, Kato T et al (2001) Regulation of drought tolerance by gene manipulation of 9-CIS-EPOXYCAROTENOID DIOXYGENASE, a key enzyme in abscisic acid biosynthesis in Arabidopsis. Plant J 27:325–333

Jacobsen SE, Olszewski NE (1993) Mutations at the *SPINDLY* locus of Arabidopsis alter gibberellin signal transduction. Plant Cell 5:887–896

Jacobsen JV, Barrero JM, Hughes T, Julkowska M, Taylor JM, Xu Q et al (2013) Roles for blue light, jasmonate and nitric oxide in the regulation of dormancy and germination in wheat grain (*Triticum aestivum* L.). Planta 238:121–138

Kanai M, Nishimura M, Hayashi M (2010) A peroxisomal ABC transporter promotes seed germination by inducing pectin degradation under the control of ABI5. Plant J 62:936–947

Kendall SL, Hellwege A, Marriot P, Whalley C, Graham IA, Penfield S (2011) Induction of dormancy in Arabidopsis summer annuals requires parallel regulation of *DOG1* and hormone metabolism by low temperature and CBF transcription factors. Plant Cell 23:2568–25580

Kepczynska E, Piekna-Grochala J, Kepczynski J (2006) Hormonal regulation of tomato seed germination at a supraoptimal temperature. Acta Physiol Plant 28:225–231

Kepczynski J, Bihun M (2002) Induction of secondary dormancy in *Amaranthus caudatus* seeds. Plant Growth Regul 38:135–140

Kepczynski J, Kepczynska E (1997) Ethylene in seed dormancy and germination. Physiol Plant 101:720–726

Kepczynski J, Bihun M, Kepczynska E (2006) Implication of ethylene in the release of secondary dormancy in *Amaranthus caudatus* L. seeds by gibberellins or cytokinin. Plant Growth Regul 48:119–126

Khan AA, Huang XL (1987) Synergistic promotion of ethylene production and germination by cytokinin and ACC in lettuce seeds exposed to high-temperature and salinity stress. HortScience 22:1126

Khan AA, Prusinski J (1989) Kinetin enhanced 1-aminocyclopropane-1-carboxylic acid utilization during alleviation of high temperatures stress in lettuce seeds. Plant Physiol 91:733–737

Kim DH, Yamaguchi S, Lim S, Oh E, Park J, Hanada A et al (2008) SOMNUS, a CCCH-type zinc finger protein in Arabidopsis, negatively regulates light-dependent seed germination downstream of PIL5. Plant Cell 20:1260–1277

Kimball S, Angert AL, Huxman TE, Venable DL (2011) Differences in the timing of germination and reproduction relate to growth physiology and population dynamics of Sonoran desert winter annuals Am J Bot 98:1773–1781

Koornneef M, Jorna ML (1982) The isolation of abscisic acid (ABA) deficient mutants by selection of induced revertants in non-germinating gibberellin sensitive lines of *Arabidopsis thaliana* (L.) Henyh. Theor Appl Genet 61:385–393

Koornneef M, Vanderveen JH (1980) Induction and analysis of gibberellin sensitive mutants in *Arabidopsis thaliana* (L) Heynh. Theor Appl Genet 58:257–263

Koornneef M, Reuling G, Karssen CM (1984) The isolation and characterization of abscisic acid-insensitive mutants of *Arabidopsis thaliana*. Physiol Plant 61:377–383

Korkmaz A (2006) Ameliorative effects of ethylene precursor and polyamines on the high temperature inhibition of seed germination in lettuce (*Lactuca sativa* L.) before and after seed storage. Seed Sci Technol 34:465–474

Kozarewa I, Cantliffe DJ, Nagata RT, Stoffella PJ (2006) High maturation temperature of lettuce seeds during development increased ethylene production and germination at elevated temperatures. J Am Soc Hortic Sci 131:564–570

Kushiro T, Okamoto M, Nakabayashi K, Yamagishi K, Kitamura S, Asami T et al (2004) The Arabidopsis cytochrome P450 *CYP707A* encodes ABA 8′-hydroxylases: key enzymes in ABA catabolism. EMBO J 23:1647–1656

Lafta A, Mou BQ (2013) Evaluation of lettuce genotypes for seed thermotolerance. HortScience 48:708–714

Lata C, Prasad M (2011) Role of DREBs in regulation of abiotic stress responses in plants. J Exp Bot 62:4731–4748

Lee SC, Cheng H, King KE, Wang WF, He YW, Hussain A et al (2002) Gibberellin regulates Arabidopsis seed germination via *RGL2*, a *GAI/RGA*-like gene whose expression is up-regulated following imbibition. Genes Dev 16:646–658

Lefebvre V, North H, Frey A, Sotta B, Seo M, Okamoto M et al (2006) Functional analysis of Arabidopsis *NCED6* and *NCED9* genes indicates that ABA synthesized in the endosperm is involved in the induction of seed dormancy. Plant J 45:309–319

Lei L, Zhu X, Wang S, Zhu M, Carver BF, Yan L (2013) *TaMFT-A1* is associated with seed germination sensitive to temperature in winter wheat. PLoS One 8:e73330

Leon-Kloosterziel KM, Gil MA, Ruijs GJ, Jacobsen SE, Olszewski NE, Schwartz SH et al (1996a) Isolation and characterization of abscisic acid-deficient Arabidopsis mutants at two new loci. Plant J 10:655–661

Leon-Kloosterziel KM, van de Bunt GA, Zeevaart JA, Koornneef M (1996b) Arabidopsis mutants with a reduced seed dormancy. Plant Physiol 110:233–240

Leubner-Metzger G (2002) Seed after-ripening and over-expression of class I β-1, 3-glucanase confer maternal effects on tobacco testa rupture and dormancy release. Planta 215:959–968

Leubner-Metzger G, Petruzzelli L, Waldvogel R, Vogeli-Lange R, Meins F (1998) Ethylene-responsive element binding protein (EREBP) expression and the transcriptional regulation of class I β-1, 3-glucanase during tobacco seed germination. Plant Mol Biol 38:785–795

Leung J, Merlot S, Giraudat J (1997) The Arabidopsis *ABSCISIC ACID-INSENSITIVE2* (*ABI2*) and *ABI1* genes encode homologous protein phosphatases 2C involved in abscisic acid signal transduction. Plant Cell 9:759–771

Leymarie J, Robayo-Romero ME, Gendreau E, Benech-Arnold RL, Corbineau F (2008) Involvement of ABA in induction of secondary dormancy in barley (*Hordeum vulgare* L.) seeds. Plant Cell Physiol 49:1830–1838

Li S, Li F, Wang J, Zhang W, Meng Q, Chen TH et al (2011) Glycinebetaine enhances the tolerance of tomato plants to high temperature during germination of seeds and growth of seedlings. Plant Cell Environ 34:1931–1943

Lim S, Park J, Lee N, Jeong J, Toh S, Watanabe A et al (2013) ABA-INSENSITIVE3, ABA-INSENSITIVE5, and DELLAs interact to activate the expression of *SOMNUS* and other high-temperature-inducible genes in imbibed seeds in Arabidopsis. Plant Cell 25:4863–4878

Lin PC, Hwang SG, Endo A, Okamoto M, Koshiba T, Cheng WH (2007) Ectopic expression of *ABSCISIC ACID 2/GLUCOSE INSENSITIVE 1* in Arabidopsis promotes seed dormancy and stress tolerance. Plant Physiol 143:745–758

Linkies A, Leubner-Metzger G (2012) Beyond gibberellins and abscisic acid: how ethylene and jasmonates control seed germination. Plant Cell Rep 31:253–270

Linkies A, Muller K, Morris K, Tureckova V, Wenk M, Cadman CSC et al (2009) Ethylene interacts with abscisic acid to regulate endosperm rupture during germination: a comparative approach using *Lepidium sativum* and *Arabidopsis thaliana*. Plant Cell 21:3803–3822

Liu YX, Koornneef M, Soppe WJJ (2007) The absence of histone H2B monoubiquitination in the Arabidopsis *hub1* (*rdo4*) mutant reveals a role for chromatin remodeling in seed dormancy. Plant Cell 19:433–444

Liu Y, Geyer R, van Zanten M, Carles A, Li Y, Horold A et al (2011) Identification of the Arabidopsis *REDUCED DORMANCY 2* gene uncovers a role for the polymerase associated factor 1 complex in seed dormancy. Plos One 6:e22241

Long SP, Ort DR (2010) More than taking the heat: crops and global change. Curr Opin Plant Biol 13:241–248

Lopez-Molina L, Mongrand S, Chua NH (2001) A postgermination developmental arrest checkpoint is mediated by abscisic acid and requires the ABI5 transcription factor in Arabidopsis. Proc Natl Acad Sci U S A 98:4782–1787

Lopez-Molina L, Mongrand B, McLachlin DT, Chait BT, Chua NH (2002) ABI5 acts downstream of ABI3 to execute an ABA-dependent growth arrest during germination. Plant J 32:317–328

Ma Y, Szostkiewicz I, Korte A, Moes D, Yang Y, Christmann A et al (2009) Regulators of PP2C phosphatase activity function as abscisic acid sensors. Science 324:1064–1068

Machabee S, Saini HS (1991) Differences in the requirement for endogenous ethylene during germination of dormant and nondormant seeds of *Chenopodium album* L. J Plant Physiol 138:97–101

Marin E, Nussaume L, Quesada A, Gonneau M, Sotta B, Hugueney P et al (1996) Molecular identification of zeaxanthin epoxidase of *Nicotiana plumbaginifolia*, a gene involved in abscisic acid biosynthesis and corresponding to the *ABA* locus of *Arabidopsis thaliana*. EMBO J 15:2331–2342

Martinez-Andújar C, Ordiz MI, Huang Z, Nonogaki M, Beachy RN, Nonogaki H (2011) Induction of 9-cis-epoxycarotenoid dioxygenase in *Arabidopsis thaliana* seeds enhances seed dormancy. Proc Natl Acad Sci U S A 108:17225–17229

Martínez-Andújar C, Pluskota WE, Bassel GW, Asahina M, Pupel P, Nguyen TT et al (2012) Mechanisms of hormonal regulation of endosperm cap-specific gene expression in tomato seeds. Plant J 71:575–586

Matilla AJ, Matilla-Vazquez MA (2008) Involvement of ethylene in seed physiology. Plant Sci 175:87–97

McGinnis KM, Thomas SG, Soule JD, Strader LC, Zale JM, Sun TP et al (2003) The Arabidopsis *SLEEPY1* gene encodes a putative F-box subunit of an SCF E3 ubiquitin ligase. Plant Cell 15:1120–1130

Merchante C, Alonso JM, Stepanova AN (2013) Ethylene signaling: simple ligand, complex regulation. Curr Opin Plant Biol 16:554–560

Mitchum MG, Yamaguchi S, Hanada A, Kuwahara A, Yoshioka Y, Kato T et al (2006) Distinct and overlapping roles of two gibberellin 3-oxidases in Arabidopsis development. Plant J 45:804–818

Mortensen SA, Sonderkaer M, Lynggaard C, Grasser M, Nielsen KL, Grasser KD (2011) Reduced expression of the *DOG1* gene in Arabidopsis mutant seeds lacking the transcript elongation factor TFIIS. FEBS Lett 585:1929–1933

Nakabayashi K, Bartsch M, Xiang Y, Miatton E, Pellengahr S, Yano R et al (2012) The time required for dormancy release in Arabidopsis is determined by *DELAY OF GERMINATION1* protein levels in freshly harvested seeds. Plant Cell 24:2826–2838

Nakajima M, Shimada A, Takashi Y, Kim YC, Park SH, Ueguchi-Tanaka M et al (2006) Identification and characterization of Arabidopsis gibberellin receptors. Plant J 46:880–889

Nakamura S, Abe F, Kawahigashi H, Nakazono K, Tagiri A, Matsumoto T et al (2011) A wheat homolog of *MOTHER OF FT* AND *TFL1* acts in the regulation of germination. Plant Cell 23:3215–3229

Nambara E, Marion-Poll A (2005) Abscisic acid biosynthesis and catabolism. Annu Rev Plant Biol 56:165–185

Nambara E, Okamoto M, Tatematsu K, Yano R, Seo M, Kamiya Y (2010) Abscisic acid and the control of seed dormancy and germination. Seed Sci Res 20:55–67

Nascimento WM, Cantliffe DJ, Huber DJ (2000) Thermotolerance in lettuce seeds: association with ethylene and endo-β-mannanase. J Am Soc Hortic Sci 125:518–524

Nascimento WM, Cantliffe DJ, Huber DJ (2001) Endo-β-mannanase activity and seed germination of thermosensitive and thermotolerant lettuce genotypes in response to seed priming. Seed Sci Res 11:255–264

Nascimento WM, Cantliffe DJ, Huber DJ (2004) Ethylene evolution and endo-β-mannanase activity during lettuce seed germination at high temperature. Sci Agric 61:156–163

Nascimento WM, Vieira JV, Silva GO, Reitsma KR, Cantliffe DJ (2008) Carrot seed germination at high temperature: effect of genotype and association with ethylene production. HortScience 43:1538–1543

Nascimento WM, Huber DJ, Cantliffe DJ (2013) Carrot seed germination and respiration at high temperature in response to seed maturity and priming. Seed Sci Technol 41:164–169

Nonogaki H (2010) MicroRNA gene regulation cascades during early stages of plant development. Plant Cell Physiol 51:1840–1846

Nonogaki H (2014) Seed dormancy and germination—Emerging mechanisms and new hypotheses. Front Plant Science 5:233

Ogawa M, Hanada A, Yamauchi Y, Kuwalhara A, Kamiya Y, Yamaguchi S (2003) Gibberellin biosynthesis and response during Arabidopsis seed germination. Plant Cell 15:1591–1604

Oh E, Yamaguchi S, Hu J, Yusuke J, Jung B, Paik I et al (2007) PIL5, a phytochrome-interacting bHLH protein, regulates gibberellin responsiveness by binding directly to the *GAI* and *RGA* promoters in *Arabidopsis* seeds. Plant Cell 19:1192–1208

Okamoto M, Kuwahara A, Seo M, Kushiro T, Asami T, Hirai N et al (2006) *CYP707A1* and *CYP707A2*, which encode abscisic acid 8′-hydroxylases, are indispensable for proper control of seed dormancy and germination in Arabidopsis. Plant Physiol 141:97–107

Papi M, Sabatini S, Altamura MM, Hennig L, Schafer E, Costantino P et al (2002) Inactivation of the phloem-specific dof zinc finger gene *DAG1* affects response to light and integrity of the testa of Arabidopsis seeds. Plant Physiol 128:411–417

Park SY, Fung P, Nishimura N, Jensen DR, Fujii H, Zhao Y et al (2009) Abscisic acid inhibits type 2C protein phosphatases via the PYR/PYL family of START proteins. Science 324:1068–1071

Penfield S (2008) Temperature perception and signal transduction in plants. New Phytol 179:615–628

Penfield S, MacGregor D (2014) Temperature sensing in plants. In: Franklin KA, Wigge PA (eds) Temperature and plant development. Wiley Blackwell, Oxford, pp 1–18

Petruzzelli L, Coraggio I, Leubner-Metzger G (2000) Ethylene promotes ethylene biosynthesis during pea seed germination by positive feedback regulation of 1-aminocyclopropane-1-carboxylic acid oxidase. Planta 211:144–149

Pinfield-Wells H, Rylott EL, Gilday AD, Graham S, Job K, Larson TR et al (2005) Sucrose rescues seedling establishment but not germination of Arabidopsis mutants disrupted in peroxisomal fatty acid catabolism. Plant J 43:861–872

Piskurewicz U, Jikumaru Y, Kinoshita N, Nambara E, Kamiya Y, Lopez-Molina L (2008) The gibberellic acid signaling repressor RGL2 inhibits Arabidopsis seed germination by stimulating abscisic acid synthesis and ABI5 activity. Plant Cell 20:2729–2745

Probert RJ (1992) The role of temperature in germination ecophysiology. In: Fenner M (ed) The ecology of regeneration in plant communities. CAB International, Melksham, pp 285–325

Qin XQ, Zeevaart JAD (2002) Overexpression of a 9-*cis*-epoxycarotenoid dioxygenase gene in *Nicotiana plumbaginifolia* increases abscisic acid and phaseic acid levels and enhances drought tolerance. Plant Physiol 128:544–551

Qin F, Kodaira KS, Maruyama K, Mizoi J, Tran LSP, Fujita Y et al (2011) SPINDLY, a negative regulator of gibberellic acid signaling, is involved in the plant abiotic stress response. Plant Physiol 157:1900–1913

Rajjou L, Duval M, Gallardo K, Catusse J, Bally J, Job C et al (2012) Seed germination and vigor. Annu Rev Plant Biol 63:507–533

Rizza A, Boccaccini A, Lopez-Vidriero I, Costantino P, Vittorioso P (2011) Inactivation of the *ELIP1* and *ELIP2* genes affects Arabidopsis seed germination. New Phytol 190:896–905

Russell L, Larner V, Kurup S, Bougourd S, Holdsworth M (2000) The Arabidopsis *COMATOSE* locus regulates germination potential. Development 127:3759–3767

Ruyter-Spira C, Al-Babili S, van der Krol S, Bouwmeester H (2013) The biology of strigolactones. Trends Plant Sci 18:72–83

Saatkamp A, Affre L, Dutoit T, Poschlod P (2011) Germination traits explain soil seed persistence across species: the case of Mediterranean annual plants in cereal fields. Ann Bot 107:415–426

Saez A, Apostolova N, Gonzalez-Guzman M, Gonzalez-Garcia MP, Nicolas C, Lorenzo O et al (2004) Gain-of-function and loss-of-function phenotypes of the protein phosphatase 2C HAB1 reveal its role as a negative regulator of abscisic acid signalling. Plant J 37:354–369

Saez A, Robert N, Maktabi MH, Schroeder JI, Serrano R, Rodriguez PL (2006) Enhancement of abscisic acid sensitivity and reduction of water consumption in Arabidopsis by combined inactivation of the protein phosphatases type 2C ABI1 and HAB1. Plant Physiol 141:1389–1399

Saini HS, Consolacion ED, Bassi PK, Spencer MS (1986) Requirement for ethylene synthesis and action during relief of thermoinhibition of lettuce seed germination by combinations of gibberellic acid, kinetin, and carbon dioxide. Plant Physiol 81:950–953

Saini HS, Consolacion ED, Bassi PK, Spencer MS (1989) Control processes in the induction and relief of thermoinhibition of lettuce seed germination: actions of phytochrome and endogenous ethylene. Plant Physiol 90:311–315

Salaita L, Kar RK, Majee M, Downie AB (2005) Identification and characterization of mutants capable of rapid seed germination at 10 degrees C from activation-tagged lines of Arabidopsis thaliana. J Exp Bot 56:2059–2069

Sawada Y, Aoki M, Nakaminami K, Mitsuhashi W, Tatematsu K, Kushiro T et al (2008) Phytochrome- and gibberellin-mediated regulation of abscisic acid metabolism during germination of photoblastic lettuce seeds. Plant Physiol 146:1386–1396

Schwartz SH, Zeevaart JAD (2010) Abscisic acid biosynthesis and metabolism. In: Davies PJ (ed) Plant hormones-biosynthesis, signal transduction, action!, 3rd edn. Springer, Netherlands, pp 137–155

Schwartz SH, Tan BC, Gage DA, Zeevaart JA, McCarty DR (1997) Specific oxidative cleavage of carotenoids by VP14 of maize. Science 276:1872–1874

Schwember AR, Bradford KJ (2010) A genetic locus and gene expression patterns associated with the priming effect on lettuce seed germination at elevated temperatures. Plant Mol Biol 73:105–118

Shukla RK, Tripathi V, Jain D, Yadav RK, Chattopadhyay D (2009) CAP2 enhances germination of transgenic tobacco seeds at high temperature and promotes heat stress tolerance in yeast. FEBS J 276:5252–5262

Siriwitayawan G, Geneve RL, Downie AB (2003) Seed germination of ethylene perception mutants of tomato and Arabidopsis. Seed Sci Res 13:303–314

Small JGC, Schultz C, Cronje E (1993) Relief of thermoinhibition in Grand Rapids lettuce seeds by oxygen plus kinetin and their effects on respiration, content of ethanol and ATP and synthesis of ethylene. Seed Sci Res 3:129–135

Steber CM, Cooney SE, McCourt P (1998) Isolation of the GA-response mutant *sly1* as a suppressor of *ABI1–1* in *Arabidopsis thaliana*. Genetics 149:509–521

Sun TP (2008) Gibberellin metabolism, perception and signaling pathways in Arabidopsis. Arabidopsis Book 6:e0103

Sung Y, Cantliffe DJ, Nagata RT (1998) Seed developmental temperature regulation of thermotolerance in lettuce. J Am Soc Hortic Sci 123:700–705

Tamura N, Yoshida T, Tanaka A, Sasaki R, Bando A, Toh S et al (2006) Isolation and characterization of high temperature-resistant germination mutants of *Arabidopsis thaliana*. Plant Cell Physiol 47:1081–1094

Tan BC, Joseph LM, Deng WT, Liu L, Li QB, Cline K et al (2003) Molecular characterization of the Arabidopsis 9-*cis*-epoxycarotenoid dioxygenase gene family. Plant J 35:44–56
Taylor NJ, Hills PN, Gold JD, Stirk WA, van Staden J (2005) Factors contributing to the regulation of thermoinhibition in *Tagetes minuta* L. J Plant Physiol 162:1270–1279
Toh S, Imamura A, Watanabe A, Nakabayashi K, Okamoto M, Jikumaru Y et al (2008) High temperature-induced abscisic acid biosynthesis and its role in the inhibition of gibberellin action in Arabidopsis seeds. Plant Physiol 146:1368–1385
Toh S, Kamiya Y, Kawakami N, Nambara E, McCourt P, Tsuchiya Y (2012) Thermoinhibition uncovers a role for strigolactones in Arabidopsis seed germination. Plant Cell Physiol 53:107–117
Toyomasu T, Tsuji H, Yamane H, Nakayama M, Yamaguchi I, Murofushi N et al (1993) Light effects on endogenous levels of gibberellins in photoblastic lettuce seeds. J Plant Growth Regul 12:85–90
Toyomasu T, Yamane H, Murofushi N, Inoue Y (1994) Effects of exogenously applied gibberellin and red light on the endogenous levels of abscisic acid in photoblastic lettuce seeds. Plant Cell Physiol 35:127–129
Tuan PA, Park SU (2013) Molecular cloning and characterization of cDNAs encoding carotenoid cleavage dioxygenase in bitter melon (*Momordica charantia*). J Plant Physiol 170:115–120
Tyler L, Thomas SG, Hu JH, Dill A, Alonso JM, Ecker JR et al (2004) DELLA proteins and gibberellin-regulated seed germination and floral development in Arabidopsis. Plant Physiol 135:1008–1019
Umezawa T, Sugiyama N, Mizoguchi M, Hayashi S, Myouga F, Yamaguchi-Shinozaki K et al (2009) Type 2C protein phosphatases directly regulate abscisic acid-activated protein kinases in Arabidopsis. Proc Natl Acad Sci U S A 106:17588–17593
Umezawa T, Nakashima K, Miyakawa T, Kuromori T, Tanokura M, Shinozaki K et al (2010) Molecular basis of the core regulatory network in ABA responses: sensing, signaling and transport. Plant Cell Physiol 51:1821–1839
Valdes VM, Bradford KJ (1987) Effects of seed coating and osmotic priming on the germination of lettuce seeds. J Am Soc Hortic Sci 112:153–156
Wang F, Perry SE (2013) Identification of direct targets of FUSCA3, a key regulator of Arabidopsis seed development. Plant Physiol 161:1251–1264
Wang KLC, Yoshida H, Lurin C, Ecker JR (2004) Regulation of ethylene gas biosynthesis by the Arabidopsis ETO1 protein. Nature 428:945–950
Wang Y, Liu C, Li K, Sun F, Hu H, Li X et al (2007) Arabidopsis EIN2 modulates stress response through abscisic acid response pathway. Plant Mol Biol 64:633–644
Wang Y, Li L, Ye T, Zhao S, Liu Z, Feng YQ et al (2011) Cytokinin antagonizes ABA suppression to seed germination of Arabidopsis by downregulating *ABI5* expression. Plant J 68:249–261
Wasternack C (2007) Jasmonates: an update on biosynthesis, signal transduction and action in plant stress response, growth and development. Ann Bot 100:681–697
Weitbrecht K, Muller K, Leubner-Metzger G (2011) First off the mark: early seed germination. J Exp Bot 62:3289–3309
Wigge PA (2013) Ambient temperature signalling in plants. Curr Opin Plant Biol 16:661–666
Willige BC, Ghosh S, Nill C, Zourelidou M, Dohmann EM, Maier A et al (2007) The DELLA domain of GA INSENSITIVE mediates the interaction with the GA INSENSITIVE DWARF1A gibberellin receptor of Arabidopsis. Plant Cell 19:1209–1220
Xiong L, Ishitani M, Lee H, Zhu JK (2001) The Arabidopsis *LOS5/ABA3* locus encodes a molybdenum cofactor sulfurase and modulates cold stress- and osmotic stress-responsive gene expression. Plant Cell 13:2063–2083
Yamaguchi S, Smith MW, Brown RGS, Kamiya Y, Sun TP (1998) Phytochrome regulation and differential expression of gibberellin 3 β-hydroxylase genes in germinating Arabidopsis seeds. Plant Cell 10:2115–2126
Yamaguchi Y, Kamiya Y, Nambara E (2007) Regulation of ABA and GA levels during seed development and maturation in *Arabidopsis*. In: Bradford KJ, Nonogaki H (eds) Seed development, dormancy and germination. Blackwell, Scientific Publishers, Oxford, pp 224–247

Yamamoto A, Kagaya Y, Usui H, Hobo T, Takeda S, Hattori T (2010) Diverse roles and mechanisms of gene regulation by the Arabidopsis seed maturation master regulator FUS3 revealed by microarray analysis. Plant Cell Physiol 51:2031–2046

Yamauchi Y, Ogawa M, Kuwahara A, Hanada A, Kamiya Y, Yamaguchi S (2004) Activation of gibberellin biosynthesis and response pathways by low temperature during imbibition of *Arabidopsis thaliana* seeds. Plant Cell 16:367–37

Yang SF, Hoffman NE (1984) Ethylene biosynthesis and its regulation in higher plants. Annu Rev Plant Physiol Plant Mol Biol 35:155–189

Yoshioka T, Endo T, Satoh S (1998) Restoration of seed germination at supraoptimal temperatures by fluridone, an inhibitor of abscisic acid biosynthesis. Plant Cell Physiol 39:307–312

Chapter 2
Regulation of Seed Dormancy Cycling in Seasonal Field Environments

W.E. Finch-Savage and S. Footitt

Seeds Select the Habitat, Climate Space and Season in Which to Germinate by Sensing and Responding to the Soil Seed Bank Environment

Seeds are the mobile phase of the plants life cycle; vegetative development is suspended as they transport the plants genetic complement through space and time. In most species this is possible because they can tolerate extreme desiccation and will survive for extended periods in the dry state. However, equally important in the natural world is their ability to exist in an imbibed dormant state, potentially for many years in the soil seed-bank (Finch-Savage and Leubner-Metzger 2006; Footitt et al. 2011). Thus, germination is delayed until they encounter an appropriate habitat, climate space and time of the year suitable for the resulting plant to survive, be competitive and reproduce. This allows multiple species to compete successfully within species-rich natural communities (Baskin and Baskin 1998, 2006; Walck et al. 2011). During their time in the soil seed-bank seeds continually adjust their dormancy status by sensing and integrating a range of environmental signals. The signals related to slow seasonal change can be used for temporal sensing to determine the time of year. In response to these signals seeds alter their depth of dormancy and their sensitivity to other spatial environmental signals. These spatial signals indicate in a more immediate way that conditions are suitable for germination and so trigger the termination of dormancy and therefore induce germination. The response to each of these signals appears to remove successive blocks to germination. However, the process usually needs to be carried out in a set order for it

W.E. Finch-Savage (✉) · S. Footitt
1School of Life Sciences, Wellesbourne Campus, University of Warwick, Warwickshire CV35 9EF, UK
e-mail: Bill.Finch-Savage@warwick.ac.uk

S. Footitt
e-mail: s.footitt@warwick.ac.uk

© Springer International Publishing Switzerland 2015

J. V. Anderson (ed.), *Advances in Plant Dormancy,* DOI 10.1007/978-3-319-14451-1_2

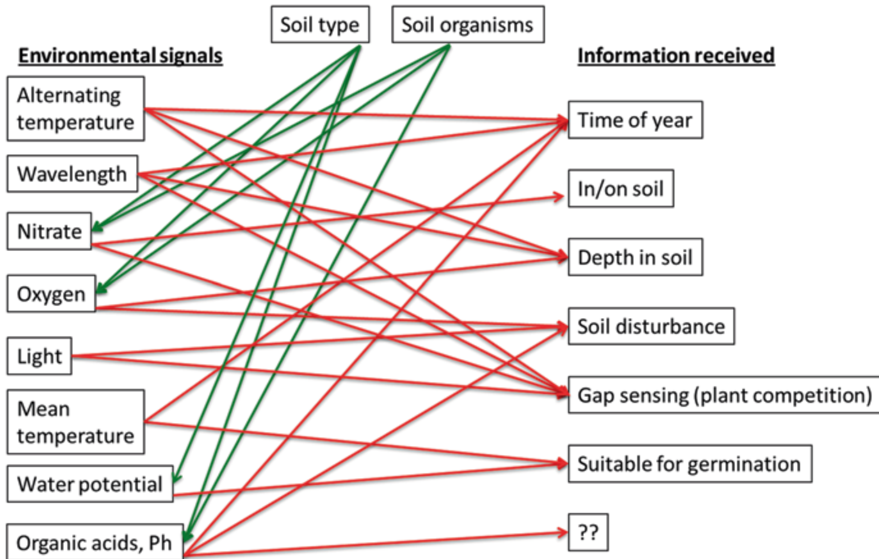


Fig. 2.1 Seeds as environmental sensors: Seeds respond to a wide range of environmental signals that can inform about the time of year (temporal signals) and the suitability of the current environment (spatial signals) for the completion of germination. Seeds are shed onto the soil and become incorporated and so they are also influenced by the physical nature of the soil and the organisms that inhabit it. The figure illustrates the range of environmental signals and how they can potentially inform the seed

to work, that is, spatial signals are only effective if temporal sensing has enhanced sensitivity to them. Thus, a dormancy continuum is proposed that is driven in both directions by environmental signals, and when all layers are removed germination occurs (Finch-Savage and Leubner-Metzger 2006; Finch-Savage and Footitt 2012). In the annual dormancy cycle, if the correct spatial window does not occur sensitivity is lost and the temporal window will close for another year.

A wide range of signals have the potential to inform the seed about its environment (Fig. 2.1). Temperature is the most important signal for temporal sensing (Probert 2000), whereas many signals can be used for spatial sensing to indicate beneficial and adverse conditions for germination, for example, depth in the soil (amplitude of diurnal temperature fluctuation, oxygen, water), soil disturbance (light, oxygen), and vegetation gaps (nitrate, light quality, the degree of diurnal temperature fluctuation) (Baskin and Baskin 2006; Finch-Savage and Leubner-Metzger 2006; Footitt et al. 2011, 2013, 2014). Response to these signals can result in dormancy cycling, which coupled with seed longevity represents a bet-hedging strategy for the short and long-term persistence of native/weed species within the soil seed-bank (Roberts 1964; Evans and Dennehy 2005; Walck et al. 2011; Footitt et al. 2014). Here we will consider the seeds response to temperature as a temporal signal and both light and nitrate as spatial signals.

The underlying sensing and resulting signaling mechanisms to the environment make seeds highly efficient in exploiting distinct habitats and climate spaces (Pons

1989; Saatkamp et al. 2011a, b; Walck et al. 2011). However, the precise response to any environmental signal differs between species, and between ecotypes within species, through adaptation to the habitat and climate space they inhabit. The resulting seasonal seed dormancy cycles and patterns of seedling emergence are well documented as a crucial component of the plants' life cycle that contributes significantly to plant fitness (Donohue 2002; Donohue et al. 2005; Huang et al. 2010; McNamara et al. 2011). It is already recognized that genetic diversity within a species contributes to variation in dormancy and germination phenology; for example in European ecotypes of Arabidopsis (Schmuths et al. 2006; Chiang et al. 2011). As such, dormancy can be seen as contributing to the persistence of genetic diversity (Walck et al. 2011; Lennon and Jones 2011). It is essential that we develop a greater understanding of the mechanisms underlying dormancy cycling and response to environmental signals to determine the impact of future climate change.

Regulation of Dormancy in the Laboratory

Despite the obvious importance of dormancy cycling in the whole life cycle of plants, very little is known about its regulation at a molecular level. In contrast, a great deal is known about mechanisms that influence dormancy loss in short-term laboratory experiments, many of which involve the screening of mutants for altered dormancy and germination (Finch-Savage and Leubner-Metzger 2006; Baskin and Baskin 1998; Finkelstein et al. 2008; Nambara et al. 2010; Graeber et al. 2012). This laboratory-based work has largely used seeds from accessions of the model species Arabidopsis that naturally have limited dormancy. In addition, the seeds used for study have been produced under optimal conditions that tend to minimize dormancy (Kendall et al. 2011). Many of the genes identified have subsequently been found to be involved in the abscisic acid (ABA) and gibberellin (GA) metabolism and signaling pathways (Kucera et al. 2005; Graeber et al. 2012). This has confirmed the central involvement of the ABA/GA balance hypothesis in the seeds ability to interpret the environment and thereby regulate dormancy and germination (Finch-Savage and Leubner-Metzger 2006; Kucera et al. 2005).

Most often, these genes/mechanisms have, for good scientific reasons, been considered in isolation, in constant and therefore simple environments. From these experiments, it is not obvious why so many different mechanisms are required and there is an apparent duplication of function and redundancy. However, in nature seeds have to operate in the complex and variable conditions of the soil seed bank that may require a complexity of subtle dormancy regulation to interpret these conditions. How this complex set of mechanisms is employed by the seed in a coordinated way to regulate dormancy cycling in variable field environments is little understood and until recently unstudied. Our approach to this has been to investigate the molecular ecophysiology of dormancy cycling in field soils using the inherently deeply dormant Arabidopsis ecotype Cape Verdi Isle (Cvi). A key feature in selecting Cvi is that it required exposure to light to remove the final layer of dormancy to allow completion of germination. This absolute requirement for light is important

Table 2.1 Genes studied in the work reported

Gene ID	Annotation
At5g57050	*ABA INSENSITIVE2 (ABI2)*
At3g24650	*ABA INSENSITIVE3 (ABI3)*
At2g40220	*ABA INSENSITIVE4 (ABI4)*
At2g36270	*ABA INSENSITIVE5 (ABI5)*
At2g29090	*CYTOCHROME P450, FAMILY 707, SUBFAMILY A, POLYPEPTIDE 2 (CYP707A2)*
At5g45830	*DELAY OF GERMINATION 1 (DOG1)*
At1g30040	*GIBBERELLIN 2-OXIDASE 2 (GA2OX2)*
At1g15550	*GIBBERELLIN 3 BETA-HYDROXYLASE 1 (GA3OX1)*
At3g05120	*GIBBERELLIN-INSENSITIVE DWARF1 (GID1A)*
At1g18100	*MOTHER OF FLOWERING TIME (MFT)*
At3g24220	*NINE-CIS-EPOXYCAROTENOID DIOXYGENASE6 (NCED6)*
At1g12110	*NITRATE TRANSPORTER 1 (NRT 1.1)*
At2g20180	*PHYTOCHROME INTERACTING FACTOR 3-LIKE 5 (PIL5)*
At4g01026	*ABSCISIC ACID RECEPTOR (PYL7)*
At4g17870	*ABSCISIC ACID RECEPTOR (PYR1)*
At1g14920	*RESTORATION ON GROWTH ON AMMONIA 2 (RGA2)*
At3g03450	*RGA-LIKE 2 (RGL2)*
At5g08590	*SNF1-RELATED PROTEIN KINASE 2.1 (SNRK 2.1)*
At1g10940	*SNF1-RELATED PROTEIN KINASE 2.4 (SNAR 2.4)*
At4g36930	*SPATULA (SPT)*
At1g30270	*CBL-INTERACTING PROTEIN KINASE 23 (CIPK23)*
At1g09570	*PHYTOCHROME A (PHYA)*

experimentally as it allows the separate study of changes in dormancy from downstream changes resulting from the germination process. Our aim was to illustrate how molecular mechanisms identified as controlling dormancy in the laboratory could be seasonally coordinated in seeds buried in field soil to fulfill this process (Footitt et al. 2011). We approached this through gene expression studies targeted at key dormancy regulating genes identified in the laboratory studies described above. We had previously studied the relative importance of these genes for dormancy cycling using full genome arrays of laboratory derived samples that built up the components of dormancy cycling (Cadman et al. 2006; Finch-Savage et al. 2007). We built the study around the dynamic ABA/GA balance and the cohorts of genes that regulate their metabolism, perception, and sensitivity via signaling networks considered central to dormancy and the control of germination completion (radical emergence through the seed coat) (Finch-Savage and Leubner-Metzger 2006; Linkies et al. 2009; Nambara et al. 2010; Bassel et al. 2011; Morris et al. 2011; Dekkers et al. 2013). The genes studied (Table 2.1) and their involvement in the regulation of dormancy are summarized in Fig. 2.2 and the remainder of this section:

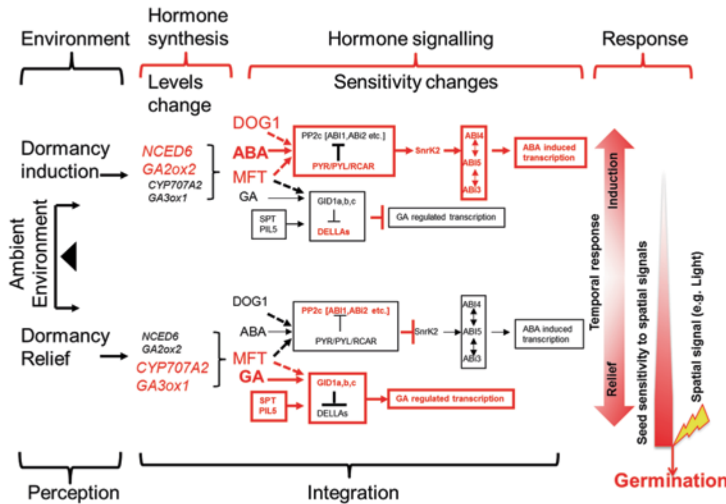


Fig. 2.2 Schematic model for the regulation of dormancy and germination by ABA and GA in response to the environment in Arabidopsis ecotype Cvi: According to this model ambient environmental signals (e.g., temperature, light and nitrate) affect the ABA/GA balance and the sensitivity to these hormones. ABA synthesis and signaling and GA catabolism dominate the induction and deepening of the dormant state, whereas GA synthesis and signaling and ABA catabolism dominate the relief of dormancy and the transition to germination. In the model, when induction or relief are induced by appropriate environmental signals the pathways indicated in red dominate. Change in the depth of dormancy in response to temporal signals alters the requirements for germination (sensitivity to spatial signals, that is, the germination environment); when these signals are perceived in the correct order, all levels of dormancy are removed and germination will proceed to completion. (Model is adapted from Finch-Savage and Leubner-Metzger 2006 and Footitt et al. 2011. Background description to these genes is given in the text)

Hormone Metabolism

GA Active GA levels increase just before radical emergence, suggesting they play a key role in the regulation of germination. The key stages in GA metabolism are now well known (Ogawa et al. 2003). GA3-oxidase is the key enzyme responsible for the final step in GA biosynthesis to produce active GAs. Subsequent degradation is via GA2-oxidase.

ABA Key genes responsible for ABA biosynthesis, degradation, and conjugation during Arabidopsis seed germination are also known and have been described (e.g., Penfield et al. 2006; Okamoto et al. 2006; Holdsworth et al. 2008; Muller et al. 2006; Piskurewicz et al. 2008). NCEDs (Nine-cis-epoxycarotenoid dioxygenases) are the primary regulatory step in ABA synthesis and subsequent inactivation is by hydroxylation (CYP707) or through conjugation with sugars (Nambara et al. 2010). The balance of these processes regulates ABA content. The influence of other hor-

mones, such as ethylene (Linkies et al. 2009), can be significant in the regulation of dormancy and germination, but in general their influence operates through the ABA/GA balance.

Hormone Signaling

Hormone signaling, through repression and de-repression, is a key component of the interacting networks regulating germination (Holdsworth et al. 2008; Kucera et al. 2005). It is now thought that on the ABA side of the balance, the ABA receptors PYR/PYL/RCAR bind to ABA to remove the repression of responses to the hormone by PP2cs (Protein phosphatase 2C; Cutler et al. 2010; Nambara et al. 2010). Removal of PP2c repression allows downstream signaling via SnRK2s to ABRE (ABA-response element) binding transcription factors (ABI3, ABI4, ABI5). On the other side of this balance, DELLA proteins (RGL2, RGA2) repress GA responses and therefore germination potential (Sun and Gubler 2004). DELLAs are degraded to remove this repression on forming a complex with the GA receptor GID1 in the presence of GA (Hartweck 2008).

Environmental Signals and Upstream Regulation

As described above, a diverse range of environmental signals, principally temperature and light, influences these hormone-signaling pathways. Key components of the interaction between these environmental signals and GA are the two phytochrome-interacting bHLH transcription factors, PIL5 and SPT. These repress germination potential, in the dark and at low temperature, respectively. PIL5 represses cell wall modifying genes, *GA3ox1* and *CYP707A2*, and enhances *GA2ox1*, *NCED6*, and *DELLA* expression, while *SPT* represses *GA3ox1* expression (Penfield et al. 2005; Ho et al. 2009). In turn, PIL5 and SPT are inactivated by DELLAs (RGL2 and RGA2) (Penfield et al. 2005). PIF (phytochrome interacting factor) proteins are released when the GID protein–GA complex binds DELLA proteins to target their degradation by the proteosome (Daviere et al. 2008).

Delay of germination 1 (DOG1) is a key regulator of dormancy (Bentsink et al. 2006) and is also closely linked to the impact of temperature on dormancy status (Footitt et al. 2013 etc.) and is thought to alter sensitivity to ABA (Teng et al. 2008). Similarly, mother of flowering time (MFT) is a proposed ABA-induced negative regulator of ABA signaling and is thought to operate as the convergence point of ABA and GA signaling pathways (Xi et al. 2010). Nakamura et al. 2011 reports that MFT expression is regulated in response to temperature and seems to transmit temperature signals to a downstream temperature-signaling cascade to regulate depth of dormancy.

Nitrate is also an important environmental signal in the soil seed bank. Seed dormancy can be released by nitrate in Arabidopsis, but it is not clear whether ni-

trate acts *per se* on seed germination or through the production of N-related signals (Alboresi et al. 2005). However, nitrate accelerates the decrease in ABA prior to completion of germination (Ali-Rachedi et al. 2004) via induction of the catabolic ABA gene *CYP707A2* (Matakiadis et al. 2009).

We have argued (Footitt et al. 2013) that the response of seeds to nitrate in the soil seed bank appears to act via CIPK23 phosphorylation/dephosphorylation of NRT1.1 and the response to light acts via PHYA. PHYA targets ABRE containing promoters and could be involved in the ABA signaling response (Chen et al. 2014).

Regulation of Dormancy Cycling in the Field Soil Seed Bank

The Arabidopsis ecotype Cvi exhibits the life cycle of a winter annual, by germinating in autumn and overwintering as a seedling rosette to produce dormant seeds in late spring that use the warmth of summer to relieve dormancy. Seeds that do not get exposed to environmental conditions that remove the final layer of dormancy and therefore induce germination completion enter a dormancy cycle and over winter as a seed. We studied the seeds' response to the soil seed bank conditions following sowing in late spring (May; Footitt et al. 2014) and autumn (October; Footitt et al. 2011, 2013). Seeds from the same harvest were used for both sowings and these were processed and stored at −80 °C to minimize physiological change. The seeds were buried under the soil surface in mesh bags and exhumed at intervals (methods described in Footitt et al. 2011) for both physiological analysis and measurement of gene expression by quantitative PCR or Nanostring technology (Footitt et al. 2011, 2013, 2014). Seed samples for the latter were exhumed and prepared in the dark.

Seeds that were sown in spring at their natural time of shedding entered a shallow dormancy cycle dominated by spatial sensing that adjusted germination potential to the maximum when soil environment was most favorable for germination and seedling emergence upon soil disturbance (Footitt et al. 2014). This behavior differed subtly from that of seeds sown in autumn and overwintered in the soil seed bank (Footitt et al. 2011, 2013) and this difference spreads the period of potential germination in the seed population (existing seed bank and newly dispersed). As soil temperature declined in autumn, seeds denied conditions required to remove all layers of dormancy and therefore germination completion (e.g., light) entered deep dormancy and the process of dormancy cycling. These spring-sown seeds then become part of the persistent seed bank. Seeds that were sown in autumn represent this cohort of the soil seed population and it is the behavior of these seeds we describe below.

Seed behavior was monitored over a complete year following sowing, and throughout soil temperature and moisture content at sowing depth were monitored. A clear seasonal temperature pattern was recorded (Fig. 2.3a) with temperature declining in winter, rising in spring, peaking in summer, and then declining toward autumn. The depth of seed dormancy, estimated as the afterripening time required

to achieve 50 % germination at 20 °C (AR50), was negatively correlated ($P < 0.001$) with this temperature pattern (Fig. 2.3a). The depth of dormancy, which was already significant, rose sharply in the imbibed seeds following sowing as temperature decreased going into winter and then declined equally quickly in spring. Interestingly, AR50 declined from more than 150 days to less than 50 days in less than one month in the moist warming soils. By mid-July seeds required only exposure to light to remove the final layer of dormancy allowing seeds to proceed to germination completion (radicle emergence). Germination potential, measured by exposing exhumed seed to light, reached a peak in all temperatures tested at this time (Fig. 2.3f). Consequently, in a parallel experiment where seeds sown in the surface layer of soil were disturbed regularly, there was a flush of seedling emergence in early August following a period of germination and pre-emergence seedling growth (Fig. 2.3f).

Over this annual cycle we followed the expression of the genes described above to shed light on the regulation of dormancy cycling and the deployment of dormancy mechanisms identified in the laboratory. Figures 2.3b–e illustrates the patterns of expression we found in key genes. Both *DOG1* and *MFT* had expression patterns that were, like depth of dormancy, negatively ($P < 0.001$) correlated with soil temperature (Fig. 2.3b). *DOG1* is not directly associated with ABA, but is altered by environmental conditions (Chiang et al. 2011) and may enhance ABA sensitivity (Teng et al. 2008). ABA concentration is linked to dormancy level in Cvi (Al-Rachedi et al. 2004), but we did not find a positive relationship between increasing AR50 and ABA concentration. Indeed, ABA concentration increased with AR50 to *c.* 50 days, but then reached a plateau as AR50 continued to increase (Footitt et al. 2011). In contrast, *DOG1* expression had a positive relationship with AR50 up to the highest recorded AR50 of *c.* 200 days. Footitt et al. (2011) suggested that ABA concentration is important to the control of dormancy as seen in the laboratory, but as deep dormancy is induced in the field, *DOG1* expression may be the dominant factor enhancing ABA sensitivity. They also suggest that MFT, as an ABA-induced germination repressor (Nakamura et al. 2011), also functions in this aspect of dormancy regulation.

On the other side of the hormone balance, the expression pattern of *GA3ox* repressors *SPT* and *PIL5* was positively correlated with temperature and therefore negatively correlated with depth of dormancy, but this correlation was only significant for SPT ($P < 0.01$; Fig. 2.3c). Their expression tended to peak when germination potential was highest. Similarly, expression of the *DELLA* genes *RGA2* and *RGL2* was low over winter (both negatively correlated with temperature ($P < 0.01$)) and increased to a peak as dormancy was lowest ($P < 0.01$) and germination potential peaked (Fig. 2.3d). At first sight, this coincidence of high germination potential and peak expression of germination repressors appears counterintuitive. However, it must be remembered that these seeds are still dormant in the soil and germination must not occur until they are exposed to the correct spatial signal (e.g., light) to indicate soil disturbance/absence of plant competition. Nevertheless, on exposure to light the response must be rapid so that germination completion and seedling emergence can take place while conditions are suitable. Thus, in winter, deep dormancy determined by sensitivity to ABA prevents any response to light. In contrast, during

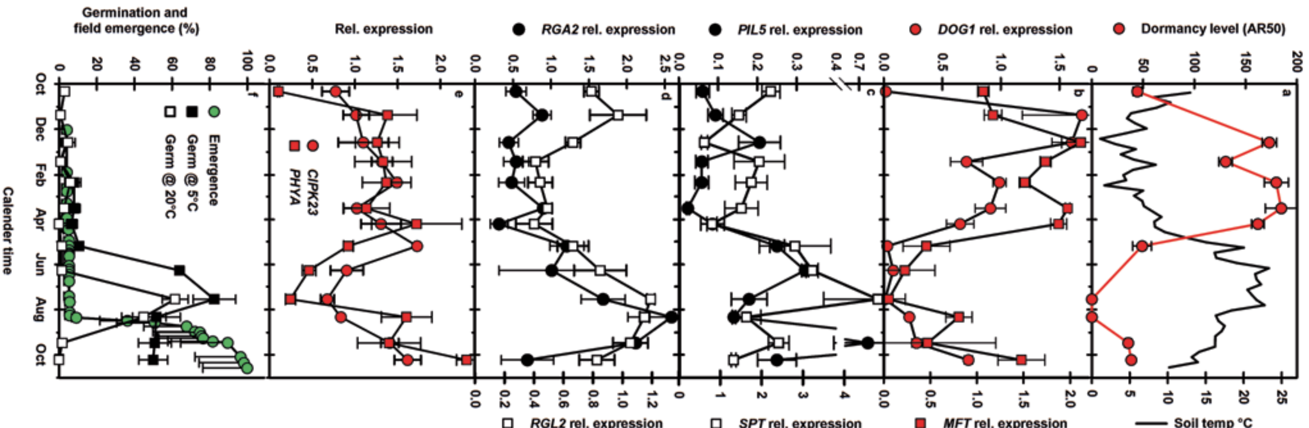


Fig. 2.3 Dormancy cycling in the soil seed bank: Changes in Arabidopsis Cvi seeds following sowing into the soil seed bank are shown. Gene expression is measured in seeds that have been flash frozen at − 80 °C immediately after exhumation in the dark. The seeds are not exposed to light until placed in germination experiments for recording germination potential following soil disturbance. **a** The patterns of mean soil temperature at sowing depth and depth of seed dormancy measured as AR50 (length of after-ripening at 20 °C that enables 50 % of the population to germinate at 20 °C). Gene expression is shown for **b** *MFT* and *DOG1*, **c** *PIL5* and *SPT*, **d** *RGA2* and *RGL2*, **e** *CIPK23* and *PHYA*. f Potential germination at 5 and 20°C and seedling emergence recorded in adjacent experimental field plots that were disturbed at regular intervals to expose the buried seeds to light. Error bars indicate SEM; $n = 3$. (The figure is adapted from Footitt et al. 2011, 2013)

summer when shallow dormancy results from GA synthesis and signaling repression, seeds are sensitive to light. In the latter case, exposure to light dramatically enhances expression of *GA3ox* (Cadman et al. 2006), resulting in synthesis of GA that binds to DELLAs removing repression of GA signaling. A temporal separation of ABA- and GA-related dormancy mechanisms is revealed, which allows accurate timing of germination completion through dormancy cycling in a seasonal environment. Throughout this cycle, the expression of other genes related to hormone synthesis and signaling were consistent with the operation of the hormone balance described in Fig. 2.2 (Footitt et al. 2011, 2013, 2014).

Temporal sensing, therefore, facilitates the completion of germination to occur at the optimum time of year. However, as discussed above this should only take place if environmental conditions are suitable as indicated by a range of spatial signals (Fig. 2.1) of which light and nitrate have received most attention and are potentially the most important (discussed in Footitt et al. 2013). The response to temporal (seasonal) signals is, therefore, to alter sensitivity to these spatial signals. Footitt et al. (2013) argue that the response to nitrate appears to act via *CIPK23* phosphorylation/dephosphorylation of *NRT1.1*. NRT1.1 is a duel affinity nitrate transporter with the low- or high-affinity function dependent upon the phosphorylation status of threonine-101 (Ho et al. 2009) and is considered to be a nutrient transceptor (duel nutrient transport/signaling function; Gojon et al. 2011). Potentially the signaling and transport function of NRT1.1 may be uncoupled through the action of CIPK23 to reduce sensitivity to nitrate and enhance dormancy (Footitt et al. 2013). They further argue from dormancy-associated expression patterns (Cadman et al. 2006; Finch-Savage et al. 2007) that the response to light is determined via PHYA. *PHYA* expression was negatively correlated with *GA3ox1* expression (Footitt et al. 2013) consistent with the reports of reduced *GA3ox1* expression and GA levels when *PHYA* is overexpressed (Jordan et al. 1995; Foo et al. 2006). Expression patterns of *PHYA* and *CIPK23* were similar being higher in winter, lower in summer, and reaching a minimum in July when germination potential peaked (Fig. 2.3e, f). Thus, in the field when this temporal and spatial sensing overlapped with ambient environmental conditions, dormancy was removed and seeds progressed to germination completion and seedling emergence.

In this work, we were able to show the temporal coordination of the major signaling networks identified in the laboratory that regulate seed dormancy in an ecological context in the field. This highlighted that seeds in the seed bank are capable of adjusting the depth of dormancy through temporal sensing (identifying the correct season and climate space for emergence) and spatial sensing (identifying signals indicating suitable conditions to terminate dormancy and complete germination). Dormancy and the expression of dormancy-related genes were highly sensitive to the soil environment, and molecular and physiological changes could be equated to changes in sensitivity to soil temperature history, nitrate, light, and gibberellins. This illustrates dormancy as a continuum with layers of dormancy being progressively removed by environmental signals until only light is required, in the absence of which seeds remain dormant and enter into another dormancy cycle as the seasons change (Footitt et al. 2011, 2013, 2014; Finch-Savage and Footitt 2012).

References

Alboresi A, Gestin C, Leydecker MT, Bedu M, Meyer C, Truong HN (2005) Nitrate, a signal relieving seed dormancy in Arabidopsis. Plant Cell Environ 28:500–512

Ali-Rachedi S, Bouinot D, Wagner MH, Bonnet M, Sotta B, Grappin P, Jullien M (2004) Changes in endogenous abscisic acid levels during dormancy release and maintenance of mature seeds: studies with the Cape Verde Islands ecotype, the dormant model of Arabidopsis thaliana. Planta 219:479–488

Baskin CC, Baskin JM (1998) Seeds—ecology, biogeography, and evolution of dormancy and germination. Academic Press, London, p. 666

Baskin CC, Baskin JM (2006) The natural history of soil seed banks of arable land. Weed Sci 54:549–557

Bassel GW, Lan H, Glaab E, Gibbs DJ, Gerjets T, Krasnogor N, Bonner AJ, Holdsworth MJ, Provart NJ (2011) Genome-wide network model capturing seed germination reveals coordinated regulation of plant cellular phase transitions. Proc Natl Acad Sci U S A 108:9709–9714

Bentsink L, Jowett J, Hanhart CJ, Koornneef M (2006) Cloning of DOG1, a quantitative trait locus controlling seed dormancy in Arabidopsis. Proc Natl Acad Sci U S A 103:17042–17047

Cadman CSC, Toorop PE, Hilhorst HWM, Finch-Savage WE (2006) Gene expression profiles of Arabidopsis Cvi seeds during dormancy cycling indicate a common underlying dormancy control mechanism. Plant J 46:805–822

Chen F, Li B, Li G, Charron J-B, Bai M, Shi X, Deng XW (2014) Arabidopsis phytochrome A directly targets numerous promoters for individualized modulation of genes in a wide range of pathways. Plant Cell 26:1949–1966

Chiang GCK, Bartsch M, Barua D, Nakabayashi K, Debieu M, Kronholm I, Koornneef M, Soppe WJJ, Donohue K, de Meaux J (2011) DOG1 expression is predicted by the seed-maturation environment and contributes to geographical variation in germination in Arabidopsis thaliana. Mol Ecol 20:3336–3349

Cutler SR, Rodriguez PL, Finkelstein RR, Abrams SR (2010) Abscisic acid: emergence of a core signaling network. Ann Rev Plant Biol 61:651–679

Daviere JM, de Lucas M, Prat S (2008) Transcriptional factor interaction: a central step in DELLA function. Curr Opin Genet Dev 18:295–303

Dekkers BJW, Pearce S, van Bolderen-Veldkamp RP, Marshall A, Widera P, Gilbert J, Drost HG, Bassel GW, Muller K, King JR, Wood ATA, Grosse I, Quint M, Krasnogor N, Leubner-Metzger G, Holdsworth MJ, Bentsink L (2013) Transcriptional dynamics of two seed compartments with opposing roles in Arabidopsis seed germination. Plant Physiol 163:205–215

Donohue K (2002) Germination timing influences natural selection on life-history characters in Arabidopsis thaliana. Ecology 83:1006–1016

Donohue K, Dorn L, Griffith C, Kim E, Aguilera A, Polisetty CR, Schmitt J (2005) Environmental and genetic influences on the germination of Arabidopsis thaliana in the field. Evolution 59:740–757

Evans MEK, Dennehy JJ (2005) Germ banking: bet-hedging and variable release from egg and seed dormancy. Q Rev Bio 80:431–451

Finch-Savage WE, Footitt S (2012) To germinate or not to germinate: a question of dormancy relief not germination stimulation. Seed Sci Res 22:243–248

Finch-Savage WE, Leubner-Metzger G (2006) Seed dormancy and the control of germination. New Phytol 171:501–523

Finch-Savage WE, Cadman CSC, Toorop PE, Lynn JR, Hilhorst HWM (2007) Seed dormancy release in Arabidopsis Cvi by dry after-ripening, low temperature, nitrate and light shows common quantitative patterns of gene expression directed by environmentally specific sensing. Plant J 51:60–78

Finkelstein R, Reeves W, Ariizumi T, Steber C (2008) Molecular aspects of seed dormancy. Ann Rev Plant Biol 59:387–415

Foo E, Platten JD, Weller JL, Reid JB (2006) PhyA and cry1 act redundantly to regulate gibberellin levels during de-etiolation in blue light. Physiol Plant 127:149–156
Footitt S, Douterelo-Soler I, Clay H, Finch-Savage WE (2011) Dormancy cycling in Arabidopsis seeds is controlled by seasonally distinct hormone signalling pathways. Proc Natl Acad Sci U S A 108:20236–20241
Footitt S, Huang Z, Clay H, Mead A, Finch-Savage WE (2013) Temperature, light and nitrate sensing coordinate Arabidopsis seed dormancy cycling resulting in winter and summer annual phenotypes. Plant J 74:1003–1115
Footitt S, Clay H, Dent K, Finch-Savage WE (2014) Environment sensing in spring-dispersed seeds of a winter annual Arabidopsis influences the regulation of dormancy to align germination potential with seasonal changes. New Phytol 202:929–939
Gojon A, Krouk G, Perrine-Walker F, Laugier E (2011) Nitrate transceptor(s) in plants. J Exp Bot 62:2299–2308
Graeber K, Nakabayashi K, Miatton E, Leubner-Metzger G, Soppe WJJ (2012) Molecular mechanisms of seed dormancy. Plant Cell Environ 35:1769–1786
Hartweck LM (2008) Gibberellin signaling. Planta 229:1–13
Ho C-H, Lin S-H, Hu H-C, Tsay Y-F (2009) CHL1 Functions as a nitrate sensor in plants. Cell 138:1184–1194
Holdsworth MJ, Bentsink L, Soppe WJJ (2008) Molecular networks regulating Arabidopsis seed maturation, after-ripening, dormancy and germination. New Phytol 179:33–54
Huang XQ, Schmitt J, Dorn L, Griffith C, Effgen S, Takao S, Koornneef M, Donohue K (2010) The earliest stages of adaptation in an experimental plant population: strong selection on QTLS for seed dormancy. Mol Ecol 19:1335–1351
Jordan ET, Hatfield PM, Hondred D, Talon M, Zeevaart JAD, Vierstra RD (1995) Phytochrome-A overexpression in transgenic tobacco—correlation of dwarf phenotype with high-concentrations of phytochrome in vascular tissue and attenuated gibberellin levels. Plant Physiol 107:797–805
Kendall SL, Hellwege A, Marrio P, Whalley C, Graham IA, Penfield S (2011) Induction of dormancy in Arabidopsis summer annuals requires parallel regulation of DOG1 and hormone metabolism by low temperature and CBF transcription factors. Plant Cell 23:2568–2580
Kucera B, Cohn MA, Leubner-Metzger G (2005) Plant hormone interactions during seed dormancy release and germination. Seed Sci Res 15:281–307
Lennon JT, Jones SE (2011) Microbial seed banks: the ecological and evolutionary implications of dormancy. Nat Rev Microbiol 9:119–130
Linkies A, Müller K, Morris K, Tureèková V, Wenk M, Cadman CSC, Corbineau F, Strnad M, Lynn JR, Finch-Savage WE, Leubner G (2009) Ethylene interacts with abscisic acid to control germination by regulating endosperm rupture: a comparative brassicaceae approach using Lepidium sativum (cress) and Arabidopsis thaliana. Plant Cell 21:3803–3822
Matakiadis T, Alboresi A, Jikumaru Y, Tatematsu K, Pichon O, Renou JP, Kamiya Y, Nambara E, Truong HN (2009) The Arabidopsis abscisic acid catabolic gene CYP707A2 plays a key role in nitrate control of seed dormancy. Plant Physiol 149:949–960
McNamara JM, Barta Z, Klaassen M, Bauer S (2011) Cues and the optimal timing of activities under environmental changes. Ecol Lett 14:183–1190
Morris K, Linkies A, Müller K, Oracz K, Wang X, Lynn JR, Leubner-Metzger G, Finch-Savage WE (2011) Regulation of seed germination in the close Arabidopsis relative Lepidium sativum (cress): a global tissue specific transcript analysis. Plant Physiol 155:1851–1870
Muller K, Tintelnot S, Leubner-Metzger G (2006) Endosperm-limited Brassicaceae seed germination: abscisic acid inhibits embryo-induced endosperm weakening of Lepidium sativum (cress) and endosperm rupture of cress and Arabidopsis thaliana. Plant Cell Physiol 47:864–877
Nakabayashi K, Bartscha M, Xianga Y, Miattona E, Pellengahra S, Yanob R, Seob M, Soppe W (2012) The time required for dormancy release in Arabidopsis is determined by DELAY OF GERMINATION1 protein levels in freshly harvested seeds. Plant Cell 24:2826–2838
Nakamura S, Abe F, Kawahigashi H, Nakazono K, Tagiri A, Matsumoto T, Utsugi S, Ogawa T, Handa H, Ishida H, Mori M, Kawaura K, Ogihara Y, Miura HA (2011) Wheat Homolog of MOTHER OF FT AND TFL1 Acts in the regulation of germination. Plant Cell 23:3215–3229

Nambara E, Okamoto M, Tatematsu K, Yano R, Seo M, Kamiya Y (2010) Abscisic acid and the control of seed dormancy and germination. Seed Sci Res 20:55–67

Ogawa M, Hanada A, Yamauchi Y, Kuwahara A, Kamiya Y, Yamaguchi S (2003) Gibberellin biosynthesis and response during Arabidopsis seed germination. Plant Cell 15:1591–1604

Okamoto M, Kuwahara A, Seo M, Kushiro T, Asami T, Hirai N, Kamiya Y, Koshiba T, Nambara E (2006) CYP707A1 and CYP707A2, which encode abscisic acid 8′-hydroxylases, are indispensable for proper control of seed dormancy and germination in Arabidopsis. Plant Physiol 141:97–107

Penfield S, Josse E-M, Kannangara R, Gilday AD, Halliday KJ, Graham IA (2005) Cold and light control seed germination through the bHLH transcription factor SPATULA. Curr Biol 15:1998–2006

Penfield S, Li Y, Gilday AD, Graham S, Graham IA (2006) Arabidopsis ABA INSENSITIVE4 regulates lipid mobilization in the embryo and reveals repression of seed germination by the endosperm. Plant Cell 18:1887–1899

Piskurewicz U, Jikumaru Y, Kinoshita N, Nambara E, Kamiya Y, Lopez-Molina L (2008) The Gibberellic acid signaling repressor RGL2 inhibits Arabidopsis seed germination by stimulating Abscisic Acid Synthesis and ABI5 activity. Plant Cell 20:2729–2745

Pons TL (1989) Breaking of seed dormancy by nitrate as a gap detection mechanism. Ann Bot-London 63:139–143

Probert RJ (2000) The role of temperature in the regulation of seed dormancy and germination. In: Fenner M (ed) Seeds: the ecology of regeneration in plant communities, 2nd edn, CABI, Wallingford, pp. 261-292

Roberts HA (1964) Emergence and longevity in cultivated soil of seeds of some annual weeds. Weed Res 4:296–307

Saatkamp A, Affre L, Dutoit T, Poschlod P (2011a) Germination traits explain soil seed persistence across species: the case of Mediterranean annual plants in cereal fields. Ann Bot 107:415–426

Saatkamp A, Affre L, Baumberger T, Dumas PJ, Gasmi A et al (2011b) Soil depth detection by seeds and diurnally fluctuating temperatures: different dynamics in 10 annual plants. Plant Soil 349:331–340

Schmuths H, Bachmann K, Weber WE, Horres R, Hoffmann MH (2006) Effects of preconditioning and temperature during germination of 73 natural accessions of Arabidopsis thaliana. Ann Bot-London 97:623–634

Sun TP, Gubler F (2004) Molecular mechanism of gibberellin signaling in plants. Ann Rev Plant Biol 55:197–223

Teng S, Rognoni S, Bentsink L, Smeekens S (2008) The Arabidopsis GSQ5/DOG1 Cvi allele is induced by the ABA-mediated sugar signalling pathway, and enhances sugar sensitivity by stimulating ABI4 expression. Plant J 55:372–381

Walck JL, Hidayati SN, Dixon KW, Thompson K, Poschlod P (2011) Climate change and plant regeneration from seed. Glob Change Biol 17:2145–2161

Xi WY, Liu C, Hou XL, Yu H (2010) MOTHER OF FT AND TFL1 regulates seed germination through a negative feedback loop modulating ABA signaling in Arabidopsis. Plant Cell 22:1733–1748

Part II
Bud Dormancy, Reviews, Research, and Perspectives

Chapter 3
Role of the Circadian Clock in Cold Acclimation and Winter Dormancy in Perennial Plants

Mikael Johansson, José M. Ramos-Sánchez, Daniel Conde, Cristian Ibáñez, Naoki Takata, Isabel Allona and Maria E. Eriksson

Abbreviations

LL	Continuous light
CDL	Critical daylength
EC	Evening complex

I. Allona (✉) · J. M. Ramos-Sánchez · D. Conde
Biotechnology and Plant Biology, Centre for Plant Biotechnology and Genomics (CBGP/UPM-NIA), Universidad Politécnica de Madrid, Campus de Montegancedo, 28223 Pozuelo de Alarcón, Madrid, Spain
e-mail: isabel.allona@upm.es

M. E. Eriksson (✉)
Plant Physiology, Umeå University, Umeå Plant Science Centre, 901 87 Umeå, Sweden
e-mail: maria.eriksson@umu.se

M. Johansson
Molecular Cell Physiology, Bielefeld University, Universitaetsstrasse 25, 33615 Bielefeld, Germany
e-mail: mikael.johansson@uni-bielefeld.de

J. M. Ramos-Sánchez
e-mail: jm.ramos@upm.es

D. Conde
e-mail: daniel.conde@upm.es

C. Ibáñez
Departamento de Biología, Universidad de La Serena, Campus Andrés Bello. Avenida Raúl Bitrán n° 1305, 1722005 La Serena, Coquimbo, Chile
e-mail: cibanez@userena.cl

N. Takata
Forest Bio-Research Center, Forestry and Forest Products Research Institute, 3809-1 Ishi, Juo, Hitachi, Ibaraki, 319-1301 Japan
e-mail: naokitakata@affrc.go.jp

© Springer International Publishing Switzerland 2015

J. V. Anderson (ed.), *Advances in Plant Dormancy,* DOI 10.1007/978-3-319-14451-1_3

LD Long day
QTL Quantitative trait loci
SD Short day
TTFL Transcriptional–translational feedback loops
ZT Zeitgeber time

Introduction

During the yearly rotation of the Earth around the sun, the earth's spin axis is tilted with respect to its orbital plane. This is what causes the seasons. When the earth's axis points toward the sun, it is summer for that hemisphere. When the earth's axis points away, winter can be expected, producing variation in photoperiod and creating different environmental seasons. This predictable pattern of photoperiodic variation is a strong cue directing the growth and development of plants. It is strongest for plants growing at temperate to boreal climes but also affects those at tropical latitudes (Thomas and Vince-Prue 1997; Borchert et al. 2005). In order to coordinate their metabolism and physiology optimally with the photoperiodic variation in the environment, many species, from bacteria to humans, have evolved internal timers which predict regular changes in environmental conditions (Nagel and Kay 2012). Timers with a self-sustained and temperature-compensated rhythm very close to the 24 h of a solar day are termed "circadian", from the Latin *circa dies* meaning "about a day". Such circadian timers or clocks are crucial if an organism is to be able to predict regular, daily changes in their environment as well as seasonal ones (Ouyang et al. 1998; Green et al. 2002; Dodd et al. 2005; Edgar et al. 2012).

Seasonal variations in light and temperature are strong cues directing growth and development of plants, particularly those perennials growing in temperate and boreal regions where woody plants must become dormant to survive the freezing temperatures of winter. Dormancy is defined as "the inability to initiate growth from meristems (and other organs and cells with the capacity to resume growth) under favorable conditions" (Rohde and Bhalerao 2007). These authors further defined the release from dormancy as the restoration of the capacity for growth and completion of their development to meristems and meristem-derived organ. Trees need to actively and accurately coordinate their periods of dormancy with the seasonal changes in their environment.

Our work has focused on understanding the circadian clock and its importance for the growth of trees in both daily and seasonal contexts. This chapter contains an update on the current model of the plant clock in an annual, thale cress (*Arabidopsis thaliana*), provides an overview of recent work on the clocks in perennial plants and its involvement in their annual growth cycles and discusses how clocks help trees withstand cold and freezing temperatures. Moreover, we give our view on areas where future work on the circadian clock is required to gain insight into the life history of a tree.

The Circadian Clock

The Clock System in Arabidopsis

A simplified way to view the clock system is to divide it into inputs, oscillator (clock), and outputs (McWatters and Devlin 2011). Light and temperature cues received by dedicated receptors set the phase of the central, self-sustained oscillator. The oscillator governs the rhythm and phases of downstream gene expression, which in turn controls diverse metabolic and physiological processes (reviewed by Salomé and McClung (2004)). The oscillatory rhythm is temperature-compensated, meaning it is buffered against temperature variation across the physiological range (12–27 °C) (Edwards 2006; Gould et al. 2006). The oscillator is built up from interconnected transcription–translation feedback loops (TTFL), simplified as central, morning, and evening loops, which together form a clock system controlling growth and development. This plant circadian system has been elucidated mainly by studies using the model annual plant Arabidopsis (*Arabidopsis thaliana*) (reviewed by McWatters and Devlin (2011); Nagel and Kay (2012); Staiger et al. (2013)).

The central clock loop of the Arabidopsis clock is made up by two Myb-transcription factors, CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY). Their expression peaks at dawn and represses expression of the dusk-phased *TIMING OF CAB EXPRESSION 1* (*TOC1*) gene (Wang and Tobin 1998; Strayer et al. 2000; Alabadí et al. 2002; Perales and Mas 2007). TOC1, in turn, represses the expression of *CCA1* and *LHY* (Gendron et al. 2012; Huang et al. 2012). *TOC1* belongs to the *PSEUDO-RESPONSE REGULATOR* (*PRR*) gene family, whose genes are known to have important clock functions. They are expressed sequentially 2–3 h apart, starting with *PRR9* just after dawn, followed by *PRR7*, *PRR5*, *PRR3* and ending at dusk with *PRR1*/*TOC1* (Strayer et al. 2000; Matsushika et al. 2000; Eriksson et al. 2003; Kaczorowski and Quail 2003; Yamamoto et al. 2003; Michael et al. 2003; Farré et al. 2005; Salomé and McClung 2005; Nakamichi et al. 2005; Para et al. 2007; Ito et al. 2009).

PRR9 and PRR7 form a so-called morning loop, which feeds back to promote the expression of their repressors *CCA1* and *LHY* (Matsushika et al. 2000; Farré et al. 2005; Locke et al. 2006; Zeilinger et al. 2006). Another gene which is repressed by CCA1 and LHY is *CCA1 HIKING EXPEDITION* (*CHE*); the CHE protein in turn inhibits the expression of *CCA1* (Pruneda-Paz et al. 2009). *CHE* expression is further modulated by TOC1, allowing the expression of *CCA1* and *LHY* to be derepressed during the night.

The evening-phased loop is formed from *TOC1, GIGANTEA* (*GI*), *EARLY FLOWERING3 (ELF3)*, *ELF4,* and *PHYTOCLOCK 1 (PCL1)/LUX ARRHYTHMO* (*LUX*); ELF3, ELF4, and PCL1/LUX form the Evening Complex (EC) (Fowler et al. 1999; Onai and Ishiura 2005; Kolmos et al. 2009; Helfer et al. 2011; Pokhilko et al. 2012; Herrero et al. 2012; Nusinow et al. 2012). The EC represses the expression of *PRR9* via direct binding of LUX to the *PRR9* promoter, while LUX also represses its own expression (Helfer et al. 2011).

CCA1- and *LHY*-like Myb-transcription factor members of the *REVEILLE* (*RVE)* gene family (Carre 2002) have been recently characterized as positive regulators of clock gene expression in Arabidopsis (Fogelmark and Troein 2014). In particular, RVE4 and RVE8 act with the NIGHT LIGHT-INDUCIBLE AND CLOCK-REGULATED1 (LNK1) and LNK2 proteins to promote the expression of *PRR5* and *TOC1* (Farinas and Mas 2011; Rawat et al. 2011; Hsu et al. 2013; Rugnone et al. 2013; Xie et al. 2014) but they may also promote *PRR9*, *GI*, *LUX,* and *ELF4* expression (Fogelmark and Troein 2014).

Clock regulation occurs at several levels beyond that of gene expression, with posttranslational regulation being of major importance for controlling protein levels, activity, and localization (Imaizumi 2010). Importantly, TOC1 protein levels are inversely proportional to the speed of the oscillator and are regulated by ZEITLUPE (ZTL), an F-box protein which, together with GI, controls TOC1 degradation (Mas et al. 2003a, b; Kim et al. 2007). ZTL also modulates clock gene expression through interaction with the transcription factor EARLY BIRD (EBI), creating a link between transcriptional regulation and the ZTL-mediated ubiquitination pathway (Johansson et al. 2011). GI is further involved in the spatial–temporal regulation of TOC1 (Kim et al. 2013), with TOC1 import to the nucleus also regulated by PRR5-dependent phosphorylation (Fujiwara et al. 2008; Wang et al. 2010). A schematic view of the circadian TTFL in *Arabidopsis* is shown in Fig. 3.1a.

In addition to these TTFL, a metabolic cyclic adenosine diphosphate ribose (cADPR) and cytosolic-free calcium ($[Ca^{2+}]_{cyt}$) stress signaling network participates in circadian oscillator function (Dodd et al. 2007). Peroxiredoxin was recently used as a circadian biomarker to show oxidation rhythms in metabolism, revealing that circadian rhythms persist in the absence of transcription from bacteria to mammals (O'Neill et al. 2011; Edgar et al. 2012).

Epigenetic regulation of the circadian clock, involving, for example, methylation of cytosine in DNA, has also been described. Hypoacetylated histones promote closed chromatin states (Kuo and Allis 1998; Finnegan and Kovac 2000), while histone hyperacetylation is associated with relaxed chromatin fibers (Grunstein 1997; Eberharter and Becker 2002). Histone methylation and acetylation are dynamic epigenetic marks necessary for the expression of circadian genes in Arabidopsis (Perales and Mas 2007; Malapeira et al. 2012). Acetylation and methylation of clock gene promoters are governed by the nicotine adenine dinucleotide (NAD^+) energy status of the cell and regulate gene expression (Malapeira et al. 2012). Transcription of *TOC1* is preceded by the acetylation of histone 3 (H3ac); however, the presence of CCA1 appears to impede H3 acetylation at the *TOC1* promoter, resulting in a decrease in *TOC1* mRNA abundance (Perales and Mas 2007). Morning expression of *LHY*, *PRR9*, *CCA1,* and *PRR7* is correlated with acetylation of lysine 56 of histone H3 (H3K56ac) (Malapeira et al. 2012). Analyses of the dynamics of histone marks suggest the sequential enrichment of different histone modifications, including acetylation and methylation, with a pattern that is in tune with the rhythmic expression of the central circadian oscillator in Arabidopsis (Malapeira et al. 2012). There is thus an intimate integration between the clock and metabolism which involves several levels of regulation, including epigenetic regulation.

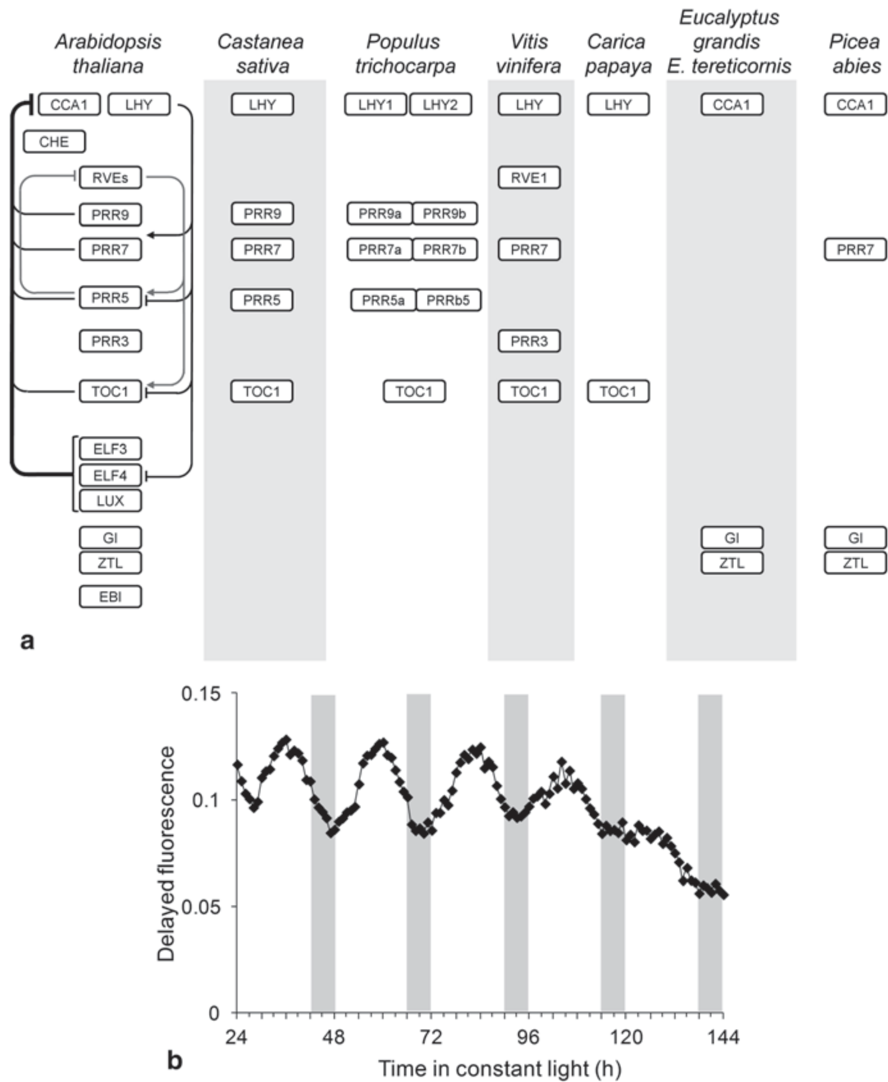


Fig. 3.1 Basic structure of the transcriptional feedback loops between key components of the circadian clock in *Arabidopsis thaliana* and selected perennial species, including clock functions revealed in *Eucalyptus grandis*. **a** Homologous components in species described in this chapter. **b** Delayed fluorescence in detached *Eucalyptus grandis* leaves on Murashige and Skoog medium without additional sugars reveals a free-running endogenous rhythm of 23.1 h ± 0.3 (SEM; $n = 8$) under continuous light (LL; 20 µmolm^{-2}s^{-1} *blue* and *red* light) after entrainment to 18 h light: 6 h dark. *White* and *gray* bars represent subjective daylight and night, respectively

The Circadian Clock in Perennials

The first clock genes identified in a woody perennial species were *CsLHY* and *CsTOC1* from chestnut, *Castanea sativa* (Ramos et al. 2005). These genes, homologues of essential components of the Arabidopsis circadian oscillator, were observed to cycle daily during vegetative growth as well as under continuous light (LL) conditions (Ramos et al. 2005). Later, homologues of *PRR9*, *PRR7,* and *PRR5* were identified in *C. sativa* (Ibáñez et al. 2008). The *C. sativa PRRs* all show a circadian pattern of expression, peaking after *CsLHY* in the order *CsPRR9* → *CsPRR7* → *CsPRR5* → *CsTOC1*, in a similar serial manner to that seen in Arabidopsis (Ibáñez et al. 2008); see Fig. 3.1a for an outline of characterized clock components in perennial species, including *C. sativa*, compared with Arabidopsis.

The core clock genes *CCA1* and *LHY* show a high degree of conservation between plant species. Phylogenetic studies in *Populus* sp. revealed two *LHY* genes, *LHY1* and *LHY2*, which were created through duplication events (Takata et al. 2009). Interestingly, the duplication of *LHY* in *Populus* occurred after the divergence between Arabidopsis and *Populus* with *AtCCA1* and *AtLHY* arising from the ancestor subsequently (Takata et al. 2009). Although genes of the *PRR* family are conserved between many angiosperm species, seven copies are found in *Populus*: two copies of each of *PRR9*, *PRR7,* and *PRR5* and one of *PRR1*/*TOC1* while the *PRR3* gene is missing (Takata et al. 2010); likewise, a cDNA library screening approach did not find *PRR3* in *C. sativa* (Ibáñez et al. 2008). Noticeably, the *TOC1* homologue in *Populus* has an earlier phase, relative to its Arabidopsis homologue (Ibáñez et al. 2010; Liu et al. 2013; Moreno-Cortés, unpublished results).

Homologues of clock genes have been identified in grapevine (*Vitis vinifera*), another deciduous perennial species, but *VvLHY* and *VvTOC1* expression do not display any circadian oscillations in berry tissues (Carbonell-Bejerano et al. 2014). However, other clock genes, including *VvPRR7, VvELF3,* and the Myb-transcription factor *VvRVE1* (a *LHY* homologue), do oscillate in the same organ (Carbonell-Bejerano et al. 2014); see Fig. 3.1a. Phylogenetic studies of clock genes and examination of their expression in perennial, vegetative tissues under various photoperiods, as well as in constant conditions, are needed to better understand the clock in grapevine.

Expression of genes involved in the circadian clock has been profiled in xylem from species of *Eucalyptus* using microarray and qPCR analysis (Fig. 3.1a). In *Eucalyptus tereticornis, EtCCA1* is expressed at dawn and *EtZTL* at zeitgeber time (ZT) 9 while, in *Eucalyptus grandis*, *EgGI* peaks from midday to dusk (Solomon et al. 2010). We detected a strong endogenous rhythm of about 23 h under LL for the *E. grandis* clock using the delayed fluorescence method (Gould et al. 2009), as shown in Fig. 3.1b. Transcriptional networks and expression profiles are also conserved in the tropical species *Carica papaya,* although many clock gene families are smaller (Zdepski et al. 2008). The ability of the circadian clock to anticipate daily changes in tropical regions, where there is only

subtle variation in photoperiods across the year, is consistent with the importance and accuracy of circadian timing.

Investigations of the clock in gymnosperms have recently begun. Homologues of *CCA1*, *GI*, *ZTL*, *PRR7,* and *TOC1* have been described in Norway spruce (*Picea abies*) (Karlgren et al. 2013). Complementation studies introducing *PaCCA1*, *PaGI,* and *PaZTL* in the corresponding mutated Arabidopsis counterparts suggest their protein functions are at least partly conserved between Arabidopsis and *P. abies*. Rhythmicity of clock-associated gene expression appears, however, to decrease rapidly under constant light in *P. abies*, suggesting light regulation of overt rhythms in the endogenous clocks of gymnosperms may differ from angiosperm clock systems (Gyllenstrand et al. 2014; overview in Fig. 3.1a.

The diurnally and circadianly regulated transcriptomes of *Arabidopsis*, rice (*Oryza sativa*, *ssp.),* and *Populus trichocarpa* show a high degree of conservation of rhythmicity and phase (Filichkin et al. 2011), with major conservation of *cis*-elements associated with timing. This underlines the similarities of circadian clock systems and their regulation across species (Zdepski et al. 2008; Takata et al. 2010; Filichkin et al. 2011).

Dormancy Regulation

Trees have acquired the capacity to time their periods of dormancy accurately by using their circadian clock to detect the critical daylength (CDL; the photoperiod which induces growth cessation and bud set). Later, when the season advances, the drop in temperature leads to a greater tolerance to cold and to leaf fall in deciduous trees (Welling and Palva 2006). The exit from dormancy is often a temperature-dependent process. A genetically determined amount of chilling hours are required to break dormancy, and further long photoperiod (also referred to as LD) and/or higher temperatures are needed to activate growth and bud burst (Rohde and Bhalerao 2007; Allona et al. 2008; Cooke et al. 2012).

Photoperiodic Induction of Growth Cessation and Bud Set

Light and temperature are the main cues entraining the circadian clock and synchronizing a plant to the time of the day and the season of the year. Photic input to the plant clock is *via* a variety of photoreceptors capable of detecting and distinguishing between different wavelengths of light.

Blue light is perceived by phototropins (phot1 and phot2), ZTL-family members (ZTL, FLAVIN-BINDING KELCH REPEAT F-BOX1 (FKF1), LOV KELCH PROTEIN2 (LKP2)) and cryptocromes (cry1 and cry2), which are also responsible for the detection of UV-A light (review in Banerjee and Batschauer (2005)). Al-

though none of these receptors have been studied in detail in trees, they have been identified in the genomes of *Populus* sp. (Hall et al. 2011; McKown et al. 2013; McKown et al. 2014) and willow (*Salix sp.)* (Ghelardini et al. 2014), and phenological and local adaptation traits have been linked to the genes encoding them. In Arabidopsis, cry1, cry2, and FKF1 are involved in the activation of flowering, firstly through multiple pathways, including those associated with circadian clock genes, which stabilize and activate the transcription factor CONSTANS (CO) (Sawa et al. 2007; Liu et al. 2008b; Song et al. 2012, 2013) and, secondly, by transcriptional activation of *FLOWERING LOCUS T* (*FT*) (Liu et al. 2008a). UV RESISTANCE LOCUS 8 (UVR8), a receptor for UV-B light, has been recently identified in Arabidopsis (Kami et al. 2010; Heijde and Ulm 2013; Heijde et al. 2013) and is also associated with abiotic stress responses.

Phytochromes (phys), which detect red and far-red light, are the most widely studied plant photoreceptors (Quail 2002). In *Populus*, there are one *PHYA* and two *PHYB* loci (Howe et al. 1998) which are involved in many phenological processes. Transgenic trees (*Populus tremula* × *P. tremuloides (Ptt)*) overexpressing oat *PHYA* are either insensitive to short day (SD) treatment or having a CDL of less than 6 h, resulting in stable auxin (indole acetic acid; IAA) and gibberellin (GA) levels under such conditions (Olsen et al. 1997). In addition, oat *PHYA* overexpressing trees are smaller than WT ones although treatment with end-of-day far-red light causes reversion of this phenotype (Olsen et al. 1997; Olsen and Junttila 2002). Moreover, *PttPHYA* down-regulated transgenic trees show early growth cessation and bud set, as well as altered expression of the circadian clock-associated genes *PttFKF1* and *PttCO* (Kozarewa et al. 2010).

The first assay of *PHYB* expression levels in *Populus* recently showed that phyB1 and phyB2 have different but overlapping functions in shade avoidance (Karve et al. 2012). Nevertheless, there are several quantitative trait loci (QTL) and association mapping studies attributing a phenological role to *PHYB* (Frewen et al. 2000; García-Gil et al. 2003; Ingvarsson et al. 2006; McKown et al. 2014; Källman et al. 2014).

Terminal buds growth arrest is the first event in the process of initiation of winter dormancy in response to shortening photoperiods (Wareing 1956; Nitsch 1957; Rohde and Bhalerao 2007). Transcriptional and metabolomic studies have demonstrated that changes in photoperiod have major consequences for plants at the transcriptional and metabolomic levels (Schrader et al. 2004; Druart et al. 2007; Ruttink et al. 2007; Hoffman et al. 2010). A study of different *P. tremula* populations originating along a latitudinal cline found the same module that controls the photoperiodic flowering pathway in Arabidopsis (CO/FT) is likely to be responsible for growth cessation in *Populus* (Böhlenius et al. 2006). This is an external coincidence model whereby CO, which is destabilized in darkness, can activate *FT* transcription (and hence initiate flowering) only during the daytime in Arabidopsis (reviewed by Kobayashi and Weigel (2007)). As a result, plants will flower only when the peak of *CO* expression coincides with light. In northern accessions of *Populus*, the maximum peak of *CO* expression occurs later in the day than in southern ones, and thus, as day light hours get shorter, this peak falls in darkness, making the CO protein

unstable and unable to activate transcription of *FT*. Absence of FT causes growth cessation and bud set of the tree (Böhlenius et al. 2006; Hsu et al. 2011).

All these processes occur in leaves, where the light signal is perceived; FT, however, moves through the phloem to the apical meristem. It has been proposed that the presence of FT activates *LIKE-APETALA1* (LAP1), an orthologue of the *Arabidopsis* floral meristem identity gene *APETALA1* (AP1), in the apical meristem (Azeez et al. 2014). In addition, *AINTEGUMENTA-LIKE 1* (*AIL1*) expression is increased following inducible activation of LAP1 (Azeez et al. 2014), suggesting transcriptional control of *AIL1* by LAP1. This entire pathway, together with the upregulation of *CYCLIN D3:2* (*CYCD3:2*) and *CYCD6:1* in an AIL1 overexpressing background (Karlberg et al. 2011), provides insights into the molecular mechanism from SD photo-reception to growth arrest by the inactivation of cell cycle regulators.

However, overexpression of *CO1* and *CO2* in *Populus* has no effect on bud set (Hsu et al. 2012), suggesting a conserved mechanism of posttranslational stabilization of CO by FKF1 similar to that observed in Arabidopsis (Song et al. 2012). In Arabidopsis, *CO* transcription is repressed by CYCLIN DOF FACTOR (CDF1), CDF2, and CDF3 (Imaizumi et al. 2005). In LD, these proteins are removed from the *CO* promoter and degraded by the GI-FKF1 complex (Sawa et al. 2007), allowing a double *CO* mRNA peak. In SD, FKF1 and GI expression do not coincide perfectly and thus the complex is less abundant, eliminating the first, daytime peak of *CO* mRNA. This pattern was observed in antisense-*PttPHYA* trees (Kozarewa et al. 2010), in which the expression of *FKF1* and *FT* was altered and there was only a single peak of *CO* expression in the dark period. This suggests *CO* was not translated into protein during the day and thus was unavailable to activate *FT*. This may explain the premature entrance into dormancy of *PttPHYA* antisense plants (Kozarewa et al. 2010). Another explanation for the absence of an effect of *CO1* and *CO2* overexpression on *Populus* phenology could be because, as in Arabidopsis, CO is degraded during the day before its natural first peak by the RING finger—containing E3 ubiquitin ligase HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENES1 (HOS1) (Lazaro et al. 2012); this mechanism would also assign a narrow window for the expression of this important protein.

Knockdown of *PttLHY1* and *PttLHY2* in hybrid aspen drastically delays bud set (Ibáñez et al. 2010). In Arabidopsis, LHY and CCA1 repress *GI* (Imaizumi 2010). Moreover, in Arabidopsis GI interacts with both *FT* promoter and some of its repressors (SHORT VEGETATIVE PHASE (SVP), TEMPRANILLO (TEM1) and TEM2) *in vivo*, leading to upregulation of *FT* expression without a change in *CO* transcription (Sawa and Kay 2011). If this mechanism is conserved in *Populus*, it would explain the altered dormancy phenotype observed in *PttLHY* knockdown trees.

Several studies suggest the role of the circadian clock for environmental adaptation in tree species. Circadian clock genes in species of *Populus* have been shown to be the targets of natural selection, as they have elevated protein evolutionary rates (Ma et al. 2010; Hall et al. 2011). Moreover, many phenological traits in *P. tremula* and *P. trichocarpa* associate strongly with genes related to the photoperiod pathway and circadian clock (Hall et al. 2011; McKown et al. 2014).

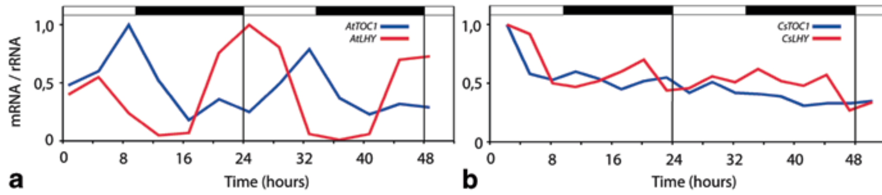


Fig. 3.2 Gene expression rhythms of *TOC1* (*blue* lines) and *LHY* (*red* lines) under field conditions. **a** Plantlets of *Arabidopsis thaliana* harvested in December in Madrid, Spain. **b** Stem material (two-year-old branches) from *Castanea sativa* plants collected under the same conditions as in **a**. Samples were collected at 3-h intervals. The experiment was performed as in Ramos et al. 2005. The *white* and *black* bars represent daylight and night, respectively

The Clock's Response to Cold, Cold Acclimation and Freezing Tolerance

The Clock's Response to Cold

So far we have focused on the role of the circadian clock in the photoperiodic regulation of winter dormancy. Since the clock acts as the link between the reception of environmental cues and physiological responses (Harmer 2009), it is interesting to examine how the oscillator responds to temperature changes. In recent years, several studies using Arabidopsis as experimental system have focused on the clock's response to cold (Dong et al. 2011; James et al. 2012; Seo et al. 2012; Kwon et al. 2014; Chow et al. 2014); very little is known, however, about the relationship between temperature and the circadian clock in perennials.

CsLHY and *CsTOC1* expression in *C. sativa* is disrupted in response to cold, leading to constitutive and arrhythmic activation of these genes (Ramos et al. 2005). Interestingly, these changes result entirely from low temperature and are independent of the photoperiod. Similar changes are also observed in the expression of other genes making up the central oscillator, including *CsPRR9, CsPRR7,* and *CsPRR5* (Ibáñez et al. 2008). Such disruption is also observed in *Populus sp.*, suggesting that cold alteration of the circadian clock is a common feature of woody plants (Ibáñez et al. 2010; Ramos-Sánchez et al. unpublished results). In addition, exposure of *C. sativa* seedlings to cold temperatures in the morning (when *CsLHY* is high and *CsTOC1* low) and in the afternoon (when *CsLHY* is low and *CsTOC1* high) resulted in both cases in upregulation of these genes (Ramos et al. 2005), suggesting transcriptional activation.

There are, however, several unanswered questions arising from such studies, including why gene expression of proteins which act as repressors of each other in *Arabidopsis* (such as LHY and TOC1 (Alabadi et al. 2001; Huang et al. 2012)) may be found at high levels at the same time in woody plants (Ramos et al. 2005), or how *CsPRR9*, *CsPRR7*, *CsPRR5,* and *CsTOC1* are all present simultaneously in *C. sativa* during the period of cold acclimation and winter (Ramos et al. 2005; Ibáñez et al. 2008). Taken together, these studies indicate there is, in perennials,

a widespread control of the disruption of the circadian clock caused by cold, possibly including layers of regulation other than the transcriptional ones. It is very likely that disruption of the circadian clock has an important biological meaning in perennials.

Similar experiments were carried out in Arabidopsis (Bieniawska et al. 2008). Plants were transferred from 20 to 4 °C and subsequently harvested every 4 h over the first, second, seventh, and fourteenth days under either LD or LL. Analysis of expression of circadian clock genes, circadianly regulated clock output genes and cold-induced genes revealed a variety of responses. Importantly, circadian clock genes and their outputs retained their rhythmic expression, albeit with lower amplitude than the controls, in the presence of light:dark cycles, with the exception of *LUX* whose expression in LD at 4 °C did not change relative to that of controls in LD at 21 °C. However, under LL at 4 °C, all rhythmic expression of circadian clock genes and their outputs was lost, resulting in constant high expression, with the sole exception of *CATALASE 3* (*CAT3)*, which showed very low amplitude (Bieniawska et al. 2008). All cold-induced genes lost their rhythmicity on the first day of exposure to cold although rhytmic expression of *C-REPEAT BINDING FACTORS* (*CBF*s) recovered next day with lower amplitude than seen in controls, while expression of *COLD-REGULATED* (*COR*) genes remained disrupted. Under conditions of LL at 4 °C, cold-induced genes showed high and arrhythmic levels of expression (Bieniawska et al. 2008). Metabolites showed similar alterations in expression and rhythmicity in response to cold: 80 % of diurnally regulated metabolites retain rhythmicity under LD conditions but lost this rhythm in constant light conditions (Espinoza et al. 2010).

Such data suggest circadian clock function in Arabidopsis also alters at 4 °C but, unlike in *C. sativa* or *Populus*, the photoperiod signal is sufficient to maintain oscillations. Noticeably, the clock disruption observed in *C. sativa* during the winter is not seen in Arabidopsis (Fig. 3.2a and 3.2b) even during the exposure of both species to the same low temperatures and short daylengths of December in Madrid, Spain (Ramos et al. 2005; Ibáñez et al. 2008; Ramos PhD thesis 2006).

Similar changes in level of expression and rhythmicity have been found for some circadian clock output genes in *C. sativa* (Berrocal-Lobo et al. 2011; Moreno-Cortés et al. 2012). One of these is *DUAL SPECIFICITY PROTEIN PHOSPHATASE 4* (*DSP4*), which encodes a protein phosphatase involved in starch degradation (Niittylä et al. 2006; Kerk et al. 2006). The catabolism of starch is important at the beginning of autumn in order to raise the levels of cryoprotective compounds, primarily sucrose, trehalose, raffinose, and stachyose (Sauter 1988; Rinne et al. 1994; Witt and Sauter 1995; Renaut et al. 2004). In this context, a GALACTINOL SYNTHASE protein (GolS1), which catalyzes the first step in the raffinose family oligosaccharide (RFO) synthesis, has been associated with endodormancy in *C. sativa* and with seasonal mobilization of carbohydrates in *Poplar* (Unda et al. 2012; Ibáñez et al. 2013). The role of *C. sativa* DSP4 on dormancy was analyzed under conditions of natural growth, revealing its transcript had a circadian rhythm during summer but was disrupted in winter. This response was also observed at the functional level of the protein (Berrocal-Lobo et al. 2011), indicating a potential role for DSP4 in

starch degradation and cold acclimation following low-temperature exposure during the activity—dormancy transition (Berrocal-Lobo et al. 2011). Such data imply that clock disruption in the cold plays an important role in protecting trees in winter.

The basic mechanisms of clock function in plants and animals are similar, although their oscillator genes are unrelated. The circadian clock of the ruin lizard (*Podarcis sicula*), a hibernating ectothermal vertebrate, is also disrupted in response to cold (Chiara Magnone et al. 2005; Vallone et al. 2007), in a similar manner to clock genes in *C. sativa* and *Populus*. Interruptions to the molecular circadian clock in the European hamster (*Cricetus cricetus*) during hibernation have also been described (Revel et al. 2007). Such similarities between evolutionary distinct organisms suggest "stopping" the circadian clock in response to cold could be part of a general adaptive strategy enabling organisms which undergo dormancy or hibernation to survive the winter.

Cold Acclimation and Freezing Tolerance

Herbaceous plants and perennials appear to share common strategies, at least in response to cold in the daily context when proper acclimation is essential for survival of bouts of cold weather. Cold acclimation is the process by which exposure to a period of low temperature allows a plant to survive a subsequent period of freezing. As elucidated by studies in Arabidopsis, it relies on regulated expression of *CBF1*, *CBF2,* and *CBF3* (Liu et al. 1998; Gilmour et al. 1998; Thomashow 1999), a group of transcription factors belonging to APETALA2/ETHYLENE-RESPONSIVE FACTOR (AP2/ERF) family of proteins (Agarwal et al. 2006). CBFs (also referred as DEHYDRATION RESPONSIVE ELEMENT BINDING PROTEINS (DREBs)) confer freezing tolerance by interacting with *cis*-acting elements in the promoters of cold-induced genes in Arabidopsis (Sakuma et al. 2002; Navarro et al. 2009; Lee and Thomashow 2012). A similar acclimation response occurs in deciduous trees (Benedict et al. 2006; Welling and Palva 2008; Navarro et al. 2009; Eriksson and Webb 2011; Fennell 2014), although trees also show a SD induction of CBFs which enhances winter hardiness and enables them to cope with the cold (Christersson 1978; Jarvis et al. 1996; Richard et al. 2000; Li et al. 2002; Schrader et al. 2004; Puhakainen et al. 2004). In *Arabidopsis thaliana*, *Populus tremula x P. tremuloides*, *Eucalyptus gunnii,* and *Prunus persica*, *CBF* gene expression is induced about 4 h after dawn (ZT4) (Dodd et al. 2006; Navarro et al. 2009; Ibáñez et al. 2010; Artlip et al. 2013), allowing plants to respond more strongly in the morning than at other times. This process is referred to as "gating" of the cold response and is primed by the circadian clock (Fowler et al. 2005; Artlip et al. 2013). *Populus* trees with reduced levels of *PttTOC1* show high and arrhythmic increases in the expression of *PttLHY1*, *PttLHY2,* and *PttCBF1* in response to cold, and also have increased freezing tolerance (Ibáñez et al. 2010). Conversely, the loss of *PttLHY1* and *PttLHY2*

expression leads to a loss of *PttCBF1* expression and reduced freezing tolerance (Ibáñez et al. 2010), implying expression of *PttLHY1* and *PttLHY2* is necessary for the induction of *PttCBF1*.

Recent work in Arabidopsis has revealed that there is a reciprocal *CBF* regulation of circadian clock components through the interaction between the *PCL1/LUX* promoter and CBF1 (Chow et al. 2014). CBF1 is responsible for maintaining oscillations of the *LUX* transcript at 4 °C in light:dark cycles (as noted in " The Clock's Response to Cold" section, *LUX* is the only circadian gene to retain its original rhythm in those conditions (Bieniawska et al. 2008)). In addition, freezing tolerance of the *lux* mutant is impaired following cold acclimation (Chow et al. 2014), as is that of the *cca1–11;lhy–21* double mutant (Dong et al. 2011). This positions the clock in the cold acclimation pathway. Moreover, Arabidopsis plants overexpressing *CBF1* have alterations in the expression of other clock components, which in turn feeds back on *CBF1* expression (Chow et al. 2014). Some of these changes in gene expression increase *CBF* expression and, perhaps, sugar levels (Bieniawska et al. 2008; Nakamichi et al. 2009). In fact, in Arabidopsis, PRR7 appears crucial for sugar sensing (Haydon et al. 2013) and PRR9, PRR7, and PRR5 are all involved in sugar metabolism (Nakamichi et al. 2009). This may be an important part of the control of cryoprotective sugars. Several carbohydrates are diurnally regulated in *Populus*, with some being increased in SD compared to LD, allowing the first stage of cold acclimation (Hoffman et al. 2010).

Chilling Requirements

In perennials, a period of accumulative chilling is often required to release buds from the state of endodormancy. This chilling requirement is similar to the vernalization requirement for flowering in many Arabidopsis accessions and other annual plants. In Arabidopsis, the MADS-box protein FLOWERING LOCUS C (FLC) is the central regulator of vernalization. FLC represses flowering, and the level of *FLC* transcript (and protein) reduces over the period of vernalization, eventually allowing flowering of late-flowering ecotypes (Sheldon et al. 2000). Two different MADS-box genes, *VERNALIZATION 1* (*VRN1*) and *VRN2,* regulate flowering time in cereals. While *VRN1* is induced by vernalization and promotes flowering, VRN2, like FLC, represses *FT* until the period of vernalization is complete (reviewed in Trevaskis et al. 2007). In perennials, *DORMANCY ASSOCIATED MADS-BOX* (*DAM*) genes seem to be regulators of bud dormancy, as suggested by studies of *Euphorbia esula* and peach (*Prunus persica*) (Li et al. 2009; Horvath et al. 2010; Jiménez et al. 2010). Interestingly, similar epigenetic regulation of these MADS-box genes has been described in Arabidopsis, barley, and peach (Bastow et al. 2004; Oliver et al. 2009; Leida et al. 2012; Kim and Sung 2013). Such diverse studies highlight the importance of MADS-box genes in regulating vernalization-dependent flowering and the central role of epigenetic regulation of these genes, and indicate their likely importance in ending bud dormancy in perennials.

Over the last decade, several transcriptomic analyses in *Populus* have led to the identification of genes involved in the epigenetic regulation of gene expression and of genes differentially expressed during active growth and winter dormancy. The entrance into dormancy of the vascular cambium enforces a global change in the transcriptome of *Populus* (Schrader et al. 2004). The homologue of *FERTILIZATION INDEPENDENT ENDOSPERM* (*FIE*) was strongly upregulated during cambial dormancy in *P. tremula*. FIE acts as part of the Polycomb Repressive Complex 2 (PRC2), which is involved in establishing the chromatin modification H3 lysine 27 trimethylation (H3K27me3). This epigenetic mark is associated with stable gene silencing. Interestingly, *FIE* and *PICKLE* (*PKL*, encode an ATP-dependent chromatin remodeling protein associated with HK27me3) are both rapidly upregulated following the perception of SD (Ruttink et al. 2007). Karlberg et al. (2010) reported that *Populus tremula* × *P. tremuloides GENERAL CONTROL NON-REPRESSIBLE 5 LIKE (GCN5-like),* an histone acetyl transferase was upregulated in the apex after short day treatment. Also, after five weeks of short days, two putative *HISTONE DEACETYLASES (HDACs)*, *HDA14* and *HDA08*, the histone lysine methyltransferase *SET DOMAIN PROTEIN 20* (*SDG20*) and a gene involved in histone ubiquitination, *HISTONE MONO-UBIQUITINATION 2 (HUB2)*, were all up-regulated, while a putative *DEMETER-LIKE* (*DML*) gene was down-regulated over the same period. The authors proposed that the down-regulation of this later gene could increase the level of DNA methylation and hence the chromatin compaction and gene silencing, after SD perception. The analysis of dormant *Populus* stem tissues found that many genes involved in chromatin remodeling were upregulated in winter compared to actively growing (summer) tissues (Ko et al. 2011). Moreover, transcriptomic analysis of *C. sativa* buds revealed higher expression of *CsHUB2* and *CsGCN5L* in dormant buds (Santamaria et al. 2011) compared to growing ones. Further genetic approaches should be performed in order to understand the connections between the circadian clock, chilling requirement and epigenetic modifications.

Future Perspectives

Winter dormancy is a process during which plants pass through different physiological states. Both the rate of dormancy development and depth of dormancy vary with the environmental signals inducing the process, especially photoperiod and temperature (Allona et al. 2008). The network of connections between light, temperature and the clock's interactions with *CBF* regulation provides a means of better understanding the regulation of cold acclimation, cryoprotection, and how and why both daylength and temperature induce cold acclimation and dormancy.

Strong similarities exist between circadian clock systems and their regulation across species, but the question of whether there is a specific control governing the disruption by cold of the circadian clock in perennials, and whether this includes layers of regulation other than transcription, requires further investigation of the circadian clock function in trees. The recent publication of the full genome

sequences of *Eucalyptus grandis*, *Picea abies,* and *Picea glauca* will increase our knowledge of circadian regulation in a nondormant tree and allow further characterization of the gymnosperm circadian clock, respectively (Birol et al. 2013; Nystedt et al. 2014; Myburg et al. 2014).

Studies by Kumar and Wigge (2010) in Arabidopsis suggest that temperature-regulated chromatin remodeling could act as a "thermometer" and that this might be important in clock regulation of growth activation in perennials. Subsequent examination and quantification of global epigenetic changes led to a picture of epigenetic regulation of winter dormancy of woody plants. *C. sativa* apical buds show higher levels of methylation of genomic DNA during bud set than bud burst while the opposite is seen for the AcH4 epigenetic mark (Santamaría et al. 2009). These contrasting patterns between epigenetic marks associated with relaxed and condensed chromatin have also been described in *Populus* stem tissue, where xylem and phloem cells show higher levels of DNA methylation and lower global levels of acetylated Lys8 in histone H4 during winter dormancy compared to the active growth season (Conde et al. 2013). The mechanism for establishing the chilling requirement and regulating growth activation has not yet been elucidated; doing so remains an important task if, as expected, it involves the clock as well as epigenetic regulation (Ríos et al. 2014).

Regulation of dormancy and, more especially, the control of growth activation at bud burst are of the greatest importance in maintaining productivity and building biomass in perennial deciduous crops, fruit- and nut-producing trees and in forest trees (Mohamed et al. 2010; Yordanov et al. 2014). We have merely begun to understand some aspects of timing, as the metabolic aspects of circadian regulation and its integration with the transcriptional oscillator and coupled epigenetic regulation are yet to be elucidated. Doing so will build a more complete picture of the timing of growth in both the annual and perennial contexts. Further knowledge of the regulation of the circadian clock during dormancy, cold acclimation and freezing tolerance will give us the means to develop crop plants better adapted to new and changing climates and thus increasing productivity of food and other plant materials.

Acknowledgments We gratefully acknowledge support from our funding agencies. Work in M.E.E.'s group was supported by Stiftelsen Nils och Dorthi Troëdssons forskningsfond, Carl Trygger Foundation for Scientific Research, the Kempe Foundations, the Swedish Governmental Agency for Innovation Systems (VINNOVA), the Swedish Research Council (VR), the Swedish Research Council Formas and the Berzelii Centre for forest biotechnology. M.E.E. is a VINNMER fellow supported by VINNOVA and the EU. Work in I.A.'s group was supported by the Spanish Ministry of Economy and Competitiveness grant AGL2011–22625/FOR and the Plant KBBE project PIM2010PKB-00702 from the EU. C.I. was supported by FONDECYT grant no. 1110831 (CONICYT—Chile). N.T. was supported by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (no. 25850124). J.M.R-S was supported by the Spanish Ministry of Education, Culture and Sport FPU Fellowship FPU12/01648. M.J. was supported by Alexander von Humboldt Fellowship. We are grateful for technical assistance with delayed fluorescence from Dr. Manuela Jurca, and to Tamara Hernandez-Verdeja and Mariano Perales for critical reading of the chapter.

References

Agarwal PK, Agarwal P, Reddy MK, Sopory SK (2006) Role of DREB transcription factors in abiotic and biotic stress tolerance in plants. Plant Cell Rep 25:1263–1274. doi:10.1007/s00299-006-0204-8

Alabadi D, Oyama T, Yanovsky MJ et al (2001) Reciprocal regulation between TOC1 and LHY/CCA1 within the Arabidopsis circadian clock. Science 293:880–883. doi:10.1126/science.1061320

Alabadí D, Yanovsky MJ, Mas P et al (2002) Critical role for CCA1 and LHY in maintaining circadian rhythmicity in Arabidopsis. Curr Biol 12:757–761

Allona I, Ramos A, Ibáñez C et al (2008) Molecular control of winter dormancy establishment in trees: a review. Span J Agric Res 6:201–10. doi:10.5424/sjar/200806S1-389

Artlip TS, Wisniewski ME, Bassett CL, Norelli JL (2013) CBF gene expression in peach leaf and bark tissues is gated by a circadian clock. Tree Physiol 33:866–877. doi:10.1093/treephys/tpt056

Azeez A, Miskolczi P, Tylewicz S, Bhalerao RP (2014) A tree ortholog of APETALA1 mediates photoperiodic control of seasonal growth. Curr Biol 24:717–724. doi:10.1016/j.cub.2014.02.037

Banerjee R, Batschauer A (2005) Plant blue-light receptors. Planta 220:498–502. doi:10.1007/s00425-004-1418-z

Bastow R, Mylne JS, Lister C et al (2004) Vernalization requires epigenetic silencing of FLC by histone methylation. Nature 427:164–167. doi:10.1038/nature02269

Benedict C, Skinner JS, Meng R et al (2006) The CBF1-dependent low temperature signalling pathway, regulon and increase in freeze tolerance are conserved in Populus spp. Plant Cell Environ 29:1259–1272

Berrocal-Lobo M, Ibáñez C, Acebo P et al (2011) Identification of a homolog of Arabidopsis DSP4 (SEX4) in chestnut: its induction and accumulation in stem amyloplasts during winter or in response to the cold. Plant Cell Environ 34:1693–1704. doi:10.1111/j.1365-3040.2011.02365.x

Bieniawska Z, Espinoza C, Schlereth A et al (2008) Disruption of the Arabidopsis circadian clock is responsible for extensive variation in the cold-responsive transcriptome. Plant Physiol 147:263–279. doi:10.1104/pp.108.118059

Birol I, Raymond A, Jackman SD et al (2013) Assembling the 20 Gb white spruce (Picea glauca) genome from whole-genome shotgun sequencing data. Bioinformatics 29:1492–1497. doi:10.1093/bioinformatics/btt178

Böhlenius H, Huang T, Charbonnel-Campaa L et al (2006) CO/FT regulatory module controls timing of flowering and seasonal growth cessation in trees. Science 312:1040–1043. doi:10.1126/science.1126038

Borchert R, Renner SS, Calle Z et al (2005) Photoperiodic induction of synchronous flowering near the Equator. Nature 433:627–629. doi:10.1038/nature03259

Carbonell-Bejerano P, Rodríguez V, Royo C et al (2014) Circadian oscillatory transcriptional programs in grapevine ripening fruits. BMC Plant Biol 14:78. doi:10.1186/1471-2229-14-78

Carre IA (2002) MYB transcription factors in the Arabidopsis circadian clock. J Exp Bot 53:1551–1557. doi:10.1093/jxb/erf027

Chiara Magnone M, Jacobmeier B, Bertolucci C et al (2005) Circadian expression of the clock gene Per2 is altered in the ruin lizard (Podarcis sicula) when temperature changes. Mol Brain Res 133:281–285. doi:10.1016/j.molbrainres.2004.10.014

Chow BY, Sanchez SE, Breton G et al (2014) Transcriptional regulation of LUX by CBF1 mediates cold input to the circadian clock in Arabidopsis. Curr Biol 24:1518–1524. doi:10.1016/j.cub.2014.05.029

Christersson L (1978) The influence of photoperiod and temperature on the development of frost hardiness in seedlings of Pinus silvestris and Picea abies. Physiol Plantarum 44:288–294. doi:10.1111/j.1399-3054.1978.tb08634.x

Conde D, González-Melendi P, Allona I (2013) Poplar stems show opposite epigenetic patterns during winter dormancy and vegetative growth. Trees 27:311–320. doi:10.1007/s00468-012-0800-x

Cooke JEK, Eriksson ME, Junttila O (2012) The dynamic nature of bud dormancy in trees: environmental control and molecular mechanisms. Plant Cell Environ 35:1707–1728. doi:10.1111/j.1365-3040.2012.02552.x

Dodd AN, Salathia N, Hall A et al (2005) Plant circadian clocks increase photosynthesis, growth, survival, and competitive advantage. Science 309:630–633. doi:10.1126/science.1115581

Dodd AN, Jakobsen MK, Baker AJ et al (2006) Time of day modulates low-temperature Ca signals in Arabidopsis. Plant J 48:962–973. doi:10.1111/j.1365-313X.2006.02933.x

Dodd AN, Gardner MJ, Hotta CT et al (2007) The Arabidopsis circadian clock incorporates a cADPR-based feedback loop. Science 318:1789–1792

Dong MA, Farré EM, Thomashow MF (2011) Circadian clock-associated 1 and late elongated hypocotyl regulate expression of the C-repeat binding factor (CBF) pathway in Arabidopsis. Proc Natl Acad Sci U S A 108:7241–7246. doi:10.1073/pnas.1103741108

Druart N, Johansson A, Baba K et al (2007) Environmental and hormonal regulation of the activity-dormancy cycle in the cambial meristem involves stage-specific modulation of transcriptional and metabolic networks. Plant J 50:557–573. doi:10.1111/j.1365-313X.2007.03077.x

Eberharter A, Becker PB (2002) Histone acetylation: a switch between repressive and permissive chromatin. Second in review series on chromatin dynamics. EMBO Rep 3:224–229. doi:10.1093/embo-reports/kvf053

Edgar RS, Green EW, Zhao Y et al (2012) Peroxiredoxins are conserved markers of circadian rhythms. Nature 485:459–464. doi:10.1038/nature11088

Edwards KD (2006) FLOWERING LOCUS C Mediates natural variation in the high-temperature response of the Arabidopsis circadian clock. Plant Cell 18:639–650. doi:10.1105/tpc.105.038315

Eriksson ME, Webb AA (2011) Plant cell responses to cold are all about timing. Curr Opin Plant Biol 1–7. doi:10.1016/j.pbi.2011.08.005

Eriksson ME, Hanano S, Southern MM et al (2003) Response regulator homologues have complementary, light-dependent functions in the Arabidopsis circadian clock. Planta 218:159–162. doi:10.1007/s00425-003-1106-4

Espinoza C, Degenkolbe T, Caldana C et al (2010) Interaction with diurnal and circadian regulation results in dynamic metabolic and transcriptional changes during cold acclimation in Arabidopsis. PLoS One 5:e14101. doi:10.1371/journal.pone.0014101

Farinas B, Mas P (2011) Functional implication of the MYB transcription factor RVE8/LCL5 in the circadian control of histone acetylation. Plant J 66:318–329. doi:10.1111/j.1365-313X.2011.04484.x

Farré EM, Harmer SL, Harmon FG et al (2005) Overlapping and distinct roles of PRR7 and PRR9 in the Arabidopsis circadian clock. Curr Biol 15:47–54. doi:10.1016/j.cub.2004.12.067

Fennell A (2014) Genomics and functional genomics of winter low temperature tolerance in temperate fruit crops. Crit Rev Plant Sci 33:125–140. doi:10.1080/07352689.2014.870410

Filichkin SA, Breton G, Priest HD et al (2011) Global profiling of rice and poplar transcriptomes highlights key conserved circadian-controlled pathways and cis-regulatory modules. PLoS One 6:e16907. doi:10.1371/journal.pone.0016907

Finnegan EJ, Kovac KA (2000) Plant DNA methyltransferases. Plant Mol Biol 43:189–201

Fogelmark K, Troein C (2014) Rethinking transcriptional activation in the Arabidopsis circadian clock. PLoS Comput Biol 10:e1003705. doi:10.1371/journal.pcbi.1003705

Fowler S, Lee K, Onouchi H et al (1999) GIGANTEA: a circadian clock-controlled gene that regulates photoperiodic flowering in Arabidopsis and encodes a protein with several possible membrane-spanning domains. EMBO J 18:4679–4688. doi:10.1093/emboj/18.17.4679

Fowler SG, Cook D, Thomashow MF (2005) Low temperature induction of Arabidopsis CBF1, 2, and 3 is gated by the circadian clock. Plant Physiol 137:961–968. doi:10.1104/pp.104.058354

Frewen BE, Chen TH, Howe GT et al (2000) Quantitative trait loci and candidate gene mapping of bud set and bud flush in populus. Genetics 154:837–845

Fujiwara S, Wang L, Han L et al (2008) Post-translational regulation of the Arabidopsis circadian clock through selective proteolysis and phosphorylation of pseudo-response regulator proteins. J Biol Chem 283:23073–23083. doi:10.1074/jbc.M803471200

García-Gil MR, Mikkonen M, Savolainen O (2003) Nucleotide diversity at two phytochrome loci along a latitudinal cline in Pinus sylvestris. Mol Ecol 12:1195–1206

Gendron JM, Pruneda-Paz JL, Doherty CJ et al (2012) Arabidopsis circadian clock protein, TOC1, is a DNA-binding transcription factor. Proc Natl Acad Sci U S A 109:3167–3172. doi:10.1073/pnas.1200355109

Ghelardini L, Berlin S, Weih M et al (2014) Genetic architecture of spring and autumn phenology in Salix. BMC Plant Biol 14:31. doi:10.1186/1471-2229-14-31

Gilmour SJ, Zarka DG, Stockinger EJ et al (1998) Low temperature regulation of the Arabidopsis CBF family of AP2 transcriptional activators as an early step in cold-induced COR gene expression. Plant J 16:433–442.

Gould PD, Locke JCW, Larue C et al (2006) The molecular basis of temperature compensation in the Arabidopsis circadian clock. Plant Cell 18:1177–1187. doi:10.1105/tpc.105.039990

Gould PD, Diaz P, Hogben C, Kusakina J, Salem R, Hartwell J, Hall AJW (2009) Delayed fluorescence as a universal tool for the measurement of circadian rhythms in higher plants. Plant J 58:893–901

Green RM, Tingay S, Wang Z-Y, Tobin EM (2002) Circadian rhythms confer a higher level of fitness to Arabidopsis plants. Plant Physiol 129:576–584. doi:10.1104/pp.004374

Grunstein M (1997) Histone acetylation in chromatin structure and transcription. Nature 389:349–352. doi:10.1038/38664

Gyllenstrand N, Karlgren A, Clapham D et al (2014) No time for spruce: rapid dampening of circadian rhythms in Picea abies (L. Karst). Plant Cell Physiol 55:535–550. doi:10.1093/pcp/pct199

Hall D, Ma X-F, Ingvarsson PK (2011) Adaptive evolution of the Populus tremula photoperiod pathway. Mol Ecol 20:1463–1474. doi:10.1111/j.1365-294X.2011.05014.x

Harmer SL (2009) The circadian system in higher plants. Annu Rev Plant Biol 60:357–377. doi:10.1146/annurev.arplant.043008.092054

Haydon MJ, Mielczarek O, Robertson FC et al (2013) Photosynthetic entrainment of the Arabidopsis thaliana circadian clock. Nature 502:689–692. doi:10.1038/nature12603

Heijde M, Ulm R (2013) Reversion of the Arabidopsis UV-B photoreceptor UVR8 to the homodimeric ground state. Proc Natl Acad Sci U S A 110:1113–1118. doi:10.1073/pnas.1214237110

Heijde M, Binkert M, Yin R et al (2013) Constitutively active UVR8 photoreceptor variant in Arabidopsis. Proc Natl Acad Sci U S A 110:20326–20331. doi:10.1073/pnas.1314336110

Helfer A, Nusinow DA, Chow BY et al (2011) LUX ARRHYTHMO Encodes a nighttime repressor of circadian gene expression in the Arabidopsis core clock. Curr Biol 21:126–133. doi:10.1016/j.cub.2010.12.021

Herrero E, Kolmos E, Bujdoso N et al (2012) EARLY FLOWERING4 recruitment of EARLY FLOWERING3 in the nucleus sustains the arabidopsis circadian clock. Plant Cell 24:428–443. doi:10.1105/tpc.111.093807

Hoffman DE, Jonsson P, Bylesjö M et al (2010) Changes in diurnal patterns within the Populus transcriptome and metabolome in response to photoperiod variation. Plant Cell Environ 33:1298–1313. doi:10.1111/j.1365-3040.2010.02148.x

Horvath DP, Sung S, Kim D et al (2010) Characterization, expression and function of DORMANCY ASSOCIATED MADS-BOX genes from leafy spurge. Plant Mol Biol 73:169–179. doi:10.1007/s11103-009-9596-5

Howe GT, Bucciaglia PA, Hackett WP et al (1998) Evidence that the phytochrome gene family in black cottonwood has one PHYA locus and two PHYB loci but lacks members of the PHYC/F and PHYE subfamilies. Mol Biol Evol 15:160–175

Hsu C-Y, Adams JP, Kim H et al (2011) FLOWERING LOCUS T duplication coordinates reproductive and vegetative growth in perennial poplar. Proc Natl Acad Sci U S A 108:10756–10761. doi:10.1073/pnas.1104713108

Hsu C-Y, Adams JP, No K et al (2012) Overexpression of constans homologs CO1 and CO2 fails to alter normal reproductive onset and fall bud set in woody perennial poplar. PLoS One 7:e45448. doi:10.1371/journal.pone.0045448

Hsu PY, Devisetty UK, Harmer SL, Chory J (2013) Accurate timekeeping is controlled by a cycling activator in Arabidopsis. eLife 2:e00473–e00473. doi:10.7554/eLife.00473

Huang W, Perez-Garcia P, Pokhilko A et al (2012) Mapping the core of the Arabidopsis circadian clock defines the network structure of the oscillator. Science 336:75–79. doi:10.1126/science.1219075

Ibáñez C, Ramos A, Acebo P et al (2008) Overall alteration of circadian clock gene expression in the chestnut cold response. PLoS One 3:e3567. doi:10.1371/journal.pone.0003567

Ibáñez C, Kozarewa I, Johansson M et al (2010) Circadian clock components regulate entry and affect exit of seasonal dormancy as well as winter hardiness in Populus trees. Plant Physiol 153:1823–1833. doi:10.1104/pp.110.158220

Ibáñez C, Collada C, Casado R et al (2013) Winter induction of the galactinol synthase gene is associated with endodormancy in chestnut trees. Trees 27:1309–1316. doi:10.1007/s00468–013-0879–8

Imaizumi T (2010) Arabidopsis circadian clock and photoperiodism: time to think about location. Curr Opin Plant Biol 13:83–89. doi:10.1016/j.pbi.2009.09.007

Imaizumi T, Schultz TF, Harmon FG et al (2005) FKF1 F-box protein mediates cyclic degradation of a repressor of CONSTANS in Arabidopsis. Science 309:293–297. doi:10.1126/science.1110586

Ingvarsson PK, García MV, Hall D et al (2006) Clinal variation in phyB2, a candidate gene for day-length-induced growth cessation and bud set, across a latitudinal gradient in European aspen (Populus tremula). Genetics 172:1845–1853. doi:10.1534/genetics.105.047522

Ito S, Kawamura H, Niwa Y et al (2009) A genetic study of the Arabidopsis circadian clock with reference to the TIMING OF CAB EXPRESSION 1 (TOC1) gene. Plant Cell Physiol 50:290–303. doi:10.1093/pcp/pcn198

James AB, Syed NH, Bordage S et al (2012) Alternative splicing mediates responses of the Arabidopsis circadian clock to temperature changes. Plant Cell 24:961–981. doi:10.1105/tpc.111.093948

Jarvis SB, Taylor MA, MacLeod MR, Davies HV (1996) Cloning and characterisation of the cDNA clones of three genes that are differentially expressed during dormancy-breakage in the seeds of Douglas Fir (Pseudotsuga menziesii). J Plant Physiol 147:559–566. doi:10.1016/S0176-1617(96)80046-0

Jiménez S, Reighard GL, Bielenberg DG (2010) Gene expression of DAM5 and DAM6 is suppressed by chilling temperatures and inversely correlated with bud break rate. Plant Mol Biol 73:157–167. doi:10.1007/s11103-010-9608-5

Johansson M, McWatters HG, Bako L et al (2011) Partners in time: EARLY BIRD associates with ZEITLUPE and regulates the speed of the Arabidopsis clock. Plant Physiol 155:2108–2122. doi:10.1104/pp.110.167155

Kaczorowski KA, Quail PH (2003) Arabidopsis PSEUDO-RESPONSE REGULATOR7 is a signaling intermediate in phytochrome-regulated seedling deetiolation and phasing of the circadian clock. Plant Cell 15:2654–2665. doi:10.1105/tpc.015065

Källman T, De Mita S, Larsson H et al (2014) Patterns of nucleotide diversity at photoperiod related genes in Norway spruce [Picea abies (L.) Karst.]. PLoS One 9:e95306. doi:10.1371/journal.pone.0095306

Kami C, Lorrain S, Hornitschek P, Fankhauser C (2010) Light-regulated plant growth and development. Curr Top Dev Biol 91:29–66. doi:10.1016/S0070-2153(10)91002-8

Karlberg A, Englund M, Petterle A et al (2010) Analysis of global changes in gene expression during activity-dormancy cycle in hybrid aspen apex. Plant Biotechnol 27:1–16. doi:10.5511/plantbiotechnology.27.1

Karlberg A, Bako L, Bhalerao RP (2011) Short day–mediated cessation of growth requires the downregulation of AINTEGUMENTALIKE1 Transcription factor in hybrid Aspen. PLoS Genet 7:e1002361. doi:10.1371/journal.pgen.1002361

Karlgren A, Gyllenstrand N, Källman T, Lagercrantz U (2013) Conserved function of core clock proteins in the gymnosperm Norway spruce (Picea abies L. Karst). PLoS One 8:e60110. doi:10.1371/journal.pone.0060110

Karve AA, Jawdy SS, Gunter LE et al (2012) Initial characterization of shade avoidance response suggests functional diversity between Populus phytochrome B genes. New Phytol 196:726–737. doi:10.1111/j.1469-8137.2012.04288.x

Kerk D, Conley TR, Rodriguez FA et al (2006) A chloroplast-localized dual-specificity protein phosphatase in Arabidopsis contains a phylogenetically dispersed and ancient carbohydrate-binding domain, which binds the polysaccharide starch. Plant J 46:400–413. doi:10.1111/j.1365-313X.2006.02704.x

Kim DH, Sung S (2013) Coordination of the vernalization response through a VIN3 and FLC gene family regulatory network in Arabidopsis. Plant Cell 25:454–469. doi:10.1105/tpc.112.104760

Kim W-Y, Fujiwara S, Suh S-S et al (2007) ZEITLUPE is a circadian photoreceptor stabilized by GIGANTEA in blue light. Nature 449:356–360. doi:10.1038/nature06132

Kim J, Geng R, Gallenstein RA, Somers DE (2013) The F-box protein ZEITLUPE controls stability and nucleocytoplasmic partitioning of GIGANTEA. Development 140:4060–4069. doi:10.1242/dev.096651

Ko J-H, Prassinos C, Keathley D, Han K-H (2011) Novel aspects of transcriptional regulation in the winter survival and maintenance mechanism of poplar. Tree Physiol 31:208–225. doi:10.1093/treephys/tpq109

Kobayashi Y, Weigel D (2007) Move on up, it's time for change–mobile signals controlling photoperiod-dependent flowering. Genes Dev 21:2371–2384. doi:10.1101/gad.1589007

Kolmos E, Nowak M, Werner M et al (2009) Integrating ELF4 into the circadian system through combined structural and functional studies. HFSP J 3:350–366. doi:10.2976/1.3218766

Kozarewa I, Ibáñez C, Johansson M et al (2010) Alteration of PHYA expression change circadian rhythms and timing of bud set in Populus. Plant Mol Biol 73:143–156. doi:10.1007/s11103-010-9619-2

Kumar SV, Wigge PA (2010) H2A.Z-containing nucleosomes mediate the thermosensory response in Arabidopsis. Cell 140:136–147. doi:10.1016/j.cell.2009.11.006

Kuo MH, Allis CD (1998) Roles of histone acetyltransferases and deacetylases in gene regulation. Bioessays 20:615–626. doi:10.1002/(SICI)1521-1878(199808)20:8<615::AID-BIES4>3.0.CO;2-H

Kwon Y-J, Park M-J, Kim S-G et al (2014) Alternative splicing and nonsense-mediated decay of circadian clock genes under environmental stress conditions in Arabidopsis. BMC Plant Biol 14:136–15. doi:10.1186/1471-2229-14-136

Lazaro A, Valverde F, Pineiro M, Jarillo JA (2012) The Arabidopsis E3 Ubiquitin Ligase HOS1 negatively regulates CONSTANS abundance in the photoperiodic control of flowering. Plant Cell 24:982–999. doi:10.1105/tpc.110.081885

Lee C-M, Thomashow MF (2012) Photoperiodic regulation of the C-repeat binding factor (CBF) cold acclimation pathway and freezing tolerance in Arabidopsis thaliana. Proc Natl Acad Sci U S A 109:15054–15059. doi:10.1073/pnas.1211295109

Leida C, Conesa A, Llácer G et al (2012) Histone modifications and expression of DAM6 gene in peach are modulated during bud dormancy release in a cultivar-dependent manner. New Phytol 193:67–80. doi:10.1111/j.1469-8137.2011.03863.x

Li C, Puhakainen T, Welling A et al (2002) Cold acclimation in silver birch (Betula pendula). Development of freezing tolerance in different tissues and climatic ecotypes. Physiol Plantarum 116:478–488. doi:10.1034/j.1399-3054.2002.1160406.x

Li Z, Reighard GL, Abbott AG, Bielenberg DG (2009) Dormancy-associated MADS genes from the EVG locus of peach [Prunus persica (L.) Batsch] have distinct seasonal and photoperiodic expression patterns. J Exp Bot 60:3521–3530. doi:10.1093/jxb/erp195

Liu Q, Kasuga M, Sakuma Y et al (1998) Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and low-temperature-responsive gene expression, respectively, in Arabidopsis. Plant Cell 10:1391–1406

Liu H, Yu X, Li K et al (2008a) Photoexcited CRY2 interacts with CIB1 to regulate transcription and floral initiation in Arabidopsis. Science 322:1535–1539. doi:10.1126/science.1163927

Liu L-J, Zhang Y-C, Li Q-H et al (2008b) COP1-mediated ubiquitination of CONSTANS is implicated in cryptochrome regulation of flowering in Arabidopsis. Plant Cell 20:292–306. doi:10.1105/tpc.107.057281
Liu W, Yuan JS, Stewart CN (2013) Advanced genetic tools for plant biotechnology. Nature Reviews Genetics 1–13. doi:10.1038/nrg3583
Locke JCW, Kozma-Bognar L, Gould PD et al (2006) Experimental validation of a predicted feedback loop in the multi-oscillator clock of Arabidopsis thaliana. Mol Syst Biol 2:59. doi:10.1038/msb4100102
Ma X-F, Hall D, Onge KRS et al (2010) Genetic differentiation, clinal variation and phenotypic associations with growth cessation across the Populus tremula photoperiodic pathway. Genetics 186:1033–1044. doi:10.1534/genetics.110.120873
Malapeira J, Khaitova LC, Mas P (2012) Ordered changes in histone modifications at the core of the Arabidopsis circadian clock. Proc Natl Acad Sci U S A 109:21540–21545. doi:10.1073/pnas.1217022110
Mas P, Alabadí D, Yanovsky MJ et al (2003a) Dual role of TOC1 in the control of circadian and photomorphogenic responses in Arabidopsis. Plant Cell 15:223–236
Mas P, Kim W-Y, Somers DE, Kay SA (2003b) Targeted degradation of TOC1 by ZTL modulates circadian function in Arabidopsis thaliana. Nature 426:567–570. doi:10.1038/nature02163
Matsushika A, Makino S, Kojima M, Mizuno T (2000) Circadian waves of expression of the APRR1/TOC1 family of pseudo-response regulators in Arabidopsis thaliana: insight into the plant circadian clock. Plant Cell Physiol 41:1002–1012
McKown AD, Guy RD, Klápště J et al (2013) Geographical and environmental gradients shape phenotypic trait variation and genetic structure in Populus trichocarpa. New Phytologist 201:1263–1276. doi:10.1111/nph.12601
McKown AD, Klápště J, Guy RD et al (2014) Genome-wide association implicates numerous genes underlying ecological trait variation in natural populations of Populus trichocarpa. New Phytologist 203:535–553. doi:10.1111/nph.12815
McWatters HG, Devlin PF (2011) Timing in plants—A rhythmic arrangement. FEBS Lett 585:1474–1484. doi:10.1016/j.febslet.2011.03.051
Michael TP, Salomé PA, Yu HJ et al (2003) Enhanced fitness conferred by naturally occurring variation in the circadian clock. Science 302:1049–1053. doi:10.1126/science.1082971
Mohamed R, Wang C-T, Ma C et al (2010) Populus CEN/TFL1 regulates first onset of flowering, axillary meristem identity and dormancy release in Populus. Plant J 62:674–688. doi:10.1111/j.1365-313X.2010.04185.x
Moreno-Cortés A, Hernández-Verdeja T, Sánchez-Jiménez P et al (2012) CsRAV1 induces sylleptic branching in hybrid poplar. New Phytologist 194:83–90. doi:10.1111/j.1469-8137.2011.04023.x
Myburg AA, Grattapaglia D, Tuskan GA et al (2014) The genome of Eucalyptus grandis. Nature 510:356–362. doi:10.1038/nature13308
Nagel DH, Kay SA (2012) Complexity in the wiring and regulation of plant circadian networks. Curr Biol 22:R648–R657. doi:10.1016/j.cub.2012.07.025
Nakamichi N, Kita M, Ito S et al (2005) PSEUDO-RESPONSE REGULATORS, PRR9, PRR7 and PRR5, together play essential roles close to the circadian clock of Arabidopsis thaliana. Plant Cell Physiol 46:686–698. doi:10.1093/pcp/pci086
Nakamichi N, Kusano M, Fukushima A et al (2009) Transcript profiling of an Arabidopsis PSEUDO RESPONSE REGULATOR arrhythmic triple mutant reveals a role for the circadian clock in cold stress response. Plant Cell Physiol 50:447–462. doi:10.1093/pcp/pcp004
Navarro M, Marque G, Ayax C et al (2009) Complementary regulation of four Eucalyptus CBF genes under various cold conditions. J Exp Bot 60:2713–2724. doi:10.1093/jxb/erp129
Niittylä T, Comparot-Moss S, Lue W-L et al (2006) Similar protein phosphatases control starch metabolism in plants and glycogen metabolism in mammals. J Biol Chem 281:11815–11818. doi:10.1074/jbc.M600519200
Nitsch JP (1957) Photoperiodism in woody plants. Proc Amer Soc Hort Sci 70:526–544

Nusinow DA, Helfer A, Hamilton EE et al (2012) The ELF4-ELF3-LUX complex links the circadian clock to diurnal control of hypocotyl growth. Nature 475:398–402. doi:10.1038/nature10182

Nystedt B, Street NR, Wetterbom A et al (2014) The Norway spruce genome sequence and conifer genome evolution. Nature 497:579–584. doi:10.1038/nature12211

Oliver SN, Finnegan EJ, Dennis ES et al (2009) Vernalization-induced flowering in cereals is associated with changes in histone methylation at the VERNALIZATION1 gene. Proc Natl Acad Sci U S A 106:8386–8391. doi:10.1073/pnas.0903566106

Olsen JE, Junttila O (2002) Far red end-of-day treatment restores wild type-like plant length in hybrid aspen overexpressing phytochrome A. Physiol Plantarum 115:448–457

Olsen JE, Junttila O, Nilsen J et al (1997) Ectopic expression of oat phytochrome A in hybrid aspen changes critical daylength for growth and prevents cold acclimatization. Plant J 12:1339–1350

Onai K, Ishiura M (2005) PHYTOCLOCK 1 encoding a novel GARP protein essential for the Arabidopsis circadian clock. Genes Cells 10:963–972. doi:10.1111/j.1365-2443.2005.00892.x

O'Neill JS, van Ooijen G, Dixon LE et al (2011) Circadian rhythms persist without transcription in a eukaryote. Nature 469:554–558. doi:10.1038/nature09654

Ouyang Y, Andersson CR, Kondo T et al (1998) Resonating circadian clocks enhance fitness in cyanobacteria. Proc Nat Acad Sci U S A 95:8660–8664

Para A, Farré EM, Imaizumi T et al (2007) PRR3 Is a vascular regulator of TOC1 stability in the Arabidopsis circadian clock. Plant Cell 19:3462–3473. doi:10.1105/tpc.107.054775

Perales M, Mas P (2007) A functional link between rhythmic changes in chromatin structure and the Arabidopsis biological clock. Plant Cell 19:2111–2123. doi:10.1105/tpc.107.050807

Pokhilko A, ndez APNAFA, Edwards KD et al (2012) The clock gene circuit in Arabidopsis includes a repressilator with additional feedback loops. Mol Syst Biol 8:1–13. doi:10.1038/msb.2012.6

Pruneda-Paz JL, Breton G, Para A, Kay SA (2009) A functional genomics approach reveals CHE as a component of the Arabidopsis circadian clock. Science 323:1481–1485. doi:10.1126/science.1167206

Puhakainen T, Li C, Boije-Malm M et al (2004) Short-day potentiation of low temperature-induced gene expression of a C-repeat-binding factor-controlled gene during cold acclimation in silver birch. Plant Physiol 136:4299–4307. doi:10.1104/pp.104.047258

Quail PH (2002) Photosensory perception and signalling in plant cells: new paradigms? Curr Opin Cell Biol 14:180–188

Ramos A, Pérez-Solís E, Ibáñez C et al (2005) Winter disruption of the circadian clock in chestnut. Proc Natl Acad Sci U S A 102:7037–7042. doi:10.1073/pnas.0408549102

Rawat R, Takahashi N, Hsu PY, et al. (2011) REVEILLE8 and PSEUDO-REPONSE REGULATOR5 form a negative feedback loop within the Arabidopsis circadian clock. PLoS Genet 7:e1001350. doi: 10.1371/journal.pgen.1001350

Renaut J, Lutts S, Hoffmann L, Hausman J-F (2004) Responses of poplar to chilling temperatures: proteomic and physiological aspects. Plant Biol (Stuttg) 6:81–90. doi:10.1055/s-2004-815733

Revel FG, Herwig A, Garidou M-L et al (2007) The circadian clock stops ticking during deep hibernation in the European hamster. Proc Nat Acad Sci 104:13816–13820. doi:10.1073/pnas.0704699104

Richard S, Morency MJ, Drevet C et al (2000) Isolation and characterization of a dehydrin gene from white spruce induced upon wounding, drought and cold stresses. Plant Mol Biol 43:1–10

Rinne P, Saarelainen A, Junttila O (1994) Growth cessation and bud dormancy in relation to ABA level in seedlings and coppice shoots of Betula pubescens as affected by a short photoperiod, water stress and chilling. Physiol Plantarum 90:451–458. doi:10.1111/j.1399-3054.1994.tb08801.x

Ríos G, Leida C, Conejero A, Badenes ML (2014) Epigenetic regulation of bud dormancy events in perennial plants. Front Plant Sci 5:247. doi:10.3389/fpls.2014.00247

Rohde A, Bhalerao RP (2007) Plant dormancy in the perennial context. Trends Plant Sci 12:217–223. doi:10.1016/j.tplants.2007.03.012

Rugnone ML, Faigón Soverna A, Sanchez SE et al (2013) LNK genes integrate light and clock signaling networks at the core of the Arabidopsis oscillator. Proc Natl Acad Sci U S A 110:12120–12125. doi: 10.1073/pnas.1302170110

Ruttink T, Arend M, Morreel K et al (2007) A molecular timetable for apical bud formation and dormancy induction in poplar. Plant Cell 19:2370–2390. doi:10.1105/tpc.107.052811

Sakuma Y, Liu Q, Dubouzet JG et al (2002) DNA-binding specificity of the ERF/AP2 domain of Arabidopsis DREBs, transcription factors involved in dehydration- and cold-inducible gene expression. Biochem Biophys Res Commun 290:998–1009. doi:10.1006/bbrc.2001.6299

Salomé PA, McClung CR (2004) The Arabidopsis thaliana clock. J Biol Rhythms 19:425–435. doi:10.1177/0748730404268112

Salomé PA, McClung CR (2005) PSEUDO-RESPONSE REGULATOR 7 and 9 are partially redundant genes essential for the temperature responsiveness of the Arabidopsis circadian clock. Plant Cell 17:791–803. doi:10.1105/tpc.104.029504

Santamaría ME, Hasbún R, Valera MJ et al (2009) Acetylated H4 histone and genomic DNA methylation patterns during bud set and bud burst in Castanea sativa. J Plant Physiol 166:1360–1369. doi:10.1016/j.jplph.2009.02.014

Santamaria ME, Rodriguez R, Canal MJ, Toorop PE (2011) Transcriptome analysis of chestnut (Castanea sativa) tree buds suggests a putative role for epigenetic control of bud dormancy. Ann Bot 108:485–498. doi:10.1093/aob/mcr185

Sauter JJ (1988) Temperature-induced Changes in Starch and Sugars in the Stem of Populus × canadensis «robusta». J Plant Physiol 132:608–612. doi:10.1016/S0176-1617(88)80263-3

Sawa M, Kay SA (2011) GIGANTEA directly activates Flowering Locus T in Arabidopsis thaliana. Proc Natl Acad Sci U S A 108:11698–11703. doi:10.1073/pnas.1106771108

Sawa M, Nusinow DA, Kay SA, Imaizumi T (2007) FKF1 and GIGANTEA Complex Formation Is Required for Day-Length Measurement in Arabidopsis. Science 318:261–265. doi:10.1126/science.1146994

Schrader J, Moyle R, Bhalerao R et al (2004) Cambial meristem dormancy in trees involves extensive remodelling of the transcriptome. Plant J 40:173–187. doi:10.1111/j.1365-313X.2004.02199.x

Seo PJ, Park MJ, Lim MH et al (2012) A Self-Regulatory Circuit of CIRCADIAN CLOCK-ASSOCIATED1 Underlies the Circadian Clock Regulation of Temperature Responses in Arabidopsis. Plant Cell 24:2427–2442. doi:10.1105/tpc.112.098723

Sheldon CC, Rouse DT, Finnegan EJ et al (2000) The molecular basis of vernalization: the central role of FLOWERING LOCUS C (FLC). Proc Nat Acad Sci U S A 97:3753–3758. doi:10.1073/pnas.060023597

Solomon OL, Berger DK, Myburg AA (2010) Diurnal and circadian patterns of gene expression in the developing xylem of Eucalyptus trees. S Afr J Bot 76:425–439. doi:10.1016/j.sajb.2010.02.087

Song YH, Smith RW, To BJ et al (2012) FKF1 conveys timing information for CONSTANS stabilization in photoperiodic flowering. Science 336:1045–1049. doi:10.1126/science.1219644

Song YH, Ito S, Imaizumi T (2013) Flowering time regulation: photoperiod- and temperature-sensing in leaves. Trends in Plant Science 1–9. doi:10.1016/j.tplants.2013.05.003

Staiger D, Shin J, Johansson M, Davis SJ (2013) The circadian clock goes genomic. Genome Biol 14:208. doi:10.1186/gb-2013-14-6-208

Strayer C, Oyama T, Schultz TF et al (2000) Cloning of the Arabidopsis clock gene TOC1, an autoregulatory response regulator homolog. Science 289:768–771

Takata N, Saito S, Tanaka Saito C et al (2009) Molecular phylogeny and expression of poplar circadian clock genes, LHY1 and LHY2. New Phytol 181:808–819. doi:10.1111/j.1469-8137.2008.02714.x

Takata N, Saito S, Saito C, Uemura M (2010) Phylogenetic footprint of the plant clock system in angiosperms: evolutionary processes of pseudo-response regulators. BMC Evol Biol 10:126. doi:10.1186/1471-2148-10-126

Thomas B, Vince-Prue D (1997) Photoperiodism in plants. Academic Press, London

Thomashow MF (1999) Plant cold acclimation: freezing tolerance genes and regulatory mechanisms. Annu Rev Plant Physiol Plant Mol Biol 50:571–599. doi:10.1146/annurev.arplant.50.1.571

Trevaskis B, Tadege M, Hemming MN et al (2007) Short vegetative phase-like MADS-box genes inhibit floral meristem identity in barley. Plant Physiol 143:225–235. doi:10.1104/pp.106.090860

Unda F, Canam T, Preston L, Mansfield SD (2012) Isolation and characterization of galactinol synthases from hybrid poplar. J Exp Bot 63:2059–2069. doi:10.1093/jxb/err411

Vallone D, Frigato E, Vernesi C et al (2007) Hypothermia modulates circadian clock gene expression in lizard peripheral tissues. Am J Physiol Regul Integr Comp Physiol 292:R160–6. doi:10.1152/ajpregu.00370.2006

Wang ZY, Tobin EM (1998) Constitutive expression of the CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) gene disrupts circadian rhythms and suppresses its own expression. Cell 93:1207–1217

Wang L, Fujiwara S, Somers DE (2010) PRR5 regulates phosphorylation, nuclear import and subnuclear localization of TOC1 in the Arabidopsis circadian clock. EMBO J 29:1903–1915. doi:10.1038/emboj.2010.76

Wareing PF (1956) Photoperiodism in woody plants. Annu Rev Plant Physiol 7:191–214. doi:10.1146/annurev.pp.07.060156.001203

Welling A, Palva ET (2006) Molecular control of cold acclimation in trees. Physiol Plantarum 127:167–181. doi:10.1111/j.1399-3054.2006.00672.x

Welling A, Palva ET (2008) Involvement of CBF transcription factors in winter hardiness in birch. Plant Physiol 147:1199–1211. doi:10.1104/pp.108.117812

Witt W, Sauter JJ (1995) In vitro degradation of starch grains by Phosphorylases and Amylases from poplar wood. J Plant Physiol 146:35–40. doi:10.1016/S0176-1617(11)81964-4

Xie Q, Wang P, Liu X et al (2014) LNK1 and LNK2 Are Transcriptional Coactivators in the Arabidopsis Circadian Oscillator. Plant Cell. doi:10.1105/tpc.114.126573

Yamamoto Y, Sato E, Shimizu T et al (2003) Comparative genetic studies on the APRR5 and APRR7 genes belonging to the APRR1/TOC1 quintet implicated in circadian rhythm, control of flowering time, and early photomorphogenesis. Plant Cell Physiol 44:1119–1130

Yordanov YS, Ma C, Strauss SH, Busov VB (2014) EARLY BUD-BREAK 1 (EBB1) is a regulator of release from seasonal dormancy in poplar trees. Proc Natl Acad Sci U S A 201405621. doi:10.1073/pnas.1405621111

Zdepski A, Wang W, Priest HD et al (2008) Conserved daily transcriptional programs in carica papaya. Trop Plant Biol 1:236–245. doi:10.1007/s12042-008-9020-3

Zeilinger MN, Farré EM, Taylor SR et al (2006) A novel computational model of the circadian clock in Arabidopsis that incorporates PRR7 and PRR9. Mol Syst Biol 2:58. doi:10.1038/msb4100101

Chapter 4
Dormancy Behaviors and Underlying Regulatory Mechanisms: From Perspective of Pathways to Epigenetic Regulation

Zongrang Liu, Hong Zhu and Albert Abbott

Introduction

Plants encounter a wide range of environmental insults both seasonally and daily during annual growth cycles. Unfavorable conditions such as periodic freezing temperature in winter, high temperature in summer, and seasonal drought and flooding, pose great challenges to growth, development, and reproduction of both annual and perennial crops. During evolution, plants adapted to different climates have developed various strategies to cope with short or long periods of stress. Perennial plants by virtue of their continued growth over many years in various climates have adapted to cyclic periods of environmental stress by going dormant (Rohde and Bhalerao 2007). Plants cease their growth or development and enter a dormant state when encountering harsh conditions and resume growth or development when more favorable conditions return (Viémont and Crabbé 2000; Arora et al. 2003). For different stress contexts, three categories of dormancy, ecodormancy, endodormancy, and paradormancy, are exhibited by plants (Lang et al. 1987). Ecodormancy is a growth arrest induced by temporary environmental stresses but this arrest is released once the stressful condition is over as exemplified by a high temperature-induced summer growth arrest. Endodormancy is the deep dormant state induced by periodic seasonal stress conditions, which unlike ecodormancy is not immediately

Z. Liu (✉)
USDA-ARS, Appalachian Fruit Research Station,
2217 Wilshire Road, Kearneysville, WV 25430, USA
e-mail: Zongrang.liu@ars.usda.gov

H. Zhu
Chinese Academy of Sciences, South China Botanical Garden,
No.723, Xingke Road, Tianhe District, Guangzhou 510650, China
e-mail: zhuhong@scbg.ac.cn

A. Abbott
Forest Health Research and Education Center, University of Kentucky,
Lexington, KY 40506, USA
e-mail: albert.abbott@uky.edu

© Springer International Publishing Switzerland 2015
J. V. Anderson (ed.), *Advances in Plant Dormancy,* DOI 10.1007/978-3-319-14451-1_4

reversible but requires that specific conditions (e.g., chilling requirement) are met. Paradormancy in most perennials, imposed by apical dominance, occurs only in lateral buds during the growing season and can be reversed by decapitation (Hillman 1984). However, in some perennials, lateral as well as underground adventitious buds are also subjected to paradormancy under these conditions (Anderson et al. 2005; Chao et al. 2006). These different types of dormancy are often inter-related and their interplay is important during endodormancy onset and maintenance. For example, during dormancy onset in peach the apical shoot meristems go through an ecodormant state (e.g., growth cessation and bud set) before entering the endodormancy stage, while the lateral growing meristems in the paradormant state directly enter endodormancy (Arora et al. 2003). For simplicity, we will exclusively discuss endodormancy in this review referring to it as dormancy from now on.

Plants grown in different climates evolved diverse dormancy strategies to cope with distinct stresses. In temperate regions, winter freezing is a major threat for plant survival and plants accordingly develop winter dormancy (Arora et al. 2003; Horvath et al. 2003; Rohde and Bhalerao 2007) while plants grown in a Mediterranean climate evolve summer dormancy to survive the hot temperatures and extreme dry conditions of summer and fall (Ofir 1986; Ofir and Kigel 1999, 2007). Similarly, many plant species grown in tropical regions with a long dry season go dormant to endure the drought stress challenges (Borchert and Rivera 2001). Developmental programming such as fruit ripening in some pear can also induce, albeit less evident, a dormancy-like condition that has value in ensuring plants successfully reproduce in a special form or survive in particular environmental conditions (EI-Sharkawy et al. 2003, 2004; Villalobos-Acuńa and Mitcham 2008). Although dormancy occurs in meristem cells, it does not occur in all meristems depending on plant growth habit, life cycle, and architectural features. For example, the root meristem in woody plants and the aerial shoot meristems in perennial herbaceous leafy spurge (*Euphorbia esula*) are not subjected to periodic dormancy regulation (Rinne et al. 2011; Anderson et al. 2005; Chao et al. 2006). Evidently, plants have evolved diverse dormancy biotypes or phenotypes reliant on intricate regulatory mechanisms.

Winter dormancy is of economic importance, and also relatively well studied. In temperate species, winter dormancy is induced by cold temperature or short photoperiod or both in the fall, and released by the cumulated effects of chilling temperatures ranging above 0 °C to 10 °C (Arora et al. 2003; Horvath et al. 2003; Rohde and Bhalerao 2007). If the chilling requirement is not met, the dormant buds cannot or very poorly break dormancy, which could lead to non-uniform flowering that compromises fruit productivity. Thus, chilling requirement is obligatory and cannot be naturally circumvented. This phenomenon is analogous to vernalization in winter annuals that require chilling for flower induction (Amasino 2004). In fact, both dormancy and vernalization share, albeit acting on different biological processes, similar growth/developmental arrest and release principles enabling plants to survive the adverse winter conditions (Arora et al. 2003; Horvath et al. 2003; Amasino 2004; Rohde and Bhalerao 2007). In both cases, the length of the effective chilling period in winter directly orchestrates dormancy release and vernalization programming (Purvis and Gregory 1952; Erez et al. 1979b). Thus, elucidating the

chilling profile, properties, action spectrum, and biological role is key to deciphering the regulatory mechanism/s that underlie dormancy release as well as elucidating how plants adapt to extreme short or long chilling periods in different climates. Vernalization and its regulation have been intensively investigated in the winter annual *Arabidopsis* (Michaels and Amasino 1999; Sheldon et al. 1999) and also in wheat to a lesser degree (Hemming et al. 2008; Shimada et al. 2009). In these plants, different regulatory pathways arrest the floral developmental program prior to winter onset and prolonged exposure to chilling temperatures progressively releases this arrest, enabling plants to competently respond to favorable environmental stimuli that induce flowering in the spring (Fig. 4.1). This chilling-mediated regulation occurs at an epigenetic level in both species (Bastow et al. 2004; Sung and Amasino 2004a; Oliver et al. 2009). Because of the obvious parallels between vernalization and chilling requirement, the knowledge and progress made in understanding the regulation of the vernalization response should be directly translatable to dormancy research.

Diverse Dormancy Phenotypes Evolved in Plants

Winter Dormancy and its Regulation

Two environmental cues, photoperiod and cold temperature, serve as seasonal signals to induce dormancy in temperate perennials in the fall. Plants cease vegetative growth and set bud to enter the ecodormant state in early fall as an initial response, followed by transition into the endodormant state in later fall or early winter (Arora et al. 2003). Plants differentially respond to environmental cues. For example, peach and poplar (*Populus tremula*) respond to both shortening photoperiod and declining temperature while apple (*Malus domestica*) and pear (*Pyrus communis*) respond only to cold temperature (Nitsch 1957; Davis 2002; Song et al. 2010). However, once dormancy is established, plants exhibit similar behavior and remain in a resting state unless exposed to prolonged chilling (0–10°C), which is obligatory for dormancy release (Arora et al. 2003). The chilling requirement, which appears to be determined by genetic factors, varies from species to species or from cultivar to cultivar, even within the same species (Arora et al. 2003). In addition to chilling, other factors infrequently appear to also regulate dormancy release. For example, chilling treatment is not enough to break dormancy in beech (*Fagus sylvatica*) but additional exposure to long photoperiod (> 13 h) after chilling is fulfilled is required for dormancy break (Heide 1993). A short-term freezing treatment during the chilling period also promotes dormancy release in two birch species (*Betula pubescens* and *Betula pendula*) (Rinne et al. 1997, 2001). Dehydration treatment of dormant underground adventitious buds of leafy spurge also accelerated dormancy break (Dogramaci et al. 2011). Even a short-term exposure (2–5 h) to high temperature (40–45°C) accelerates the break of dormant apple, peach, and poplar buds (Wang

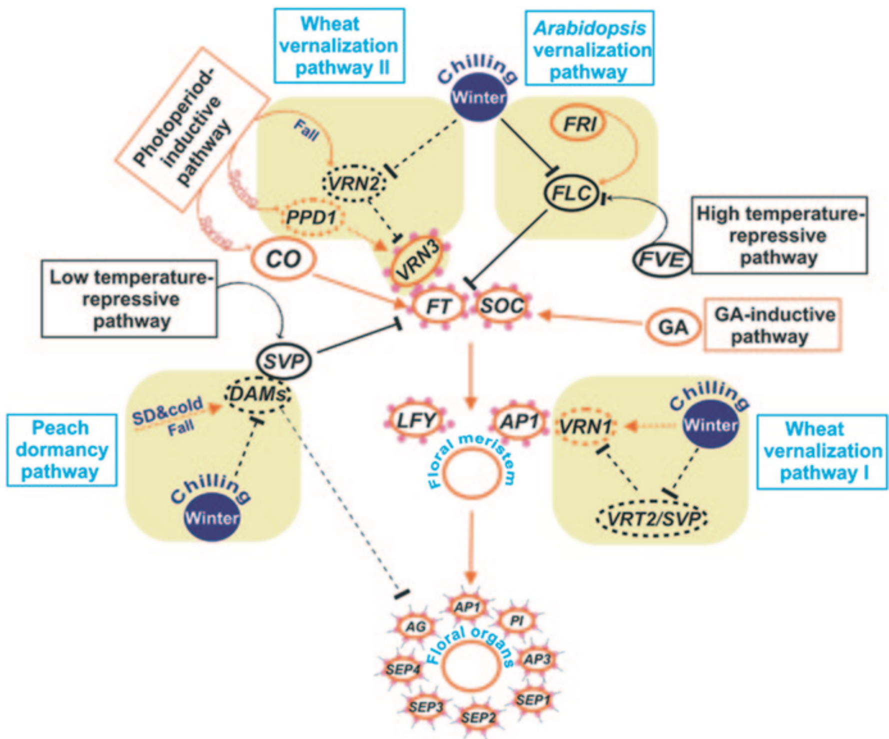


Fig. 4.1 Floral regulatory pathways and their relevance to vernalization in winter annuals and dormancy in temperate perennials. *Arabidopsis* floral integrators, *FT* and *SOC*, interact with and activate floral meristem identity genes *AP1*, *LFY* and others that promote the transition from vegetative to floral meristem, and subsequently give rise to sepal, petal, stamen and carpel structures by interacting with floral organ identity genes *AP3, AP1, PI, AG, SEPs* and others. In autonomous flowering *Arabidopsis* (or summer *Arabidopsis*), long-day photoperiod and GA induce flowering through the *CO-FT* and *GA-SOC* pathways, respectively. Low (5 °C to less than 23 °C) and high temperatures (>23 °C) inhibit flowering through the *SVP-FT* and *FVE-FLC-FT* repressive pathways, respectively. Three vernalization and dormancy pathways characterized in *Arabidopsis*, wheat and peach and their relevance to floral regulatory pathways are highlighted. In *Arabidopsis*, vernalization represses *FLC* constitutively through *FRI* in the *FRI-FLC-FT* pathway and induces flowering the following spring. In wheat, vernalization promotes flowering by releasing the *PPD1-VRN3/FT* pathway from repression by *VRN2* that down-regulates *VRN3/FT* expression in fall, and also activates *VRN1/AP1* that prior to winter, is repressed by *VRT2* coding for an SVP-like protein. In peach, short photoperiod and cold temperature in the fall up-regulate several *DAMs* coding for SVP-like proteins, concomitantly inducing dormancy, and chilling down-regulates the same *DAMs*, progressively releasing dormancy throughout winter or earlier spring. Flowering or growth activators are marked in *orange* (either *circle or lines*) while repressors are represented in *black*. Solid lines indicate either gene activation (*arrows*) or repression (*T symbol*) in *Arabidopsis* while *dashed lines* indicates the same gene interactions in wheat vernalization and peach dormancy pathways

and Faust 1994; Wisniewski et al. 1994). This high temperature-mediated dormancy release appears to be stage-dependent because the buds at early and later dormant stages are prone to break while those in deeper dormant stages rarely do (Nee 1986; Shirazi 1992; Siller-Cepeda et al. 1992; Wisniewski et al. 1994).

Summer Dormancy and Regulation

Many plants grown in regions with long dry and hot summers develop summer dormancy in order to avoid severe damage resulting from extreme water deficit, high temperature or both. Under the arid summer climate of southern California, a few species of perennial grasses (e.g., *Poa scabrella*, *Poa bulbosa*) become dormant even when supplied with water throughout the dry summer (Laude 1953; Ofir and Kigel 2007). Similarly, perennial herbaceous plants originating in bi-seasonal Mediterranean climates with an active growing season during the mild, rainy winter, and spring become dormant during the often long, hot, and dry summer and fall seasons. In contrast to winter dormancy species, long photoperiod and high temperatures in early summer induce plants to enter deep dormancy (Ofir and Kigel 1999), and exposure to temperatures of 40°C for two months releases plants from dormancy (Ofir 1986). Thus, summer dormancy is distinct from winter dormancy in terms of the environmental inducing and releasing factors, and deals with different stresses.

Drought Dormancy and Regulation

In tropical regions that lack conspicuous temperature fluctuation, there are no drastic seasonal changes and resident plants are able to grow all year around. However, in many regions such as Guanacaste, Cost Rica, where the growing season alternates with a long dry season that occurs from December to April, stem-succulent tree species develop dormancy-mediated water storage strategies to survive the extremely dry season. Trees that store a large quantity of water in a succulent stem during the growing season rapidly shed their leaves and enter a deep dormant state early in the dry season. The dormant but well-hydrated trees remain leafless for several months, and neither irrigation nor abnormal dry-season rain induces bud break, indicating that dormancy entry and exit is regulated by factors other than moisture conditions. Field and laboratory examination indicated that a subtle change in photoperiod appears to dictate the dormancy entry and exit process (Borchert and Rivera 2001). A photoperiod shorter than 12 h in December (early dry season) induces bud dormancy while a photoperiod of about 13 h in April (a few weeks before the major rains) triggers bud burst. Evidently, as short as a 1 h change of photoperiod orchestrates dormancy entry and exit. At low latitudes, where annual variation of daylight is less than 1 h, bud dormancy is induced and released by variations in photoperiod of less than 30 min (Borchert and Rivera 2001). Seasonal rainfall in the tropics is tightly linked with zenithal position of the sun. Declining and increasing photoperiod thus reflect the imminent end of the rainy and drought seasons, respectively. Hence, plants in tropical areas delicately exploit the subtle changes of photoperiod to regulate the growth to dormant transition for surviving the extreme dry season.

Fruit ripening Dormancy and Regulation

Winter pear (*Pyrus communis* L.) fruit-ripening arrest and resumption resembles the dormancy entry and exit process. Many pears, such as apple and peach, develop and ripen continuously during summer through fall, and become edible at harvest. However, winter pear is an exception. Fruit is not fully ripe and edible at harvest, and it appears that the ripening program is arrested at an early stage as judged by lack of a buttery-juicy texture, a key feature of ripe pear fruit. Yet, the fruit, after storage at a chilling temperature for 1–2 months, immediately resumes ripening when transferred to ambient temperature becoming edible in a few days (Villalobos-Acuńa and Mitcham 2008). Biochemical and molecular studies demonstrated that application of ethylene can substitute chilling treatment and restore fruit ripening suggesting that chilling treatment renders the under ripe fruit capable of producing ethylene, a key ripening promoting hormone, following the return to ambient conditions (EI-Sharkawy et al. 2003, 2004; Villalobos-Acuńa and Mitcham 2008). Evidently ethylene synthesis or production is developmentally suppressed, leading to arrest of fruit ripening in fall while a long period of chilling treatment can attenuate or erase such suppression. Thus in winter pear, ripening regulation has parallels to winter dormancy except that the fruit developmental program appears to trigger ripening arrest.

Dormancy Occurrence and Manifestation

Dormancy involves multiple and complex phenomenon, manifested in different forms or appearances that occur in the annual growth life cycle. Dormancy primarily happens in meristems to slow or arrest vegetative and/or reproductive growth in order to survive adverse stresses. It can also take place in seeds to control life cycle initiation in response to changes in environmental conditions. However, when and where dormancy occurs and to what extent tissues or organs are subjected to dormancy primarily depend on plant growth habit, structure and growth cycle as well as the geo-climates where plants reside. Annual species, for example, complete their life cycle during favorable seasons and pass unfavorable seasons as seeds, whereas perennial plants that live for multiple life cycles survive unfavorable conditions by arresting meristem growth and form special protective structures (e.g., bud scales) that are far more tolerant to stresses than growing meristems (Arora et al. 2003; Rinne et al. 2011). Even within perennials, woody and herbaceous species exhibit distinct growth patterns and life cycles. Woody species such as apple that live for several decades have above-ground parts directly exposed to winter temperatures, and only the above-ground meristems (both vegetative and floral) undergo winter dormancy (Arora et al. 2003) while the under-ground meristem (e.g., root) enters temporary growth suppression but is not dormant in winter as demonstrated by a quick reversal of the suppressed root growth by warm ambient temperature rather than chilling temperature (Rinne et al. 2011). On the contrary, perennial herbaceous

species lose almost all the above-ground parts in winter or hot summer, and only the under-ground parts survive; therefore, dormancy happens in the under-ground meristems. For example, underground adventitious buds (crown and root buds) of leafy spurge plants maintain paradormancy during active growth of the main aerial shoot, from early spring to late September, and generally enter endodormancy in October when the aerial parts of the plants have senesced or been killed by frost. The endodormant state is broken in November to early December and the underground adventitious buds transition to an ecodormant state; thus, sprouting is constrained by harsh environmental conditions until spring (Anderson et al. 2005; Chao et al. 2006). Hence, woody and herbaceous perennials share distinct spatio-temporal difference of dormancy behaviors.

Formation of dormant organs is another type of dormancy response to environmental stresses, which has been adopted by many geophyte species that initiate and develop special underground storage bulbs, tubers, corms, and rhizomes where the meristematic buds are embedded (Le Nard and De Hertogh 1993). In response to stress signals, bulbous plants switch their growth or development courses to initiate and form these bulbous organs that are considered concomitantly dormant organs during organ initiation. Bulbous storage organs and propagules remain dormant during the stressful season before regrowth. Many plants (e.g., iris and tulip bulbs and freesia corms) that have adapted to extreme continental climates with very hot, dry summers, cold winters, and springs with brief rain showers, adopt summer dormancy while others such as *Gladious hybridus*, *Lilium* spp. that adapt to a typical temperate climate with cold, dry winters adopt winter dormancy (Phillips and Rix 1989; Dole 2003). In general, winter dormancy in bulbous organs is broken by chilling and summer dormancy by heat (Ofir 1986; Rossi 1990; Dole 2003). Although both types of dormancy are generally induced by temperature, photoperiod also appears to act as a dormancy inducer in a few geophyte species. For example, a day length of 11–12 h or less triggers tuberous root formation in Dahlia hybrids (*D. coccinea* Cav. x *D. pinnata* Cav.), and the formed roots remain in dormancy unless exposed to chilling temperature for at least six weeks (Moser and Hess 1968).

Distinct dormancy behaviors of vegetative and floral buds in the same plant represent another feature of bulbous plants. The apical meristem in a mature tulip bulb is converted to a floral bud in summer, and high temperatures of summer immediately drive the formed floral buds into dormancy while the lateral buds remain vegetative (Boonekamp et al. 1990; Saniewski et al. 2000). In fall and winter, chilling temperatures release floral buds from dormancy while inducing a concomitant transformation of vegetative buds into dormant bulbs (Okubo 2000). Thus, two parallel but opposite dormancy responses occur in floral and vegetative buds: High temperature induces floral buds to enter dormancy and low temperature induces dormant floral buds to exit the dormant state, and vegetative buds enter the dormant state. Thus, bulbous species such as tulip have evolved sophisticated strategies enabling both sexual and asexual organs to survive the extremes of hot summers and cold winters.

Chilling, A Dormancy Break and Biological Regulator

Chilling is a compulsory requirement for breaking winter dormancy and cannot be substituted or omitted. Although chilling temperatures (0–15 °C) are necessary to break dormancy or drive the vernalization process, only a narrow spectrum of low temperatures (5–7.2 °C) are most effective (Erez et al. 1979b; Arora et al. 2003). Evidently, chilling temperature is an active biological regulator rather than a stress signal.

Chilling releases cells from arrested growth and elongation. One obvious dormancy feature is the lack of cell growth activity; thus, dormancy appears to arrest cell division by directly acting on the cell cycle checkpoint. For example, dormant potato tubers and terminal vegetative buds of woody perennials arrest at G1 phase of the cell cycle (Cottignies 1986; Campbell et al. 1996). Chilling releases this arrest, enabling cells to resume normal growth. In contrast, dormant tulip flower buds arrest at the floral stem elongation stage (Coleman 1987; Le Nard and De Hertogh 1993), and a chilling treatment of 0–9 °C for 10–12 weeks drives the arrested stems to slowly and progressively elongate through critical developmental stages, before major elongation can proceed in the following spring (Le Nard and De Hertogh 1993). Thus, chilling in this case regulates cell elongation processes.

Chilling drives floral developmental programming during the winter period. For example, dormant peach floral buds appear to progressively complete their differentiation and development throughout the chilling period as evidenced by observations that non-chilled peach floral buds remain almost undeveloped even at 20–23 °C for a few months. However, the fully chilled buds undergo visible morphological changes during the same period (Yamane et al. 2011b), suggesting that chilling stimulates the floral bud developmental programming. In fact, androecium/stamens were found to slowly develop throughout the entire chilling period while the gynoecium went through clear differentiation in late winter or near the end of dormancy (Luna et al. 1991). At the cellular level, the archesporal cells and epidermis, endothecium, middle layers, microsporangium walls and tapetum in the anther become distinct, followed by formation of the pollen mother cells that enter meiosis by later winter (Reinoso et al. 2002a; Julian et al. 2011). These results indicate that microsporogenesis and microgametogenesis occur during the chilling period, which are otherwise suppressed by warm temperature. Chilling effect on gynoecium/carpel development was also studied and a major developmental event occurs near the end of chilling period where two ovules form in the unicarpelar gynoecium, the site where sporogenesis and gametogenesis occur (Luna et al. 1990, 1991, 1993). Meanwhile, vascular connections between floral primordia and branch wood are completed by late winter, another indicator of floral development. The role of chilling in female organ differentiation is further elaborated by a study of high-chill cherry cultivars grown in a warmer region (e.g., Shanghai, China) that provides about 80 % of the required chilling period for dormancy break. The cherry floral buds do not finish gynoecium development, with ovule formation severely impaired or abolished while sepal, petal and anthers develop normally and pollen grains are viable (Wang et al. 2004; Zhang et al. 2014). Thus, chilling strongly influences female organ development at critical developmental stages near the end of

the chilling period, and the female organ remains under-developed if buds at these stages do not experience sufficient chilling. Apparently chilling does not simply release growth arrest but plays a role as a critical regulator of floral development.

Chilling requirement varies not only among cultivars or species, but also within different tissues or organs of the same plant. It is known that peach cultivars originating from different geo-climates have a wide spectrum of chilling requirement, ranging from 100 chilling hours (CH) for cultivars grown in warm regions to 1500 CH for those grown in regions with a long winter (Erez and Lavee 1971; Arora et al. 2003). In the peach cultivar "Redskin," lateral leaf buds require 1200 CH while terminal leaf buds require less than 500 CH for bud break (Erez et al. 1979a). Comparison of the chilling requirements of terminal and lateral leaf and floral buds indicated that terminal buds required the shortest chilling period followed by the lateral leaf buds, with the floral buds requiring the longest chilling period for dormancy break (Scalabrelli and Couvillon 1986). The same work also demonstrated that the lateral buds are most responsive to 7.2 °C chilling temperature while both floral and terminal buds are responsive to a relatively wide range of chilling temperatures (Scalabrelli and Couvillon 1986). Even within floral buds, stamens require a shorter chilling period than the gynoecium, as evidenced by cherry floral buds grown in a region where the winter provides only about 80 % of the required chilling. In this case, stamens produce 100 % viable pollen but the ovules are sterile or growth-impaired (Wang et al. 2004; Zhang et al. 2014). The cause of this tissue-specific chilling requirement remains unknown but bud developmental status, course and speed during the dormancy/chilling period may play a role. Indeed, terminal vegetative buds are complete and well developed before entry into dormancy (Luna et al. 1991) while development of dormant floral counterparts are far from complete but continue to proceed throughout the chilling period (Luna et al. 1990, 1991, 1993; Zhang et al. 2014). Conceivably, only floral buds that finish critical developmental stages in chilling conditions are capable of growth and flowering the following spring. Interestingly, in contrast to the terminal leaf buds, lateral leaf buds are not fully developed prior to chilling and this may be attributed to apical dominance suppression. This is consistent with the earlier observation that dormant lateral buds burst sooner or require fewer chilling hours when treated at high chilling temperatures with daily alternation between 6 °C for 16 h and 15ºC for 8 h compared to those at constant 4 °C (Erez et al. 1979a). These results suggest that lateral buds develop faster at higher chilling temperatures than at lower ones. Given that active developmental programming occurs during the chilling period, chilling likely acts as a biological regulator rather than a simple "on and off" switch.

Vernalization/chilling accelerate the plant vegetative to floral meristem switch. Many winter annual (e.g., winter *Arabidopsis*, wheat) and biennial species (e.g., celery, carrot) require a long period of exposure to low, non-freezing temperature (0–10 °C) for flowering induction (Amasino 2004). In the case of winter annuals, vegetative rosettes develop in the fall that overwinter and then flower the following spring to produce seed. However, winter annuals and biennials exhibit distinct differences in response to cold with the former responding to cold at all stages of development while the latter responds only at specific stages. Nevertheless, chilling during vernalization and chilling requirement for dormancy release act similarly as

biological regulators; in both cases, plants sense the duration of chilling temperature during fall and winter to determine when the meristem switch occurs (vernalization) or when dormancy begins or finishes (chilling requirement).

Chilling requirement exhibits an obligatory, slowly accruing time period. Chilling specifically targets meristematic cells in a cell-autonomous manner. During vernalization, meristematic cells sense and perceive chilling, and start division and growth to form tissues that appear to be critical for floral induction in the following spring (Metzger 1996). During dormancy release, chilling progressively releases arrested cell growth in peach vegetative buds as well as drives developmental programming through critical stages in peach floral organs, which are otherwise inhibited by warm temperature (Erez et al. 1979a, 1979b; Luna et al. 1990, 1991, 1993). The other feature is that chilling, unlike photoperiod, acts slowly, and it usually takes a few weeks to several months before it is manifested. Chilling also displays a fixed time requirement that can be accumulated dependent on the length of chilling period. For example, the chilling period (4–6˚C) interrupted by daily 8–12 h warm temperature (20–24˚C) fails to release dormancy in peach leaf buds (Erez et al. 1979b) or to vernalize "Petkus" rye for flower induction (Purvis and Gregory 1952) even though a total required chilling period or duration is met. This indicates that the effect of a short period of chilling is reversible under warm condition. However, a chilling period with a cycle of at least 2 chill days (4–6˚C) followed by a warm day (20–24˚C) behaves as effectively as an uninterrupted chill treatment period does in both vernalizing "Petkus" rye (Purvis and Gregory 1952) and releasing peach bud dormancy (Erez et al. 1979b). Hence, the chilling effect resulting from less than 12 chilling hours is unstable, not accumulated and is reversed by intermediate warm temperature exposure, while the effect of longer than 48 chilling hours becomes fixed or stabilized and is able to accumulate. In this case, the chilling effect is no longer reversible by periods of warm temperature for both vernalization and dormancy release. Considering that chilling acts stably and slowly in a quantitative and accumulated fashion, it mimics the epigenetically stable and accumulated nature of induced DNA and histone methylation or modification (Strahl and Allis 2000; Law and Jacobsen 2010), suggesting that a significant component of chilling-mediated regulation likely occurs at epigenetic level. In this regard, the fact that the chilling-induced meristem developmental switch and dormancy release are maintained only in one generation but cannot be passed onto the next further supports its epigenetic regulatory characteristic.

Floral Induction Pathways and Vernalization

Floral Regulation in Annual Species

The fact that vernalization is able to induce flowering indicates that prolonged chilling must activate or repress one of the floral induction pathways. In *Arabidopsis*, flower regulation has been extensively characterized and the key genes and

regulatory pathways have been elaborated in detail. Two floral meristem identity genes, *APETATA1* (*AP1*) and *LEAFY* (*LFY*), act redundantly to specify and initiate the floral meristem switch (Alvarez-Buylla et al. 2010) that progressively gives rise to sepal, petal stamen and carpel primordia. At least eight genes including *AP1*, *APETATA3* (*AP3*), *PISTILLATA* (*PI*), *AGAMOUS* (*AG*) and four *SEPALATAs* (*SEPs*) are involved in the regulation of four flower whorls, with *AP1* alone specifying sepal, *AP1* together with *PI* and *AP3* specifying petal, *AP3*, *PI* and *AG* specifying stamen, and *AG* alone specifying carpel identity (Irish 2010). Although *SEP1* and *SEP2* are required for the formation of all floral whorls, *SEP3* appears to be involved in petal, stamen and carpel formation while *SEP4* only in carpel formation (Alvarez-Buylla et al. 2010). *LFY* together with co-factors sets the spatial limits of expression of *AP3*, *PI* and *AG*. *LFY* also regulates the expression of *SEP1*, *SEP2* and *SEP3* (Alvarez-Buylla et al. 2010; Irish 2010). Both *AP1* and *LFY* closely interact with and redundantly regulate the floral organ identity genes to ensure flower initiation and later development proceeds continuously. *LFY*- and *AP1*-mediated floral initiation and regulation are highly conserved among plants and constitute a fundamental floral regulatory framework, in which both environmental and endogenous cues converge to orchestrate plant flowering (Fig. 4.1).

Upstream of the floral meristem identity genes are a group of genes known as floral integrators such as *FLOWER LOCUS T* (*FT*) and *SUPPRESSOR OF CONSTANS 1*(*SOC1*) that perceive and integrate environmental and developmental cues to regulate *AP1* and *LFY*, which in turn orchestrate flowering time. In nature, seasonal photoperiod and temperature as well as plant physiological change either individually or collectively dictate plant developmental pace and flowering time. Genetic and molecular studies in *Arabidopsis* have deciphered how these environmental cues or hormone signals regulate floral formation and flowering time. *Arabidopsis* is facultative to long day, and *CONSTANS* (*CO*), a key circadian component, perceives the seasonal change of photoperiod and transduces a regulatory signal to activate *FT* (Turck et al. 2008). *CO* transcription rises and falls over the course of a day producing an unstable protein in short photoperiods while under long photoperiods, *CO* transcription is extended in the longer daylight phase and CO protein is stabilized, thereby increasing *FT* expression (Hepworth et al. 2002; Wenkel et al. 2006). This photoperiod-*CO-FT* inductive pathway (Fig. 4.1) is generally conserved among long-day plants. In parallel, gibberellic acid (GA), a plant hormone that also acts as a flowering inducer in *Arabidopsis* directly interacts with *SOC* instead of *FT* and the activated *SOC* concomitantly up-regulates *LFY* and *AP1* to trigger floral formation (Moon et al. 2003, Fig. 4.1).

Two temperature-mediated flowering repression pathways in *Arabidopsis* have also been delineated (Fig. 4.1). One of them is the *FLC*-mediated repression of *FT* expression. In mutants with de-repressed *FLC*, plant flowering is delayed for a few months (Kim et al. 2004). Earlier studies demonstrated that *FLC* influences the period of the circadian clock, a number of temperature-responsive genes, and flowering at high but not low temperatures (Edwards et al. 2006; Salathia et al. 2006; Penfield 2008; Lee et al. 2013), indicating that *FLC* becomes functional only at high temperatures. Molecular analyses showed that *FLC* down-regulates the floral integrators *FT* and *SOC* by directly binding to their gene promoters (Helliwell et al.

2006; Searle et al. 2006). *FLC* is repressed during reproduction transition at the shoot apex (Kim et al. 2004), and this repression is mediated by *FVE* because *fve* mutation leads to constitutive expression of *FLC*, and delayed flowering (Kim et al. 2004). Since *FVE* codes for a homolog of the mammalian retinoblastoma-associated protein, a component of a histone deacetylase (HDAC) complex, *FVE*-mediated repression of *FLC* must act through histone acetylation and deacetylation of chromatin that may sense or respond to the fluctuation of ambient temperatures. Nevertheless, dynamic control of *FLC* by *FVE* represents a unique example of thermal regulation of flowering time in *Arabidopsis* (Fig. 4.1).

The other temperature-mediated flowering repression pathway in *Arabidopsis* is mediated by *SHORT VEGETATIVE PHASE* (*SVP)*. Genetic studies showed that mutants of *SVP* in *Arabidopsis* flower earlier at lower temperatures (Lee et al. 2013). Molecular analysis indicated that SVP forms a protein complex with FLM-β, one of the splicing variants from *FLOWERING LOCUS M* (*FLM*), to repress the expression of *FT* and *SOC* and flowering (Lee et al. 2013). But SVP protein is stabilized at 5 ˚C to 23 ˚C but destabilized at 27 ˚C or higher (Lee et al. 2013). Clearly, *SVP*-mediated flower repression is, in contrast to *FLC* repression, functional at lower temperature range. Interestingly, its stability is also influenced by other proteins or interacting partners. SVP protein accumulation is enhanced by *EARLY FLOWERING 3* (*ELF3*) (Yoshida et al. 2009) but reduced by *LATE ELONGATED HYPOCOTYL* (*LHY*) and *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*) (Fujiwara et al. 2008), suggesting that these genes either positively or negatively regulate SVP protein stability. Given that all three factors are components of circadian clock and photoperiod regulatory pathways, SVP stability and function may be under circadian or photoperiod regulation. *SVP* and *FLC* function in a partially redundant fashion by physically interacting with each other in a protein complex possibly with other MADS factors (Fujiwara et al. 2008; Li et al. 2008). Like *FLC*, *SVP* represses *FT* in leaves and *SOC1* in meristem (Jang et al. 2009). Thus, *SVP-FT/SOC1* and *FLC-FT/SOC1* serve in two parallel pathways to regulate flowering timing under different temperature regimes. The two pathways do, however, share distinct features. First, *SVP* but not *FLC* represses flower whorl B, C and E genes (Gregis et al. 2009). Second, *FLC* but not *SVP* is up-regulated in response to cool temperature (16ºC) (Blazquez et al. 2003; Lee et al. 2007) or intermittent temperatures fluctuating between 4ºC and 23ºC mimicking autumn temperature fluctuations (Kim et al. 2004). Third, *SVP* is stable at lower temperature (5–23ºc) while *FLC* is inactive at 16ºC or below but active at 23ºC or above (Lee et al. 2013), Hence, it becomes evident that these two repression pathways operate in different temperature ranges to complementarily regulate flowering time, and are accordingly termed as lower and higher temperature-mediated repressive pathways (Fig. 4.1).

Vernalization-Requiring Plants Hijack Different Flower Regulatory Pathways

Although most *Arabidopsis* plants used for research are rapid cycling and flower without a chilling requirement, winter *Arabidopsis* requires a chilling period for

normal flowering. Genetic analysis indicated that this chilling requirement is determined by two loci that were later identified as *FRIGIDA* (*FRI*) and *FLC* (Michaels and Amasino 1999; Sheldon et al. 1999). *FRI* acts solely to up-regulate *FLC* (Michaels and Amasino 2001), and FLC represses *FT*, *FD* and *SOC1* through direct binding to their *cis* regulatory regions (Helliwell et al. 2006; Searle et al. 2006). Flower production in plants without vernalization was induced by ectopic expression of these downstream floral integrators, thus bypassing the repressive effect of *FLC* and the vernalization requirement (Lee et al. 2000; Michaels et al. 2005) and delineating *FRI-FLC-FT/SOC* as the vernalization-mediated flowering regulatory pathway. Hence, the level of *FLC* expression is the primary determinant for vernalization requirement in *Arabidopsis*. *FLC* is down-regulated by prolonged cold exposure during vernalization (Sheldon et al. 2000). Once *FLC* transcription is stably repressed, its expression remains low even after plants are returned to warm conditions (Sheldon et al. 2000). This provides a "winter memory" and allows rapid flowering as temperature and daylight hours increase in spring. The "winter memory" is erased in the developing embryo, allowing the next generation plant to require vernalization (Sheldon et al. 2008). Although *SVP*, such as *FLC*, acts as a flowering repressor (Fig. 4.1), it does not play a role in the vernalization process because mutation or over-expression of *SVP* does not change or affect cold requirement for induction of flowering in winter *Arabidopsis* (Michaels and Amasino 1999; Sheldon et al. 1999; Bastow et al. 2004; Sung and Amasino 2004a). Hence, winter *Arabidopsis* specifically requires the *FLC*-mediated flower repression pathway to delay flowering until the following spring, thus avoiding winter damage.

Winter wheat and barley share similar flower arrest and promotion principles with *Arabidopsis* but exploit different strategies (Fig. 4.1). At least four genes, *VRN1*, *VRN2*, *VRN3* and *PPD1*, were genetically identified as key regulators for vernalization-induced flowering. The detailed analyses of different lines or cultivars revealed that *PPD1*, *VRN1* and *VRN3* act as flowering promoters while *VRN2* is a repressor (Hemming et al. 2008; Shimada et al. 2009). *VRN1* codes for floral meristem identity gene AP1, *VRN2* for a CO-like protein, *VRN3* for the floral integrator FT and *PPD1* for the circadian clock-regulated PRR7 factors, respectively (Turner et al. 2005; Yan et al. 2003, 2004, 2006). It appears that *VRN2*, *VRN3* and *PPD1* work in the same pathway while *VRN1* acts in a different one (Hemming et al. 2008; Shimada et al. 2009). Both *PPD1* and *VRN2* regulate *VRN3*/*FT* but in opposite manners to their *Arabidopsis* orthologues. *PPD1,* such as *CO* in *Arabidopsis*, senses and transduces the seasonal change of photoperiod cues as a regulatory signal to activate *FT*, while *VRN2* (*CO*-like gene) acts as a transcriptional repressor instead of an activator to repress *FT* expression in wheat. It is evident that *CO* in wheat has diverged from its counterpart in *Arabidopsis*, acting as a transcriptional repressor instead of a transcriptional activator, while *PPD1* is a new photoperiod sensor substituting for the *CO* function in wheat (Fig. 4.1). *VRN2* remains at a high level of expression prior to winter but declines during vernalization and is maintained a low level even after vernalization (Yan et al. 2004). Thus, the unique *VRN2* expression pattern puts the *PPD1-FT* flowering induction pathway under vernalization control, i.e., it is suppressed by *VRN2* prior to winter and only becomes competent following

a winter chilling period. On the contrary, the regulation of *VRN1*/*AP1* is relatively simple and is exclusively under control of vernalization, having expression at low levels prior to winter, up-regulation during vernalization and remaining high when plants are exposed to warm temperature following vernalization (Yan et al. 2003). This is opposite to that observed for *VRN2* and *Arabidopsis FLC*. Wheat *VEGETATIVE TO REPRODUCTIVE TRANSITION2* (*VRT2)* is orthologous to flower repressor *SVP* of *Arabidopsis*; however, it displays an exact opposite expression pattern to *VRN1* during vernalization (Kane et al. 2005). The detailed molecular analysis indicated that the *VRT2* product could bind the *VRN1* promoter and repress its expression (Kane et al. 2007), implicating *VRT2* as a repressor of *VRN1* prior to winter. Again, winter wheat and other temperate cereals modulate flowering regulatory pathways by imposing on them two unique chilling-responsive components (e.g., *PPD1*&*VRN*2-*VRN3* and *VRT2/SVP*-*VRN1/AP1*) with distinct regulatory strategies for precisely controlling flowering time and reproduction in response to seasonal change (Fig. 4.1).

Vernalization/Chilling-Mediated Gene Repression or Activation is Through Epigenetic Modification

Because chilling-mediated gene repression or activation can be stably maintained or memorized in the following seasons but does not pass onto the next generation, its regulation must occur partially or completely at the epigenetic level. Indeed, the changes in histone modification at key genes were detected during vernalization of both winter *Arabidopsis* and cereals (Bastow et al. 2004; Sung and Amasino 2004a; Oliver et al. 2009). It is known that trimethylation of lysine 4 at histone 3 (H3K4me3) is associated with active gene expression while trimethylation of lysine 27 at histone 3 (K3K27me3) is associated with a gene-repressed state. At *VRN1* chromatin in winter barley, the level of H3K4me3 is very low before vernalization but substantially increases during and after the winter chilling period while the repressive marker H3K27me3 shows the opposite trend (Oliver et al. 2009), indicating that vernalization simultaneously promotes H3K4me3 and represses H3K37me3 modifications. Thus, a high H3K27me3 and low H3K4me3 chromatin state prior to winter represses *VRN1/AP1* while the low H3K27me3 and high H3K4me3 state promotes *VRN1/AP1*expression. Histone methylation occurs at the regions spanning the gene promoter and first exon and intron but is particularly enriched at the 5' end of the first exon and adjacent 1st intron. A large deletion (up to 5.2 kb) at the 5' first intron results in significant decrease in H3K27me3 level and concomitant increase of *VRN1* expression in vernalized plants, suggesting that the first intron is critical for H3K27me3 modulation of *VRN1* expression (Oliver et al. 2009). No change of histone methylation state at either *VRN2* or *VRN3* chromatin was detected during chilling period (Oliver et al. 2009), suggesting that they may not be regulated by histone methylation.

Repression of *FLC* by the prolonged chilling temperature in winter *Arabidopsis* is also regulated by several epigenetic regulatory complexes that modify histones. Vernalization results in an increase in two repressive modifications at *FLC* chromatin: dimethylation of lysine 9 at histone 3 (H3K9me2) and H3K27me3 (Bastow et al. 2004; Sung and Amasino 2004a). These histone methylation markers require the PLANT-HOMEODOMAIN POLYCOMB REPRESOR COMPLEX 2 (PHD-PRC2), whose components include SWINGER (an E(Z) histone methyltransferase homologue) and vernalization-essential *VRN1*, *VRN2* (note that these *VRN1* and *VRN2* are different from wheat *VRN1* and *VRN2*), *VIN3* and *VIN3-LIKE 1* (*VIL1*) (Gendall et al. 2001; Bastow et al. 2004; Sung and Amasino 2004b; Greb et al. 2007). The PRC2 complex is thought to initiate H3K27me3 in a region close to the first exon and with a subsequent spread across the *FLC* genomic region during vernalization (Gendall et al. 2001; Bastow et al. 2004; Sung and Amasino 2004b; Finnegan and Dennis 2007; Greb et al. 2007; Angel et al. 2011); accumulation of H3K27me3 quantitatively correlates with the chilling units or period (Finnegan and Dennis 2007). The fact that both H3K9me2 and H3K27me3 in most of the *vrn1*, *vrn2*, *vin3*, *vil1*, *vrn5* mutants were not enriched at the *FLC* locus following a sufficient cold treatment suggests that the PRC2 complex can also methylate H3K9 (Bastow et al. 2004; Sung and Amasino 2004a). The vernalization-mediated stable enrichment of H3K27 methylation at *FLC* chromatin also requires the methylation of arginine 3 of H4 (H4R3me) (Schmitz et al. 2008). In addition, FVE also interacts with PRC2 complex to elevate H3K27 methylation at *FLC* chromatin during the regulation of flowering timing (Pazhouhandeh et al. 2011).

Recent evidence suggests that H3K27me3 at *FLC* is mediated by a long intronic non-coding RNA (COLDAIR). Association of COLDAIR triggers PRC2 targeting to *FLC*, a situation that leads to *FLC* repression during vernalization (Swiezewski et al. 2009; Heo and Sung 2011). Expression of *FLC* is partially controlled by miRNAs since mutations within the miRNA biogenesis genes *DICER-LIKE 1* (*DCL1*) and *DICER-LIKE 3* (*DCL3*) can lead to delayed flowering due to excessively high expression of *FLC* in these mutant backgrounds (Schmitz et al. 2007). Thus, long non-coding and miRNAs both play roles in the vernalization-mediated flowering.

Several histone modifications that actively promote *FLC* expression and an early flowering phenotype have also been identified during the analysis of autonomous flower regulatory pathways in *Arabidopsis*. Many of the altered flowering mutants (either late or early flowering phenotypes) display altered epigenetic states or modifications such as H3K4 trimethylation (H3K4me3), H3K36 di- and trimethylation (H3K36me2 and H3K36me3), H2B monoubiquitination (H2Bub) and deposition of H2A.Z at *FLC* chromatin (He 2009; Kim et al. 2009). Interestingly, *H2Bub1* is also required for maintaining the dormant state of seeds and mutation of *hub1* and *2* responsible for monoubiquitination of H2B led to partial abolishment of *Arabidopsis* seed dormancy that requires cold treatment (stratification) for dormancy release (Liu et al. 2007). The role of deposition of H2A.Z at *FLC* chromatin for maintaining active *FLC* transcription has been demonstrated in the mutants of *arp6* and *pie1* that encode components of the SWR1 complex involved in deposition of H2A.Z in chromatin (March-Diaz et al. 2007; Yoshida et al. 2010). Intriguingly, these mutants

also lost their sensitivity to ambient temperatures, behaving similarly to *fve* and *svp* mutants (Kim et al. 2004; Lee et al. 2007). This suggests that H2A.Z could serve as a potential molecular thermo-sensor. Whether H2A.Z mediates modification of chromatin state at *FLC* during dormancy remains an interesting topic. Evidently, epigenetic regulation of *FLC* is complex and it appears that multiple factors interact coordinately to establish a dynamic epigenetic balance at *FLC* chromatin to modulate flowering timing and response to cold temperature.

Global DNA methylation status also appears to influence the vernalization requirement. Although PRC2-mediated methylation of H3K27 at *FLC* chromatin is a primary determinant for vernalization, DNA methylation status of the genome also affects vernalization. Early research demonstrated that plants treated with the cytosine methyltransferase inhibitor 5-azacytidine (5-aza-C) promoted flowering in the vernalization-requiring *Arabidopsis* ecotype C24 (Burn et al. 1993; Dennis et al. 1998; Finnegan et al. 1998) and in wheat (Brock and Davidson 1994). This treatment was sufficient to substitute for the vernalization process. In *Arabidopsis*, there are at least 12 genes coding for methyl-CpG binding domain (*MBD*) proteins, and these proteins interact with other proteins to form chromatin modifier complexes that repress expression of many genes (Berg et al. 2003; Springer and Kaeppler 2005). For example, AtMBD7 interacts with arginine methyltransferase (PRMT11) (Scebba et al. 2007) and AtMBD5, 6 and 7 proteins co-localize *in vivo* and bind *in vitro* to the DECREASE IN DNA METHYLATION 1 (DDM1) protein (Zemach et al. 2008). Loss-of-function studies showed that the *atmbd9* mutant displays a pleiotropic phenotype that leads to a decrease in histone acetylation and an increase in DNA methylation at the *FLC* locus (Peng et al. 2006; Yaish et al. 2009). As a result, *FLC* transcript level declines, resulting in an early flowering phenotype. *AtMBD8* is also involved in controlling flowering in the winter ecotype C24, and mutation of *AtMBD8* leads to flowering delay under both long- and short-day photoperiod. While *FLC* expression is not affected in *atmbd8-1*, the expression of *FT* and *SOC1*, which are major flowering activators, is down-regulated in the mutant (Stangeland et al. 2009). The mechanism by which the expression of these genes is decreased in the *atmbd8-1* mutant has not yet been determined. Hence, although DNA methylation does not directly regulate *FLC*, it likely regulates genes that affect the H3K27 methylation process or *FLC*-repressed genes such as *FT*, *SOC* or other flower-promoting genes.

Dormancy and its Regulation in Temperate Woody Perennials

Dormancy in Peach is Controlled by a Group of **DAM** *Homologues*

Earlier research on dormancy has implicated plant hormones, especially GA and abscisic acid (ABA), as key dormancy regulators that orchestrate cell growth and cell

division-related processes (Hansen et al. 1999; Arora et al. 2003; Molmann et al. 2005; Rohde et al. 2007). In addition, water status of plant meristems is also critical for dormancy regulation. It was reported that bound water in peach buds increased with endodormancy, and both short photoperiod and low temperature could induce the conversion of water from a free to a bound state (Erez et al. 1998). Bud dormancy release has also been extensively studied to identify and understand dormancy-breaking signals (Quamme et al. 1995; Faust et al. 1997; Jian et al. 1997). With the advent of genomics tools, great progress has been made in the area of vernalization research and extensive investigations on dormancy regulatory mechanisms has generated a wealth of information delineating the general regulatory elements in perennials (Arora et al. 2003; Horvath et al. 2003; Rohde and Bhalerao 2007). However, to achieve a definitive picture of the genetic mechanism(s) involved in regulating dormancy, we need to use mutants, genomic tools and transgenic plants.

Identification and characterization of a peach ever-growing (*evg*) mutant, in which dormancy entry and exit is impaired, revealed for the first time that dormancy in peach and perhaps other woody perennials is controlled by a specific locus (Rodriguez-A et al. 1994), providing a critical genetic basis for dormancy research. In the *evg* mutant, apical shoot growth does not cease growth and enter dormancy regardless of temperature and photoperiod or seasonal changes in different geo-climates or regions. The *evg* cultivar can flower multiple times and bear two major crops a year in warmer regions (e.g., Tetela, Mexican) (Rodriguez-A et al. 1994). On the contrary, the low-chill peach cultivars (< 250 CU) cease growth and set buds in fall and flower once a year even when grown in the same area (Rodriguez-A et al. 1994). Thus, the *evg* mutant completely loses the typical features of dormancy behavior. Reciprocal crosses demonstrated that the *evg* trait is recessive compared to the deciduous (*DE*) trait, and controlled by a single locus as seen by a typical 3:1 segregation ratio of *DE* to *evg* growth habit in F2 progeny of crosses between *DE* and *evg* cultivars (Rodriguez-A et al. 1994). Due to lack of adequate genomics resources for peach, identifying and characterizing the *EVG* gene/s was extremely difficult and no major breakthrough occurred until significant genome information and genomics tools for peach became available.

The genetic nature of the *EVG* locus was first elucidated by Albert Abbott's group. After a decade of effort on genetic and physical mapping of the peach genome, the *EVG* locus was mapped to a 1-cM interval in the peach genome (Wang et al. 2002a, 2002b) and the *evg* mutation corresponded with a large deletion containing a tandem duplication comprised of six copies of a highly conserved gene coding for MADS-box protein (Bielenberg et al. 2004). These genes were accordingly termed as *DORMANCY-ASSOCIATED MADS-BOX* (*DAM*) gene 1, 2, 3, 4, 5 and 6, respectively (Bielenberg et al. 2008). In the *evg* mutant, the deletion removes four of the six duplicated genes and silences the other two. Gene expression analysis in terminal vegetative buds in wild-type plants indicated that *DAM1*, *DAM2* and *DAM4* are up-regulated during seasonal elongation cessation and bud formation while *DAM5* and *DAM6* are up-regulated during dormancy development and then down-regulated during the winter chilling period (Li et al. 2009; Jiménez et al. 2010). Interestingly, *DAM1* and *DAM6* also displayed increased expression

in response to short photoperiod (Li et al. 2009). All of these results suggest that six *DAM* genes are collectively involved in dormancy onset and exit, with *DAM1*, *DAM2* and *DAM4* likely involved in vegetative growth cessation and terminal bud formation (ecodormant stage) and *DAM5* and *DAM6* involved in dormancy development (transition from ecodormancy to endodormancy) including maintenance and release. *DAM5* and *DAM6* are similarly up-regulated in lateral leaf, floral and lateral floral buds throughout fall, and down-regulated during winter, again coincident with dormancy development and release (Yamane et al. 2011a, b). The application of the dormancy-breaking reagent cyanimide not only induces early lateral leaf bud break but also down-regulates *DAM5* and *DAM6* (Yamane et al. 2011a), again suggesting that they play a key role in dormancy release. Direct evidence for *DAM*s' role in dormancy regulation is from a transgenic study (Sasaki et al. 2011) where ectopic expression of *DAM6* in poplar promoted growth cessation and bud set under environmental conditions that maintain rapid vegetative growth in controls. It was also found that transformation rate is very low, and the transgenic lines have much shorter shoots, confirming *DAM6* as a growth repressor. Once the transgenic lines enter dormancy, they remain dormant much longer than control plants (Sasaki et al. 2011). A major QTL trait responsible for chilling requirement in peach is closely associated with the *DAM5* and *6* loci as well (Fan et al. 2010; Zhebentyayeva et al. 2014). Collectively, these results confirm *DAM5* and *DAM6* as key dormancy regulators. However, the fact that peach cultivars carrying the mutation of both *DAM5* and *DAM6* still proceed into normal dormancy entry and exit, albeit they require only a short period of for dormancy release (Yamane et al. 2011c; Zhebentyayeva et al. 2014), strongly suggests that *DAM*s other than *DAM5* and *DAM6* are also involved in dormancy regulation.

*DAM*s are also likely to be involved in the regulation of seed dormancy in peach. The seed chilling requirement is often correlated with bud chilling requirement in temperate fruit trees, and a cultivar that requires a longer chilling period for bud dormancy break also requires a longer chilling period for seed dormancy break (Westwood and Bjornstad 1968; Kester 1969; Pasternak and Powell 1980; Kretzschmar et al. 2011). Hence, bud and seed dormancy likely share a common regulatory mechanism. Conceivably, all or specific *DAM*s could play a role in stratification through differential regulation during seed dormancy entry or release. Consistent with this prediction, the expression of *DAM1* and *DAM6* in peach seeds is substantially decreased during stratification (moist chilling of seeds) while *DAM4* and *DAM5* remain relatively unchanged (Leida et al. 2012b). Thus, two of six *DAM*s are differentially regulated during seed dormancy release or chilling treatment.

DAM-mediated dormancy regulation is conserved in other temperate woody and even herbaceous perennials. Differential regulation of *DAM*s by chilling temperatures during dormancy release has been reported in Japanese apricot (*Prunus mume*) (Sasaki et al. 2011), Japanese pears (*Pyrus pyrifolia* Nakai) (Saito et al. 2013), potato (Campbell et al. 2008), raspberry (*Rubus idaeus*) (Mazzitelli et al. 2007) and kiwifruit (*Actinidia spp*) (Wu et al. 2014). Ectopic expression of apricot *DAM6* in poplar (*Populus tremula* × *Populus tremuloides*) promotes dormancy onset in the transgenic lines (Sasaki et al. 2011), indicating that apricot and poplar likely share the similar regulatory factor or mechanism. Even the herbaceous perennial leafy

spurge shows a *DAM*-dependent regulation of dormancy entry and exit (Horvath et al. 2010).

Peach DAM*s Exploit Arabidopsis* SVP *Repression Function but Exhibit Distinct Properties*

Phylogenetic analyses demonstrated that all six *DAM* genes clustered in the same genomic region are closely related to the *Arabidopsis floral repressor SHORT VEGETATIVE PHASE* (*SVP*) (Jiménez et al. 2009), one of the two key genes that dictates flowering repressive pathways in *Arabidopsis* (Fig. 4.1). Recent genetic and molecular analyses unveiled several unique regulatory properties of *SVP* at the transcriptional and post-translational levels, which could contribute to the complexity of the *DAM*-mediated dormancy regulatory mechanism evolved in peach and other woody species. First, *SVP* serves as a floral repressor and effectively functions at low temperatures (Lee et al. 2013, Fig. 4.1), consistent with dormancy onset and exit occurring only under low-temperature condition. Second, SVP protein physically interacts with flower whorl B, C and E genes to possibly regulate floral organ formation and development in *Arabidopsis* (Gregis et al. 2009); and such interaction is critical for *DAM*-mediated arrest of floral organ development prior to winter (Luna et al. 1990, 1991, 1993; Reinoso et al. 2002a, 2002b; Julian et al. 2011). Third, SVP protein as a master transcriptional regulator can bind and regulate minimally a 1000 genes in *Arabidopsis* that are involved in an array of biological processes including floral development, growth regulator signaling, basic cellular and metabolic processes, protein modification, reproduction, morphology, and others (Gregis et al. 2013). This probably bestows *DAM* genes the ability to simultaneously coregulate a large number of genes during dormancy onset and release (Leida et al. 2012a, b; Zhong et al. 2013), ensuring that the dormancy process proceeds correctly both sequentially and temporarily. Fourth, SVP protein stability is sensitive to temperature fluctuation, becoming unstable at higher temperature (Lee et al. 2013), making it impossible for *DAM*s to induce dormancy during the growing season even when they are accidently activated due to gene and protein mutations or transient low temperature. Finally, SVP protein stability is also influenced by circadian clock or photoperiod factors (Fujiwara et al. 2008; Yoshida et al. 2009), providing an additional layer of regulatory complexity for dormancy induction. Thus, it is not difficult to understand why peach adopts *SVP* rather than *FLC* as its dormancy regulator to deal with this complex regulatory process. It is also noteworthy that *SVP* and *DAM*s do not share identical functions, with the former primarily repressing flowering transition in *Arabidopsis* while the latter promotes dormancy of the already-established floral meristems in peach. Additionally, *DAM* genes arrest vegetative growth in peach, which has not been reported for *SVP* in *Arabidopsis*. Evidently, peach *DAM*s exploit the *SVP* repression function and inherent regulatory features but evolve novel, distinct properties to meet the complex regulatory needs of dormancy onset, development and release of both vegetative and floral buds in peach (Fig. 4.1, 4.2).

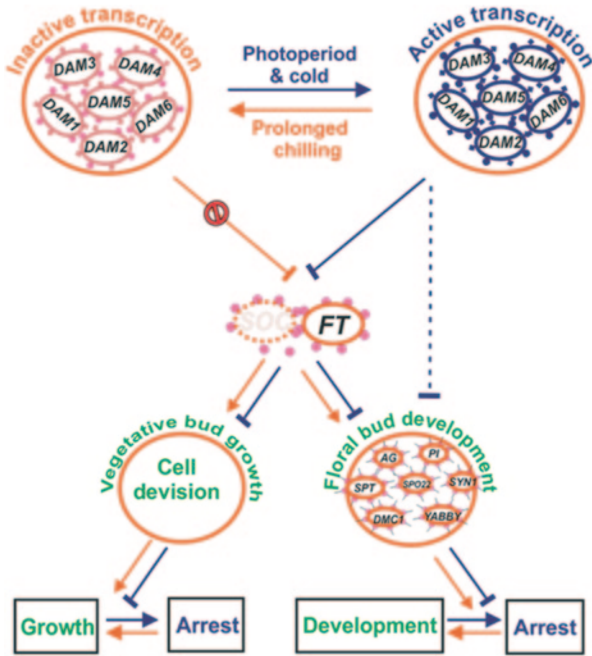


Fig.4.2 Schematic diagram of *DAMs*-mediated dormancy regulation in peach. *DAM*s that are up-regulated by photoperiod and cold temperature in fall are likely regulated through genetic and epigenetic regulation (e.g., DNA and histone modification), repress *FT* and possibly *SOC* or others to arrest or slow down cell growth and development, leading plants to enter the endodormant state. During winter, chilling temperature progressively down-regulates *DAM*s via possible DNA methylation and repressive histone methylation (e.g., H3K27), gradually releasing both vegetative and floral buds from growth arrest and enabling them to resume growth in response to warm temperature in the spring. *DAM*s may directly interact with, or compete with, *FT* or *SOC*, to repress genes regulating floral organ development. *Blue lines* represent gene interactions to promote dormancy and *orange lines* to release dormancy while the *dashed lines* indicate the potential repression of the floral organ identity genes by *DAMs*. The symbol "Φ" represents the blockage of the *DAMs*-mediated repression of *FT* and *SOC*

DAM *Genes and* FT *Constitute a Major Dormancy Regulatory Module/Pathway in Peach and Others*

In *Arabidopsis*, *SVP*, such as *FLC*, interacts with floral integrators *FT* and *SOC*, to repress flowering (Fig. 4.1) and this interaction is through direct binding of SVP factor to the *FT* and *SOC* promoters; however, little is known about whether DAM proteins directly interact with *FT* to regulate dormancy in peach and other woody species. The behavior of *FT*-over-expressed transgenic poplar suggests that *FT* plays an important role in dormancy regulation. Over-expression of poplar *FT (PtFT)* shortens the aspen's juvenile phase and induces earlier flowering under long-day photoperiods (Hsu et al. 2006), while down-regulation of *PtFT* by RNAi

leads to growth cessation and bud set independently of photoperiod (Rohde et al. 2011). More convincing evidence comes from observations on the lack of dormancy behavior in transgenic plum (*P. domestica*), a close relative of peach. Transgenic lines over-expressing *PtFT* not only show earlier flowering but also continuous vegetative growth and flowering in the greenhouse regardless of seasonal changes (Srinivasan et al. 2012). Even under field condition, these lines continuously flower and grow vegetatively until late fall with no growth cessation or vegetative terminal bud formation (Ralph Scorza, personal communication). This indicates that over-expressed *PtFT* in plum can override *DAM* gene function and must accordingly act downstream of *DAM*. Whether DAM protein regulates *FT* through direct binding to the *FT* promoter as seen with SVP in *Arabidopsis* or through protein–protein interaction remains to be determined. In addition, little is known about whether other floral integrators such as *SOC* also act downstream of *DAM*. Nevertheless, the rudiment of *DAM-FT* as a main dormancy regulatory pathway in peach and other temperate woody species seems likely (Fig. 4.2).

It is known that cold temperature and photoperiod induce dormancy and should act upstream of the *DAM-FT* pathway. How these signals are perceived and the response of the *DAM*s is modulated is not fully understood yet. Earlier experiments implicate PHYTOCHROME A (PHYA) and CO as potential photoperiod mediators in poplar, likely relaying signals to regulate *DAM* genes (Bohlenius et al. 2006; Kozarewa et al. 2010). Indeed, extensive analysis of the entire *DAM* regions identified dozens of binding sites for circadian CCA1 and cold-responsive CBF factors (data not shown), respectively, suggesting that circadian clock/photoperiod and cold signals may directly act on and regulate *DAM*s. Given that there are multiple *DAM*s sharing the feature of large introns that often serve as regulatory sites in many *MADS-box* genes (e.g., *AG*, *FLC*), further characterization of protein-DNA binding and interaction at the *DAM* gene regions will provide insight into how *DAM*s are regulated by upstream regulators and/or environmental cues.

Epigenetic Regulation of DAM*s During Dormancy Onset and Exit*

Given that dormancy, such as vernalization, is an epigenetic phenomenon, it must be regulated at least in part by epigenetic mechanisms that modify DNA or histone states. The research on dormancy regulation at the epigenetic level lags far behind vernalization research in *Arabidopsis*, and the detailed regulatory mechanisms remain largely unknown (Ríos et al. 2014). Although a series of transcriptome studies have identified numerous genes related to DNA or histone modifications or chromatin remodeling (Ruttink et al. 2007; Karlberg et al. 2010; Santamaria et al. 2011), whether these genes play an epigenetic regulatory role remains to be validated or elaborated at the molecular level. The first evidence to implicate epigenetic regulation in dormancy came from the analysis of acetylated H4 histone and genomic DNA methylation patterns during bud set and burst in chestnut (*Castanea sativa*), with a higher level of DNA methylation and a lower level of acetylated H4 histone detected in dormant buds with respect to actively growing tissues (Santamaria et al.

2009). It is still unknown whether these modifications occur at specific genomic regions or groups of genes such as the *DAM*s. Leida et al (2012a) recently analyzed H3K4me3 and H3K27me3 marks on *DAM6* chromatin in peach floral buds and revealed an opposite methylation pattern between two marks, with H3K4me3 declining and H3K27me3 increasing during the chilling period that becomes particularly substantial in the region near the translation start codon. As expected, high-chill cultivars display an increased level of H3K27me3 at the end of dormancy compared to low-chill cultivars (Leida et al. 2012a), and the level of H3K27me3 is inversely proportional to *DAM6* expression, consistent with its gene repression role. It is noted that H3K27me3 accumulation happens at the very end of the chilling period but not throughout the entire chilling period, which appears to be different from the progressive H3K27me3 accumulative pattern at *FLC* chromatin during vernalization (Angel et al. 2011). Currently, there is no report of epigenetic modification occurring at the rest of *DAM*s loci. Nevertheless, at least *DAM6* in peach appears, such as *FLC* in *Arabidopsis*, to be regulated by chilling temperature through histone methylation. However, the role of DNA methylation in the regulation of *DAMs* remains limited. In pear, three *DAM* paralogs display a similar up- and down-regulation pattern during dormancy establishment and release (Saito et al. 2013), resembling peach *DAM*s regulation (Li et al. 2009), but no evident change of DNA methylation in buds was detected during dormancy onset, maintenance and release (Saito et al. 2013).

*DAM*s might be regulated by other histone modifications as well. Hydrogen cyanamide (HC) that inhibits catalase activity serves as a potent bud dormancy breaking agent, and it is generally believed that this chemical agent induces oxidative stress that accelerates dormancy break (Halaly et al. 2008). Prior work also indicated that such treatment stimulates the consumption of oxygen and the respiratory activity that further depletes oxygen sources in tissue (Brennan et al. 1978). The oxidative stress in turn regulates gene expression and this regulation primarily occurs at the histone acetylation level (Tsuji et al. 2006) as evidenced by the fact that submerging plants or depleting oxygen increases H3 acetylation and H3K4me3, two transcriptionally active chromatin marks. Conceivably, chilling might, such as HC, influence oxidative stress in buds, which may in turn affect histone acetylation and methylation state on the *DAM*s' chromatin.

Compared to dormancy release, little is known about the regulatory mechanisms underlying dormancy induction by cold in peach or other woody species. In strawberry, exposure of plants to 12 or 15 °C for up to 4 weeks can induce genome-wide up-methylation but methylation induced by 15 °C is photoperiod dependent and occurs only under short photoperiod but not long photoperiod (Zhang et al. 2012). This suggests that both cold temperature and photoperiod are involved in concomitant methylation and dormancy induction. Detection of increased levels of DNA methylation in dormant chestnut buds further supports the role of DNA methylation in dormancy induction (Santamaria et al. 2009). Cold temperatures might also regulate histone modification through the mode of action of ABA because cold-induced dormancy is concomitant with ABA increase and ABA alone acts as a sole dormancy inducer in many species (Hansen et al. 1999; Arora et al. 2003; Molmann

et al. 2005; Rohde et al. 2007). ABA has been shown to down-regulate *Arabidopsis HISTONE DEACETYLASE 2C* (*AtHD2C*) (Sridha and Wu 2006), and up-regulate *HIGH EXPRESSION OF OSMOTIC STRESS RESPONSIVE GENES 15* (*HOS15)* that codes for a H4 deacetylase (Zhu et al. 2007). Thus, cold temperature might influence different epigenetic regulatory mechanisms during dormancy induction.

There is little information on the mechanism underlying short photoperiod-induced bud dormancy and its possible epigenetic regulation. Currently, there is no report on this subject; however, photoperiod-induced epigenetic changes during seed dormancy might provide clues for understanding the mechanism underlying bud dormancy induction. Seeds, such as buds, enter dormant state when matured, and breaking the seed dormancy requires chilling and/or red light, depending on the species. The seeds of some but not all lettuce cultivars are very sensitive to light for germination, and even a single pulse of a red light is enough to break seed dormancy (Berrie et al. 1966). A recent study in *Arabidopsis* revealed that light directly activates genes involved in GA synthesis in the seed through the regulation of histone modification (Cho et al. 2012). In the dark, the histone arginine in chromatin of *GA-3ox1/GA30x2,* coding for a key GA synthesis enzyme, is constantly up-methylated, and this increased arginine methylation (a repressed gene mark) represses *GA3ox1/GA30x2* expression, subsequently repressing GA production and seed germination. However, in light, the level of the histone arginine methylation is decreased, thereby de-repressing *GA3ox1/GA30x2* and GA production. Molecular analysis revealed that light-mediated down-methylation of histone arginine is achieved by *JUMONJI* 20 (*JMJ20*) and *JUMONJI 22* (*JMJ22*), both coding for functionally redundant histone arginine demethylases. In light, Phytochrome B (PHYB) is activated (Shinomura et al. 1994) and the activated PHYB up-regulates *JMJ20* and *JMJ22* that in turn accelerates the demethylation of the histone arginine and release *GA3ox1/GA30x2* from repression. Given that PHYA appears to be required for dormancy induction by photoperiod (Kozarewa et al. 2010), bud dormancy in woody or other perennials might exploit photoperiod-mediated histone modification as one of the mechanisms to regulate dormancy induction.

References

Alvarez-Buylla ER, Benítez M, Corvera-Poiré A, Chaos Cador A, de Folter S, Gamboa de Buen A, Garay-Arroyo A, García-Ponce B, Jaimes-Miranda F, Pérez-Ruiz RV, Piñeyro-Nelson A, Sánchez-Corrales YE (2010) Flower development. Arabidopsis Book 8:e0127

Amasino R (2004) Vernalization, competence, and the epigenetic memory of winter. Plant Cell 16:2553–2559

Anderson JV, Gesch RW, Jia Y, Chao WS, Horvath DP (2005) Seasonal shift in dormancy status, carbohydrate metabolism and related gene expression in crown buds of leafy spurge. Plant Cell Environ 28:1567–1578

Angel A, Song J, Dean C, Howard MA (2011) Polycomb-based switch underlying quantitative epigenetic memory. Nature 476:105–108

Arora R, Rowland LJ, Tanino K (2003) Induction and release of bud dormancy in woody perennials: a science comes of age. HortScience 38:911–921

Bastow R, Mylne JS, Lister C, Lippman Z, Martienssen RA, Dean C (2004) Vernalization requires epigenetic silencing of FLC by histone methylation. Nature 427:164–167
Berg A, Meza TJ, Mahić M, Thorstensen T, Kristiansen K, Aalen RB (2003) Ten members of the *Arabidopsis* gene family encoding methyl-CpG-binding domain proteins are transcriptionally active and at least one, *AtMBD11*, is crucial for normal development. Nucleic Acids Res 31:5291–5304
Berrie AMM (1966) The effect of temperature and light on the germination of lettuce seeds. Physiol Plant 19:429–436
Bielenberg DG, Wang Y, Fan S, Reighard GL, Scorza R, Abbott AG (2004) A deletion affecting several gene candidates is present in the peach evergrowing mutant. J Hered 95:436–444
Bielenberg DG, Wang Y, Li ZG, Zhebentyayeva T, Fan SH, Reighard GL, Scorza R, Abbott AG (2008) Sequencing and annotation of the evergrowing locus in peach [*Prunus persica* (L.) Batsch] reveals a cluster of six MADS-box transcription factors as candidate genes for regulation of terminal bud formation. Tree Genet Gen 4:495–507
Blazquez MA, Ahn JH, Weigel D (2003) A thermosensory pathway controlling flowering time in *Arabidopsis thaliana*. Nat Genet 33:168–171
Bohlenius H, Huang T, Charbonnel-Campaa L, Brunner AM, Jansson S, Strauss SH, Nilsson O (2006) *CO/FT* regulatory module controls timing of flowering and seasonal growth cessation in trees. Science 312:1040–1043
Boonekamp PM, Beijersbergen JCM, Franssen JM (1990) The development of flowering assays for cold-treated tulip bulbs. Acta Hortic 266:177–181
Borchert R, Rivera G (2001) Photoperiodic control of seasonal development and dormancy in tropical stem-succulent trees. Tree Physiol 21:213–221
Brennan T, Willemsen R, Rudd T, Frenkel C (1978) Interaction of oxygen and ethylene in the release of ragweed seeds from dormancy. Bot Gazette 139:46–49
Brock RD, Davidson JL (1994) 5-Azacytidine and gamma-rays partially substitute for cold treatment in vernalizing winter-wheat. Environ Exp Bot 34:195–199
Burn JE, Bagnall DJ, Metzger JD, Dennis ES, Peacock WJ (1993) DNA methylation, vernalization, and the initiation of flowering. Proc Natl Acad Sci U S A 90:287–291
Campbell MA, Suttle JC, Sell TW (1996) Changes in cell cycle status and expression of p34cdc2 kinase during potato tuber meristem dormancy. Physiol Plant 98:743–752
Campbell M, Segear E, Beers L, Knauber D, Suttle J (2008) Dormancy in potato tuber meristems: chemically induced cessation in dormancy matches the natural process based on transcript profiles. Funct Integr Genomics 8:317–328
Chao WS, Serpe MD, Anderson JV, Gesch RW, Horvath DP (2006) Sugars, hormones and environment affect the dormancy status in underground adventitious buds of leafy spurge (*Euphorbia esula*). Weed Sci 54:59–68
Cho JN, Ryu JY, Jeong YM, Park J, Song JJ, Amasino RM, Noh B, Noh YS (2012) Control of seed germination by light-induced histone arginine demethylation activity. Dev Cell 22:736–748
Coleman WK (1987) Dormancy release in potato tubers: a review. Amer Potato J 64:57–68
Cottignies A (1986) The hydrolisis of starch as related to the interruption of dormancy in the ash bud. J Plant Physiol 123:381–388
Davis SJ (2002) Photoperiodism: the coincidental perception of the season. Curr Biol 12:R841–843
Dennis ES, Bilodeau P, Burn J, Finnegan EJ, Genger R, Helliwell C, Kang BJ, Sheldon CC, Peacock WJ (1998) Methylation controls the low temperature induction of flowering in *Arabidopsis*. Symp Soc Exp Biol 51:97–103
Dogramaci M, Horvath DP, Christoffers MJ, Anderson JV (2011) Dehydration and vernalization treatments identify overlapping molecular networks impacting endodormancy maintenance in leafy spurge crown buds. Funct Integr Genomics 11:611–626
Dole JM (2003) Research approaches for determining cold requirements for forcing and flowering of geophytes. HortScience 38:341–346
Edwards KD, Anderson PE, Hall A, Salathia NS, Locke JCW, Lynn JR, Straume M, Smith JQ, Millar AJ (2006) *FLOWERING LOCUS C* mediates natural variation in the high-temperature response of the *Arabidopsis* circadian clock. Plant Cell 18:639–650

El-Sharkawy I, Jones B, Li ZG, Lelievre JM, Pech JC, Latche A (2003) Isolation and characterization of four ethylene perception elements and their expression during ripening in pears with/without cold storage. J Exp Bot 54:1615–1626

El-Sharkawy I, Jones B, Gentzbittel L, Lelievre JM, Pech JC, Latche A (2004) Differential regulation of ACC synthase genes in cold-dependent and independent ripening in pear fruit. Plant Cell Environ 27:1197–1210

Erez AG, Lavee S (1971) The effect of climatic conditions on dormancy development of peach buds. Proc Amer Soc Hort Sci 96:711–714

Erez A, Couvillon A, Hendershott CH (1979a) Quantitative chilling enhancement and negation in peach buds by high temperatures in a daily cycle. J Amer Soc Hort Sci 104:536–540

Erez A, Couvillon A, Hendershott CH (1979b) The effect of cycle length on chilling negation by high temperatures in dormant peach leaf buds. J Amer Soc Hort Sci 104:573–576

Erez A, Faust M, Line MJ (1998) Changes in water status in peach buds on induction, development and release from dormancy. Sci Hortic 73:111–123 (Amsterdam)

Fan S, Bielenberg DG, Zhebentyayeva TN, Reighard GL, Okie WR, Holland D, Abbott AG (2010) Mapping quantitative trait loci associated with chilling requirement, heat requirement and bloom date in peach (*Prunus persica*). New Phytol 185:917–930

Faust M, Erez A, Rowland LJ, Wang SY, Norman HA (1997) Bud dormancy in perennial fruit trees: physiological basis for dormancy induction, maintenance, and release. HortScience 32:623–629

Finnegan EJ, Dennis ES (2007) Vernalization-induced trimethylation of histone H3 lysine 27 at FLC is not maintained in mitotically quiescent cells. Curr Biol 17:1978–1983

Finnegan EJ, Genger RK, Kovac K, Peacock WJ, Dennis ES (1998) DNA methylation and the promotion of flowering by vernalization. Proc Natl Acad Sci U S A 95:5824–5829

Fujiwara S, Oda A, Yoshida R, Niinuma K, Miyata K, Tomozoe Y, Tajima T, Nakagawa M, Hayashi K, Coupland G, Mizoguchi T (2008) Circadian clock proteins LHY and CCA1 regulate SVP protein accumulation to control flowering in *Arabidopsis*. Plant Cell 20:2960–2971

Gendall AR, Levy YY, Wilson A, Dean C (2001) The *VERNALIZATION 2* gene mediates the epigenetic regulation of vernalization in *Arabidopsis*. Cell 107:525–535

Greb T, Mylne JS, Crevillen P, Geraldo N, An HL, Gendall AR, Dean C (2007) The PHD finger protein VRN5 functions in the epigenetic silencing of *Arabidopsis FLC*. Curr Biol 17:73–78

Gregis V, Sessa A, Dorca-Fornell C, Kater MM (2009) The *Arabidopsis* floral meristem identity genes AP1, AGL24 and SVP directly repress class B and C floral homeotic genes. Plant J 60:626–637

Gregis V, Andrés F, Sessa A, Guerra RF, Simonini S, Mateos JL, Torti S, Zambelli F, Prazzoli GM, Bjerkan KN, Grini PE, Pavesi G, Colombo L, Coupland G, Kater MM (2013) Identification of pathways directly regulated by *SHORT VEGETATIVE PHASE* during vegetative and reproductive development in *Arabidopsis*. Genome Biol 14:R56

Halaly T, Pang X, Batikoff T, Crane O, Keren A, Venkateswari J, Ogrodovitch A, Sadka A, Lavee S, Or E (2008) Similar mechanisms might be triggered by alternative external stimuli that induce dormancy release in grape buds. Planta 228:79–88

Hansen E, Olsen JE, Junttila O (1999) Gibberellins and subapical cell divisions in relation to bud set and bud break in Salix pentandra. J Plant Growth Regul 18:167–170

He YH (2009) Control of the transition to flowering by chromatin modifications. Mol Plant 2:554–564

Heide OM (1993) Dormancy release in beech buds (*Fagus sylvatica*) requires both chilling and long days. Physiol Plant 89:187–191

Helliwell CA, Wood CC, Robertson M, Peacock WJ, Dennis ES (2006) The *Arabidopsis* FLC protein interacts directly in vivo with SOC1 and FT chromatin and is part of a high-molecular-weight protein complex. Plant J 46:183–192

Hemming MN, Peacock WJ, Dennis ES, Trevaskis B (2008) Low-temperature and daylength cues are integrated to regulate *FLOWERING LOCUS T* in barley. Plant Physiol 147:355–366

Heo JB, Sung S (2011) Vernalization-mediated epigenetic silencing by a long intronic noncoding RNA. Science 331:76–79

Hepworth SR, Valverde F, Ravenscroft D, Mouradov A, Coupland G (2002) Antagonistic regulation of flowering-time gene *SOC1* by *CONSTANS* and *FLC* via separate promoter motifs. EMBO J 21:4327–4337

Hillman JR (1984) Apical dominance. In: Wilkins MB (ed) Advanced plant physiology. Pitmann, London, pp. 127–148

Horvath DP, Anderson JV, Chao WS, Foley ME (2003) Knowing when to grow: signals regulating bud dormancy. Trends Plant Sci 8:534–540

Horvath DP, Sung S, Kim D, Chao W, Anderson J (2010) Characterization, expression and function of *DORMANCY ASSOCIATED MADS-BOX* genes from leafy spurge. Plant Mol Biol 73:169–179

Hsu CY, Liu YX, Luthe DS, Yuceer C (2006) Poplar FT2 shortens the juvenile phase and promotes seasonal flowering. Plant Cell 18:1846–1861

Irish VF (2010) The flowering of *Arabidopsis* flower development. Plant J 61:1041–1028

Jang S, Torti S, Coupland G (2009) Genetic and spatial interactions between *FT*, *TSF* and *SVP* during the early stages of floral induction in *Arabidopsis*. Plant J 60:614–625

Jian LC, Li PH, Sun LH, Chen THH (1997) Alterations in ultrastructure and subcellular localization of Ca^{2+} in poplar apical bud cells during the induction of dormancy. J Exp Bot 48:1195–1207

Jiménez S, Lawton-Rauh AL, Reighard GL, Abbott AG, Bielenberg DG (2009) Phylogenetic analysis and molecular evolution of the dormancy associated *MADS-box* genes from peach. BMC Plant Biol 9:81

Jiménez S, Reighard GL, Bielenberg DG (2010) Gene expression of *DAM5* and *DAM6* is suppressed by chilling temperatures and inversely correlated with bud break rate. Plant Mol Biol 73:157–167

Julian C, Rodrigo J, Herrero M (2011) Stamen development and winter dormancy in apricot (*Prunus armeniaca*). Ann Bot 108:617–625

Kane NA, Danyluk J, Tardif G, Ouellet F, Laliberte JF, Limin AE, Fowler DB, Sarhan, F (2005) TaVRT-2, a member of the StMADS-11 clade of flowering repressors, is regulated by vernalization and photoperiod in wheat. Plant Physiol 138:2354–2363

Kane NA, Agharbaoui Z, Diallo AO, Adam H, Tominaga Y, Ouellet F, Sarhan F (2007) TaVRT2 represses transcription of the wheat vernlization gene TaVRT1. Plant J 51:670–680

Karlberg A, Englund M, Petterle A, Molnar G, Sjödin A, Bakó L, Bhalerao RP (2010) Analysis of global changes in gene expression during activity-dormancy cycle in hybrid aspen apex. Plant Biotechnol 27:1–16

Kester DE (1969) Pollen effects on chilling requirements of almond and almond hybrid seeds. J Am Soc Hort Sci 94:318–321

Kim HJ, Hyun Y, Park JY, Park MJ, Park MK, Kim MD, Kim HJ, Lee MH, Moon J, Lee I, Kim J (2004) A genetic link between cold responses and flowering time through FVE in *Arabidopsis thaliana*. Nat Genet 36:167–171

Kim DH, Doyle MR, Sung S, Amasino RM (2009) Vernalization: winter and the timing of flowering in plants. Annu Rev Cell Dev Biol 25:277–299

Kozarewa I, Ibáñez C, Johansson M, Ogren E, Mozley D, Nylander E, Chono M, Moritz T, Eriksson ME (2010) Alteration of *PHYA* expression change circadian rhythms and timing of bud set in *Populus*. Plant Mol Biol 73:143–156

Kretzschmar A, Brighenti L, Rufato L, Pelizza T, Silveira F, Miquelutti D, Faoro I (2011) Chilling requirement for dormancy bud break in European pear. Acta Hortic 909:85–87

Lang GA, Early JD, Martin GC, Darnell RL (1987) Endo-, para-, and ecodormancy: physiological terminology and classification for dormancy research. HortScience 22:371–377

Laude HM (1953) The nature of summer dormancy in perennial grasses. Bot Gaz 114:282–292

Law JA, Jacobsen SE (2010) Establishing, maintaining and modifying DNA methylation patterns in plants and animals. Nat Rev Genet 11:204–220

Le Nard M, De Hertogh AA (1993) Bulb growth and development and flowering. In: De Hertogh A, Le Nard M (eds) The physiology of flower bulbs. Elsevier, Amsterdam, pp. 29–43

Lee H, Suh SS, Park E, Cho E, Ahn JH, Kim SG, Lee JS, Kwon YM, Lee I (2000) The AGAMOUS-LIKE 20 MADS domain protein integrates floral inductive pathways in *Arabidopsis*. Gene Dev 14:2366–2376

Lee JH, Yoo SJ, Park SH, Hwang I, Lee JS, Ahn JH (2007) Role of SVP in the control of flowering time by ambient temperature in *Arabidopsis*. Gene Dev 21:397–402

Lee JH, Ryu HS, Chung KS, Posé D, Kim S, Schmid M, Ahn JH (2013) Regulation of temperature-responsive flowering by MADS-box transcription factor repressors Science 342:628–632

Leida C, Conesa A, Llácer G, Badenes ML, Ríos G (2012a) Histone modifications and expression of *DAM6* gene in peach are modulated during bud dormancy release in a cultivar-dependent manner. New Phytol 193:67–80

Leida C, Conejero A, Arbona V, Gómez-Cadenas A, Llácer G, Badenes ML, Ríos G (2012b) Chilling-dependent release of seed and bud dormancy in peach associates to common changes in gene expression. PLoS ONE 7:e35777

Li D, Liu C, Shen L, Wu Y, Chen H, Robertson M, Helliwell CA, Ito T, Meyerowitz E, Yu H (2008) A repressor complex governs the integration of flowering signals in *Arabidopsis*. Dev Cell 15:110–120

Li ZG, Reighard GL, Abbott AG, Bielenberg DG (2009) Dormancy-associated MADS genes from the *EVG* locus of peach [*Prunus persica* (L.) Batsch] have distinct seasonal and photoperiodic expression patterns. J Exp Bot 60:3521–3530

Liu Y, Koornneef M, Soppe WJ (2007) The absence of histone H2B monoubiquitination in the *Arabidopsis* hub1 (rdo4) mutant reveals a role for chromatin remodeling in seed dormancy. Plant Cell 19:433–444

Luna V, Lorenzo E, Reinoso H, Tordable MC, Abdala G, Pharis RP, Bottini R (1990) Dormancy in peach (*Prunus persica* L.) flower buds. I. Floral morphogenesis and endogenous gibberellins at the end of the dormancy period. Plant Physiol 93:20–25

Luna V, Reinoso H, Lorenzo E, Bottini R, Abdala G (1991) Dormancy in peach (*Prunus persica* L.) flower buds. II. Comparative morphology and phenology in floral and vegetative buds, and the effect of chilling and gibberellin A3. Trees 5:244–246

Luna V, Soriano MD, Bottini R, Sheng CX, Pharis RP (1993) Dormancy in peach (*Prunus persica* L.) flower buds. III. Levels of endogenous gibberellins, abscisic acid, indole-3-acetic acid, and naringenin during dormancy of peach flower buds. Acta Hortic 329:264–267

March-Diaz R, Garcia-Dominguez M, Florencio FJ, Reyes JC (2007) SEF, a new protein required for flowering repression in *Arabidopsis*, interacts with PIE1 and ARP6. Plant Physiol 143:893–901

Mazzitelli L, Hancock RD, Haupt S, Walker PG, Pont SDA, McNicol J, Cardle L, Morris J, Viola R, Brennan R, Hedley PE, Taylor MA (2007) Co-ordinated gene expression during phases of dormancy release in raspberry (*Rubus idaeus* L.) buds. J Exp Bot 58:1035–1045

Metzger JD (1996) A physiological comparison of vernalization and dormancy chilling requirement. In: Lang GA (ed) Plant dormancy: physiology, biochemistry, and molecular biology. CABI, New York, pp 147–155

Michaels SD, Amasino RM (1999) *FLOWERING LOCUS C* encodes a novel MADS domain protein that acts as a repressor of flowering. Plant Cell 11:949–956

Michaels SD, Amasino RM (2001) Loss of *FLOWERING LOCUS C* activity eliminates the late-flowering phenotype of *FRIGIDA* and autonomous pathway mutations but not responsiveness to vernalization. Plant Cell 13:935–942

Michaels SD, Himelblau E, Kim SY, Schomburg FM, Amasino RM (2005) Integration of flowering signals in winter-annual *Arabidopsis*. Plant Physiol 137:149–156

Molmann JA, Asante DKA, Jensen JB, Krane MN, Ernstsen A, Junttila O, Olsen JE (2005) Low night temperature and inhibition of gibberellin biosynthesis override phytochrome action and induce bud set and cold acclimation, but not dormancy in PHYA overexpressors and wild-type of hybrid aspen. Plant Cell Environ 28:1579–1588

Moon J, Suh SS, Lee H, Choi KR, Hong CB, Paek NC, Kim SG, Lee I (2003) The SOC1 MADS-box gene integrates vernalization and gibberellin signals for flowering in *Arabidopsis*. Plant J 35:613–623

Moser BC, Hess CE (1968) The physiology of tuberous root development in dahlia. Proc Am Soc Hort Sci 93:595–603

Nee CC (1986) Overcoming bud dormancy with hydrogen cyanamide. Timingg and mechanisms. Dissertation, Oregon State University

Nitsch JP (1957) Photoperiodism in woody plants. Proc Am Soc Hort Sci 70:526–544

Ofir M (1986) Seasonal changes in the response to temperature of summer-dormant *Poa bulbosa* L. bulbs. Ann Bot 58:81–89

Ofir M, Kigel J (1999) Photothermal control of the imposition of summer dormancy in *Poa bulbosa*, a perennial grass geophyte. Physiol Plant 105:633–640

Ofir M, Kigel J (2007) Regulation of summer dormancy by water deficit and ABA in *Poa bulbosa* ecotypes. Ann Bot 99:293–299

Okubo H (2000) Growth cycle and dromancy in plants. In: Viemont JD, Crabble J (eds) Dormancy in plants from whole plant behaviour to cellular control. CABI Publishing, Wallingford, pp. 1–22

Oliver SN, Finnegan EJ, Dennis ES, Peacock WJ, Trevaskis B (2009) Vernalization-induced flowering in cereals is associated with changes in histone methylation at the *VERNALIZATION1* gene. Proc Natl Acad Sci U S A 106:8386–8391

Pasternak G, Powell LE (1980) Chilling requirements of apple seeds from cultivars having low and high chilling requirements for shoot growth. HortScience 15:408

Pazhouhandeh M, Molinier J, Berr A, Genschik P (2011) MSI4/FVE interacts with CUL4-DDB1 and a PRC2-like complex to control epigenetic regulation of flowering time in *Arabidopsis*. Proc Natl Acad Sci USA 108:3430–3435

Penfield S (2008) Temperature perception and signal transduction in plants. New Phytol 179:615–628

Peng MS, Cui YH, Bi YM, Rothstein SJ (2006) AtMBD9: a protein with a methyl-CpG-binding domain regulates flowering time and shoot branching in *Arabidopsis*. Plant J 46:282–296

Phillips R, Rix M (1989) Shrubs. Pan Books, London

Purvis ON, Gregory FG (1952) Studies in vernalization of cereals. XII. The reversibility by high temperature of the vernalized condition in petkus winter rye. Ann Bot 16:1–21

Quamme HA, Su WA, Veto LJ (1995) Anatomical features facilitating supercooling of the flower within the dormant peach flower bud. J Am Soc Hortic Sci 120:814–822

Reinoso H, Luna V, Daurfa C, Pharis R, Bottini R (2002a) Dormancy in peach (*Prunus persica*) flower buds. VI. Effects of gibberellins and an acylcyclohexanedione (trinexapac-ethyl) on bud morphogenesis in field experiments with orchard trees and on cuttings. Can J Bot 80:664–674

Reinoso H, Luna V, Pharis RP, Bottini R (2002b) Dormancy in peach (*Prunus persica*) flower buds. V. Anatomy of bud development in relation to phenological stage. Can J Bot 80:656–663

Rinne PLH, Hänninen H, Kaikuranta P, Jalonen JE, Repo T (1997) Freezing exposure releases bud dormancy in *Betula pubescens* and *B. pendula*. Plant Cell Environ 20:1199–1204

Rinne PLH, Kaikuranta PM, van der Schoot C (2001) The shoot apical meristem restores its symplasmic organization during chilling-induced release from dormancy. Plant J 26:259–264

Rinne PLH, Welling A, Vahala J, Ripel L, Ruonala R, Kangasjärvi J, van der Schoot C (2011) Chilling of dormant buds hyperiduced *FLOWERING LOCUS T* and recruits GA-inducible 1,3-β-glucanases to reopen signal conduits and release dormancy in *Populus*. Plant Cell 23:130–146

Ríos G, Leida C, Conejero A, Badenes ML (2014) Epigenetic regulation of bud dormancy events in perennial plants. Front Plant Sci 5:247

Rodriguez-A J, Sherman WB, Scorza R, Wisniewski M, Okie WR (1994) Evergreen peach, its inheritance and dormant behavior. J Am Soc Hortic Sci 119:789–792

Rohde A, Bhalerao RP (2007) Plant dormancy in the perennial context. Trends Plant Sci 12:217–223

Rohde A, Ruttink T, Hostyn V, Sterck L, Van Driessche K, Boerjan W (2007) Gene expression during the induction, maintenance, and release of dormancy in apical buds of poplar. J Exp Bot 58:4047–4060

Rohde A, Bastien C, Boerjan W (2011) Temperature signals contribute to the timing of photoperiodic growth cessation and bud set in poplar. Tree Physiol 31:472–482

Rossi R (1990) Guía de Bulbos (in Spanish). Grijalbo, Barcelona

Ruttink T, Arend M, Morreel K, Storme V, Rombauts S, Fromm J, Bhalerao RP, Boerjan W, Rohde A (2007) A molecular timetable for apical bud formation and dormancy induction in poplar. Plant Cell 19:2370–2390

Saito T, Bai S, Ito A, Sakamoto D, Saito T, Ubi BE, Imai T, Moriguchi T (2013) Expression and genomic structure of the *dormancy-associated MADS box* genes *MADS13* in Japanese pears (*Pyrus pyrifolia* Nakai) that differ in their chilling requirement for endodormancy release. Tree Physiol 33:654–667

Salathia N, Davis SJ, Lynn JR, Michaels SD, Amasino RM, Millar AJ (2006) *FLOWERING LOCUS C*-dependent and -independent regulation of the circadian clock by the autonomous and vernalization pathways. BMC Plant Biol 6:10

Saniewski M, Kawa-Miszcak L, Wegrzynowicz-Lesiak E, Okubo H (2000) Role of ABA, gibberellins and auxin in dormancy and dormancy release of tulip bulbs. In: Viemont JD, Crabble J (eds) Dormancy in plants from whole plant behaviour to cellular control. CABI Publishing, Wallingford, pp. 227–244

Santamaría ME, Hasbún R, Valera MJ, Meijón M, Valledor L, Rodríguez JL, Toorop PE, Cañal MJ, Rodríguez R (2009) Acetylated H4 histone and genomic DNA methylation patterns during bud set and bud burst in *Castanea sativa*. J Plant Physiol 166:1360–1369

Santamaría ME, Rodríguez R, Cañal MJ, Toorop PE (2011) Transcriptome analysis of chestnut (*Castanea sativa*) tree buds suggests a putative role for epigenetic control of bud dormancy. Ann Bot 108:485–498

Sasaki R, Yamane H, Ooka T, Jotatsu H, Kitamura Y, Akagi T, Tao R (2011) Functional and expressional analyses of *PmDAM* genes associated with endodormancy in Japanese apricot (*Prunus mume*). Plant Physiol 157:485–497

Scalabrelli G, Couvillon GA (1986) The effect of temperature and bud type on rest composition and the GDH ºC requirement for budbreak in "Redhaven" peach. J Amer Soc Hort Sci 111:537–540

Scebba F, De Bastiani M, Bernacchia G, Andreucci A, Galli A, Pitto L (2007) PRMT11: a new *Arabidopsis* MBD7 protein partner with arginine methyltransferase activity. Plant J 52:210–222

Schmitz RJ, Hong L, Fitzpatrickt KE, Amasino RM (2007) DICER-LIKE 1 and DICER-LIKE 3 Redundantly Act to promote flowering via repression of *FLOWERING LOCUS C* in *Arabidopsis thaliana*. Genetics 176:1359–1362

Schmitz RJ, Sung S, Amasino RM (2008) Histone arginine methylation is required for vernalization-induced epigenetic silencing of FLC in winter-annual Arabidopsis thaliana. Proc Natl Acad Sci USA 105:411–416

Searle I, He Y, Turck F, Vincent C, Fornara F, Krober S, Amasino RA, Coupland G (2006) The transcription factor FLC confers a flowering response to vernalization by repressing meristem competence and systemic signaling in *Arabidopsis*. Genes Dev 20:898–912

Sheldon CC, Burn JE, Perez PP, Metzger J, Edwards JA, Peacock WJ, Dennis ES (1999) The *FLF* MADS box gene: A repressor of flowering in *Arabidopsis* regulated by vernalization and methylation. Plant Cell 11:445–458

Sheldon CC, Rouse DT, Finnegan EJ, Peacock WJ, Dennis ES (2000) The molecular basis of vernalization: the central role of *FLOWERING LOCUS C* (*FLC*). Proc Natl Acad Sci USA 97:3753–3758

Sheldon CC, Hills MJ, Lister C, Dean C, Dennis ES, Peacock WJ (2008) Resetting of *FLOWERING LOCUS C* expression after epigenetic repression by vernalization. Proc Natl Acad Sci USA 105:2214–2219

Shimada S, Ogawa T, Kitagawa S, Suzuki T, Ikari C, Shitsukawa N, Abe T, Kawahigashi H, Kikuchi R, Handa H, Murai K (2009) A genetic network of flowering-time genes in wheat leaves, in which an APETALA1/FRUITFULL-like gene, *VRN1*, is upstream of *FLOWERING LOCUS T*. Plant J 58:668–681

Shinomura T, Nagatani A, Chory J, Furuya M (1994) The induction of seed germination in *Arabidopsis thaliana* is regulated principally by phytochrome B and secondarily by phytochrome A. Plant Physiol 104:363–371
Shirazi AM (1992) Relationship of 'near-lethal' stress on dormancy, cold hardiness and recovery of red-osier dogwood. Dissertation, Oregon State University
Siller-Cepeda JH, Fuchigami LH, Chen THH (1992) Glutathione content in peach buds in relation to development and release of rest. Plant Cell Physiol 33:867–872
Song YH, Ito S, Imaizumi T (2010) Similarities in the circadian clock and photoperiodism in plants. Curr Opin Plant Biol 13:594–603
Springer NM, Kaeppler SM (2005) Evolutionary divergence of monocot and dicot methyl-CpG-binding domain proteins. Plant Physiol 138:92–104
Sridha S, Wu K (2006) Identification of AtHD2C as a novel regulator of abscisic acid responses in *Arabidopsis*. Plant J 46:124–133
Srinivasan C, Dardick C, Callahan A, Scorza R (2012) Plum (*Prunus domestica*) trees transformed with Poplar *FT1* result in altered architecture, dormancy requirement, and continuous flowering. PLoS One 7:e40715
Stangeland B, Rosenhave EM, Winge P, Berg A, Amundsen SS, Karabeg M, Mandal A, Bones AM, Grini PE, Aalen RB (2009) AtMBD8 is involved in control of flowering time in the C24 ecotype of *Arabidopsis thaliana*. Physiol Plant 136:110–126
Strahl BD, Allis CD (2000) The language of covalent histone modifications. Nature 403:41–45
Sung SB, Amasino RM (2004a) Vernalization and epigenetics: how plants remember winter. Curr Opin Plant Biol 7:4–10
Sung SB, Amasino RM (2004b) Vernalization in *Arabidopsis thaliana* is mediated by the PHD finger protein VIN3. Nature 427:159–164
Swiezewski S, Liu FQ, Magusin A, Dean C (2009) Cold-induced silencing by long antisense transcripts of an *Arabidopsis* Polycomb target. Nature 462:799–802
Tsuji H, Saika H, Tsutsumi N, Hirai A, Nakazono M (2006) Dynamic and reversible changes in histone H3-Lys4 methylation and H3 acetylation occurring at submergence-inducible genes in rice. Plant Cell Physiol 47:995–1003
Turck F, Fornara F, Coupland G (2008) Regulation and identity of florigen: *FLOWERING LOCUS T* moves center stage. Annu Rev Plant Biol 59:573–594
Turner A, Beales J, Faure S, Dunford RP, Laurie DA (2005) The pseudoresponse regulator Ppd-H1 provides adaptation to photoperiod in barley. Science 310:1031–1034
Viémont J, Crabbé J (2000) Growth cycle and dormancy in plants. CABI, Belgium
Villalobos-Acuńa M, Mitcham EJ (2008) Ripening of European pears: the chilling dilemma. Postharvest Biol Technol 49:187–200
Wang SY, Faust M (1994) Changes in the antioxidant system associated with bud break in 'Anna' apple (*Malus domestica* Borkh) buds. J Am Soc Hort Sci 119:735–741
Wang Y, Garay L, Georgi LL, Reighard GL, Scorza R, and Abbott AG (2002a) Development of bacterial artificial chromosome contigs in the *evergrowing* gene region in peach [*Prunus persica* (L.) Batsch]. Acta Hort 592:183–189
Wang Y, Georgi LL, Reighard GL, Scorza R, Abbott AG (2002b) Genetic mapping of the *evergrowing* gene in peach [*Prunus persica* (L.) Batsch]. J Hered 93:352–358
Wang SP, Yuan CJ, Dai YT, Shu H, Yang TY, Zhang CX (2004) Development of flower organs in sweet cherry in Shanghai area. Acta Hortic Sinica 31:357–359
Wenkel S, Turck F, Singer K, Gissot L, Le Gourrierec J, Samach A, Coupland G (2006) CONSTANS and the CCAAT box binding complex share a functionally important domain and interact to regulate flowering of *Arabidopsis*. Plant Cell 18:2971–2984
Westwood MN, Bjornstad HO (1968) Chilling requirement of dormant seeds of 14 pear species as related to their climatic adaption. Proc Am Soc Hort Sci 92:141–149
Wisniewski M, Santer JJ, Stepien V, Fuchigami LH (1994) Effects of near-lethal heat stress on endodormancy and ecodormancy of peach and hybrid poplar. HortScience 29:511
Wu R, Wang T, McGie T, Voogd C, Allan AC, Hellens RP, Varkonyi-Gasic E (2014) Overexpression of the kiwifruit SVP3 gene affects reproductive development and suppresses anthocyanin

biosynthesis in petals, but has no effect on vegetative growth, dormancy, or flowering time. J Exp Bot 65:4985–4995

Yaish MWF, Peng MS, Rothstein SJ (2009) AtMBD9 modulates *Arabidopsis* development through the dual epigenetic pathways of DNA methylation and histone acetylation. Plant J 59:123–135

Yamane H, Ooka T, Jotatsu H, Hosaka Y, Sasaki R, Tao R (2011a) Expressional regulation of PpDAM5 and PpDAM6, peach (*Prunus persica*) dormancy-associated MADS-box genes, by low temperature and dormancy-breaking reagent treatment. J Exp Bot 62:3481–3488

Yamane H, Ooka T, Jotatsu H, Sasaki R, Tao R (2011b) Expression analysis of *PpDAM5* and *PpDAM6* during flower bud development in peach (*Prunus persica*). Sci Hortic (Amsterdam) 129:844–848

Yamane H, Tao R, Ooka T, Jotatsu H, Sasaki R, Yonemori K (2011c) Comparative analyses of dormancy-associated *MADS-box* Genes, *PpDAM5* and *PpDAM6*, in low- and high-chill peaches (*Prunus persica* L.). J Jpn Soc Hortic Sci 80:276–283

Yan L, Loukoianov A, Tranquilli G, Helguera M, Fahima T, Dubcovsky J (2003) Positional cloning of the wheat vernalization gene *VRN1*. Proc Natl Acad Sci USA 100:6263–6268

Yan L, Loukoianov A, Blechl A, Tranquilli G, Ramakrishna W, SanMiguel P, Bennetzen JL, Echenique V, Dubcovsky J (2004) The wheat *VRN2* gene is a flowering repressor down-regulated by vernalization. Science 303:1640–1644

Yan L, Fu D, Li C, Blechl A, Tranquilli G, BonafedeM, Sanchez A, Valarik M, Yasuda S, Dubcovsky J (2006) The wheat and barley vernalization gene *VRN3* is an orthologue of *FT*. Proc Natl Acad Sci USA 103:19581–19586

Yoshida R, Fekih R, Fujiwara S, Oda A, Miyata K, Tomozoe Y, Nakagawa M, Niinuma K, Hayashi K, Ezura H, Coupland G, Mizoguchi T (2009) Possible role of EARLY FLOWERING 3 (ELF3) in clock-dependent floral regulation by SHORT VEGETATIVE PHASE (SVP) in *Arabidopsis thaliana*. New Phytol 182:838–850

Yoshida T, Shimada K, Oma Y, Kalck V, Akimura K, Taddei A, Iwahashi H, Kugou K, Ohta K, Gasser SM, Harata M (2010) Actin-related protein Arp6 influences H2A.Z-dependent and -independent gene expression and links ribosomal protein genes to nuclear pores. PLoS Genet 6:e1000910

Zemach A, Gaspan O, Grafi G (2008) The three methyl-CpG-binding domains of AtMBD7 control its subnuclear localization and mobility. J Biol Chem 283:8406–8411

Zhang L, Wang Y, Zhang X, Zhang M, Han D, Qiu C, Han Z (2012) Dynamics of phytohormone and DNA methylation pattern changes during dormancy induction in strawberry (*Fragaria* × *ananassa* Duch.). Plant Cell Rep 31:155–165

Zhang L, Wang L, Li B, Whiting MD, Liu Z, Singer S, Xu W, Wang S, Zhang C (2014) The effect of hydrogen cyanamide on floral organ development of sweet cherry in a warm region. In submission

Zhebentyayeva TN, Fan S, Chandra A, Bielenberg DG, Reighard GL, Okie WR, Abbott AG (2014) Dissection of chilling requirement and bloom date QTLs in peach using a whole genome sequencing of sibling trees from an F2 mapping population. Tree Genet Gen 10:35–51

Zhong W, Gao Z, Zhuang W, Shi T, Zhang Z, Ni Z (2013) Genome-wide expression profiles of seasonal bud dormancy at four critical stages in Japanese apricot. Plant Mol Biol 83:247–264

Zhu J, Jeong J, Zhu Y, Sokolchik I, Miyazaki S, Zhu JK, Hasegawa PM, Bohnert HJ, Shi H, Yun DJ, Bressan RA (2007) Involvement of *Arabidopsis* HOS15 in histone deacetylation and cold tolerance. Proc Natl Acad Sci USA 105:4945–4950

Chapter 5
Recent Advances in Genetics and Molecular Control of Bud Dormancy in Pipfruits

Vítor da Silveira Falavigna, Diogo Denardi Porto, Carolina Pereira Silveira and Luís Fernando Revers

Introduction

Temperate fruit crops have great economic importance worldwide and their production is closely related to bud dormancy, given that a well-adjusted dormancy cycle is crucial for the achievement of their full genetic potential. This process is regulated by environmental inputs, mainly chilling temperatures and photoperiodic changes, which are required for dormancy establishment and release (Horvath et al. 2003; Rohde and Bhalerao 2007). Bud dormancy is usually divided into paradormancy, endodormancy and ecodormancy, which refers to a failure of meristem growth under favorable conditions caused by signals derived from outside of the bud (but from the same plant), from the bud itself or from the environment, respectively (Lang et al. 1987). Dormancy entrance is characterized by growth cessation, bud set and leaf senescence. Once dormant, plants often need to be exposed to extended periods of cold (temperatures below 7.2 °C) to overcome it and the fulfillment of this chilling requirement (CR) culminates in ecodormancy (Horvath et al. 2003). The mechanisms regulating dormancy release are highly heritable and finely tuned, with each genotype being strongly influenced by its region of origin, suggesting a

Authors Vítor da Silveira Falavigna and Diogo Denardi Porto contributed equally to this work.

L. F. Revers (✉) · C. P. Silveira
Laboratory of Plant Molecular Genetics, Centro Nacional de Pesquisa de Uva e Vinho, Empresa Brasileira de Pesquisa Agropecuária, Rua Livramento, 515, P.O. Box 130, Bento Gonçalves, RS 95700–000, Brazil
e-mail: luis.revers@embrapa.br

V. da S. Falavigna
Graduate Program in Cell and Molecular Biology, Centro de Biotecnologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS 91501–970, Brazil

D. D. Porto
Centro de Pesquisa Agropecuária Trópico Semiárido, Empresa Brasileira de Pesquisa Agropecuária, Petrolina, PE 56302–970, Brazil

© Springer International Publishing Switzerland 2015

J. V. Anderson (ed.), *Advances in Plant Dormancy,* DOI 10.1007/978-3-319-14451-1_5

strong genetic control of this trait (Dennis 1987; Howe et al. 2000; Labuschagné et al. 2002; Jackson 2003; Campoy et al. 2011).

The direct relationships between bud dormancy and cold exposure gain importance when considering the recently proposed models for global warming. These models predict a rise in global mean temperatures and milder winters, which could result in difficulties for the production of temperate fruit crops (Arora et al. 2003; Campoy et al. 2011; Kirtman et al. 2013). Thereby, the importance of understanding the regulation of dormancy progression is gaining momentum with the main objective of maintaining sustainable crop yields in a changing environment. In this context, a wide range of approaches, from the genetic to the genomic perspective, are being used in several perennial crops as study models. In fact, although the main controlling mechanisms are still unknown or only partially explained, research advances in plant dormancy, especially in peach and poplar, are unveiling key regulators of this process.

Despite worldwide efforts applied to studying the dormancy process, only recently have two of the most economically important temperate fruit crops, apples and pears (FAO 2012), been explored for this agronomic trait. These pipfruits gain their name because of the small hard seeds (pips) in the center of the fruit (Palmer 2012), which differ from seeds of other Rosaceae species, such as peaches and strawberries. In addition, pipfruits also diverge in bud dormancy regulation because instead of being triggered by photoperiodic changes the main regulator of this process is exposure to low temperatures (Heide and Prestrud 2005). The Central and Western Asian origin of the pipfruits could explain their partial insensitivity to photoperiod, given that temperature in these regions varies more strongly than day length in comparison with other latitudes. Therefore, temperature would be a more reliable marker of the cold season than light quality to synchronize their phenology to the environment (Campoy et al. 2011).

Several advanced molecular models for bud dormancy control have been proposed (Horvath 2009; Campoy et al. 2011; van der Schoot and Rinne 2011; Rinne et al. 2011). However, they are based on species in which photoperiodic changes play a major role in dormancy induction and the peculiarities of this process in pipfruits are not addressed by these models. This review intends to help fill this gap, discussing the recent findings in genetics and genomics of bud dormancy control in pipfruits. The better understanding of this process may permit the development of new strategies that could help the generation of cultivars better adapted to each regional cultivation scenario.

Linkage Mapping of Dormancy-Related Traits

A major approach in the discovery of genes controlling phenological characteristics, such as bud dormancy, is to determine the association between the presence or absence of the trait of interest (phenotypes) and the profiles of molecular markers (genotypes) across individuals of a segregating population, a strategy known as linkage mapping (Mackay et al. 2009). Linkage mapping from experimental populations is very widespread in herbaceous crops, such as wheat and rice, but this is

not the case for tree crops, such as apple and pear (Troggio et al. 2012). The main reasons are the high costs of maintaining a population of trees suitable for linkage mapping and their long juvenile period, especially when working with adult traits such as fruit quality or dormancy of reproductive buds (Flachowsky et al. 2009; Grattapaglia and Kirst 2008; Neale and Kremer 2011; Myles 2013).

One of the first attempts to assess the heritable components of tree bud phenology was done using populations of *Populus* sp. hybrids (Bradshaw and Stettler 1995). At the time, the consensus among geneticists was that characters with broad phenotype distributions, such as time of bud flush, were controlled by a large number of genes, each one with small effects. The authors found that most of the variation for bud phenology observed in their experimental population (84.7 %) was explained by five quantitative trait loci (QTL) distributed in five linkage groups. However, it remained an open question whether each identified QTL represented one gene with a major effect or a cluster of genes with minor effects. This question was addressed by the refinement of the QTL analysis and the mapping of candidate genes for the control of bud phenology (Frewen et al. 2000). The authors found two genes potentially related to dormancy regulation to be coincident with the confidence intervals of two major QTLs, namely *PHYTOCHROME B* (*PHYB*) and *ABSCISIC ACID INSENSITIVE* (*ABI*) homologs. Both were shown to be involved in timing of bud set and bud development (Olsen et al. 1997; Rohde et al. 2002). These first studies demonstrated that most of the genetic control of bud phenology could be mapped to a few genomic intervals.

Dormancy-associated traits, due to their quantitative nature, are often a subject of quantitative genetics disciplines. Bud dormancy-related phenotypes exhibiting a classical Mendelian segregation, which are more straightforward to map than QTLs (Mackay 2001), are very rare. An invaluable research opportunity was explored from the mapping of the *evergrowing* (*evg*) locus of peach (Bielenberg et al. 2008). The *evg* mutants are non-dormant, i.e., they do not stop growing even when exposed to short photoperiods or low temperatures, and the *evg* trait segregates as a single recessive gene. Sequencing of the *evg* locus revealed a cluster of six MIKC-type MADS-box genes, thereafter called *Dormancy-Associated MADS-box* (*DAM*) genes.

When the genetic control of bud dormancy in peach was characterized by quantitative genetics approach, two major QTLs (explaining more than 30 % of the phenotypic variation) were found, and one of them overlapped with the *evg* locus on linkage group one (LG1, Fan et al. 2010). Further high-resolution mapping of this QTL and next-generation resequencing of the genomes from extreme phenotype individuals indicated *DAM* genes as the most probable genetic elements underlying the effects of the LG1 QTL (Zhebentyayeva et al. 2014).

Both peach and poplar are self-compatible and fast-growing trees; hence, true F2 populations can be established in relatively short timeframes (Fan et al. 2010; Faria et al. 2011). Linkage mapping in F2 generation is virtually impossible for self-incompatible species; therefore, alternative cross strategies are needed to obtain segregant populations. The high level of heterozygosity commonly found in self-incompatible species can be used as leverage for the generation of linkage maps by

the two-way pseudo-testcross approach (Grattapaglia and Sederoff 1994). The main idea behind this strategy is to follow the 1:1 segregation of genotypes from markers that are heterozygous in only one parent. It follows that two linkage maps are constructed, one for each parent, and the maps can be integrated through markers that are present in both parental lines. The two-way pseudo-testcross is a convenient, simple-to-implement and robust strategy for linkage mapping of tree species in the F1 generation and does not depend on prior genetic information from the parental lines.

Apple and pear are self-incompatible species with a long juvenile period, and these limitations have hampered genetic understanding and improvement of both crops (Jackson 2003). The first controlled crosses of apple trees for breeding purposes date from 1806, and apple breeders usually select genotypes carrying desired traits from the F1 progenies (Kellerhals 2009). Many of the target traits to be introgressed to apple cultivars are related to disease resistance, tree architecture, flowering and fruit quality (Korban and Tartarini 2009). Pear breeding also typically involves generation of genetic variation by crossing, aiming to improve fruit quality, disease resistance, storage ability, among other traits (Yamamoto and Chevreau 2009).

Breeding and academic research of slow growing trees, such as apple and pear, can benefit greatly from the knowledge obtained using molecular markers linked to heritable traits. For apple, a considerable range of molecular and genetic data is publicly available, as well as a high-quality whole genome draft (Velasco et al. 2010). Among the many tools and databases available, a noteworthy resource is the apple 8K single nucleotide polymorphism (SNP) array developed by the International RosBREED Consortium (Chagné et al. 2012). The SNPs that compose the chip were chosen after analyzing the resequencing data from 27 cultivar accessions, representing most of the genetic variation available for apple germplasm. Afterwards, due to the lack of SNP markers described for pear and the high collinearity between apple and pear genomes, approximately 1000 newly discovered SNPs from pear were added to the chip, collectively totaling nearly 9000 markers (Montanari et al. 2013). One limitation of this platform, however, is the unexpected segregation patterns for a large number (more than half) of markers (Troggio et al. 2013). The reason for this anomaly is the high level of paralogy exhibited by the apple genome, probably caused by a recent whole genome duplication event (Velasco et al. 2010). In practical terms, a great number of probes anneal in paralogous sites, resulting in distorted genotype proportions in the experimental population. This can be minimized by the use of stringent quality filtering of observed genotype distributions, in order to select only reliable markers.

In apple, several linkage mapping studies have already been done specifically for the characterization of dormancy traits. The experimental population for dormancy-related QTL analysis in apple is set up from the offspring of a crossing between individual cultivars differing in CR. There are many apple cultivars with various ranges of CR, and this trait, as for *Populus* sp., is largely genetically controlled, most likely as a single dominant gene for the low CR trait (Hauagge and Cummins 1991). In an early QTL identification attempt following the two-way pseudo-

testcross strategy, Conner et al. (1998) found eight regions distributed in seven linkage groups as highly associated with timing of bud break. However, the linkage map constructed did not include markers that could be transferred to the reference apple genetic map and, hence, the numbering of linkage groups is not the standard for apple genetic studies.

In a more recent study, van Dyk et al. (2010) performed map construction and QTL analysis for dormancy traits from populations in South Africa derived from crosses between individuals from 'Anna' (very low CR) and 'Golden Delicious' (high CR) and from 'Anna' and 'Sharpe's Early' (high CR). The maps, constructed from F1 genotypes employing 320 simple sequence repeat (SSR) markers, were composed of 17 linkage groups (LGs), corresponding to the number of apple chromosomes. The single QTL found was positioned on LG9 and explained around 40% of the variation in the timing of both vegetative and floral bud break (van Dyk et al. 2010). In a similar approach, Celton et al. (2011) constructed maps from crossings between 'Starkrimson' and 'Granny Smith' and between X3263 and 'Belrène,' the last consisting of a population of more than 300 individuals. The QTL analysis of timing of bud break revealed several associations for this trait across the genome, the major one being on LG9, in close agreement with the confidence interval found by van Dyk et al. (2010). The region of interest was defined as the first 4 million base pairs from chromosome 9 in the apple genome, a region identical to the one found in an independent linkage mapping of dormancy-related traits performed by our own group (Tessele et al. manuscript in preparation). Candidate gene analysis of this region revealed enrichment for functional classes such as stimulus, biological regulation, signaling, programmed cell death and cell cycle control (Celton et al. 2011). These segregant populations were established in very divergent climatic conditions, yet shared the same genomic region as containing most of the genetic control of the timing of bud break. These findings suggest that variation in dormancy-related traits in apple has a strong genetic component. In addition, the overlap of genomic intervals for QTLs identified from different progenies suggests a common underlying genetic mechanism as responsible for the variation of the trait. The next step, therefore, is to further characterize the major QTLs for apple bud dormancy-related traits, as already carried on for peach (Zhebentyayeva et al. 2014). A consensus approach among molecular geneticists is to genotype the same population used for QTL identification using a high number of markers located in the region of interest, which is often called fine mapping or high-resolution mapping (Mackay 2001).

The availability of the next-generation sequencing technologies and high-quality genomes now allows the discovery of new molecular markers with low relative cost. DNA resequencing was carried out for parental individuals from the population segregating for dormancy traits established by our group, and as a result, more than 80,000 SNPs were discovered (Alencar et al. 2011). After validation, these new markers will be fundamental for the fine characterization of the apple dormancy-related traits QTLs.

Despite its significant economic importance, pear does not benefit from the same range of genetics and genomics resources as apple. Molecular markers have been

used in pear for the determination of genetic diversity, association with genes of agronomical interest, and construction of linkage maps (Yamamoto and Chevreau 2009). The first pear genetic map was constructed from a cross between Japanese (*P. pyrifolia*) cultivars using random amplified polymorphic DNA (RAPD) markers (Iketani et al. 2001). Yamamoto et al. (2002) assembled a pear map including simple sequence repeats (SSR) markers shared between apple and pear. The transferability of these markers allowed the comparison of maps from apple and pear, and indicated a high level of synteny between the two genomes. The close evolutionary relationship between the two species was clearly demonstrated with the recent publication of the genomes of the Japanese (Wu et al. 2013) and the European (Chagné et al. 2014) pears. In fact, the high transferability of molecular markers between pear and apple allowed a combination of SNPs from both species to be arrayed in the same platform for the genotyping of the two crops interchangeably (Montanari et al. 2013).

Various QTL identification attempts have been made in pear and yielded DNA markers closely associated with disease resistance, fruit storage and leaf traits (Yamamoto and Chevreau 2009; Sun et al. 2009). However, to the best of our knowledge, no QTL mapping for dormancy-related traits were performed in pear to date. Indeed, due to their genetic similarities, much of what is being discovered in apple may be applied to dormancy in pear. This statement is in agreement with the findings reported by Celton et al. (2009), which confirmed the ready transferability of SSR markers from *Malus* to *Pyrus*.

Molecular Control of Bud Dormancy Progression

Bud dormancy is a complex process that includes a range of states, degrees of development and the outgrowth that is tightly synchronized with seasonal changes. The elucidation of molecular networks responsible for the control of bud dormancy progression has been almost exclusively done on systems induced by photoperiodic changes (Böhlenius et al. 2006; Li et al. 2009; Jiménez et al. 2010; Doğramaci et al. 2010). Some components of photoperiod perception are known to play roles in dormancy regulation, such as PHYA (PHYTOCHROME A), CONSTANS (CO) and FT (FLOWERING LOCUS T). In annual plants such as *Arabidopsis thaliana*, flowering occurs in response to long-day photoperiods, with CO and FT controlling photoperiod perception and flowering time, respectively (Amasino and Michaels 2010). In *Populus* trees, Böhlenius et al. (2006) reported that *PtFT1* also controls the short-day-photoperiod-induced growth cessation and bud set. In an independent study, Hsu et al. (2011) identified two *FT* paralogs (*FT1* and *FT2*) in poplar and indicated that their expressions are temporally and spatially separated. These authors demonstrated that *FT1* expression during winter coincides with the transition of vegetative to reproductive phases, whereas *FT2* promotes vegetative growth and inhibition of bud set in response to warm temperatures and long days. In agreement to these findings, Kotoda et al. (2010) reported that apple also has two *FT* genes,

and Srinivasan et al. (2012) showed that the overexpression of a poplar *FT* in plum (*Prunus* x *domestica*) impaired dormancy entrance.

The expression of genes regulated by photoperiod is interconnected in a cascade of events, where PHYA, along with other circadian clock components, regulates CO, which in turns induces *FT* transcription leading to flowering. Furthermore, the signaling cascade regulated by photoperiod perception is closely connected to the cold temperature perception pathway, involving several related transcription factors (Amasino and Michaels 2010). However, the role of temperature perception in bud set and in induction of bud dormancy is still poorly understood. Some genes that play key roles in photoperiod perception involved in crosstalk with the temperature pathway could act as temperature sensors, such as the phytochromes (Franklin 2009). In *A. thaliana*, temperature regulates flowering through the vernalization pathway, which is mediated by the FLOWERING LOCUS C (FLC). FLC is a MADS-domain transcriptional regulator that represses two floral integrators, *FT* and *SOC1*, inhibiting flowering at low temperatures (Helliwell et al. 2006). Interestingly, there is a feedback loop involving SOC1 and FLC regulation, which may prevent premature flowering under cold conditions (Seo et al. 2009). Hereupon, SOC1 negatively regulates the cold response pathway through the direct repression of C-repeat binding factor/dehydration-responsive element-binding protein (CBF/DREB1) transcription factors, which are responsible for most of the cold-induced gene expression in plants (Seo et al. 2009; Thomashow 2010). On the other hand, the expression of *CBF/DREB1* increases *FLC* expression that in turn represses *FT* and *SOC1*, thereby delaying flowering (Seo et al. 2009). The crosstalk between temperature and photoperiod pathways in dormancy regulation was markedly demonstrated by Wisniewski et al. (2011), which reported that the ectopic expression of a peach *CBF* in apple triggered dormancy induction by short-day photoperiod. The same transgenic plants were further evaluated over three growing seasons demonstrating increased cold tolerance, delayed growth and altered dormancy phenology under field conditions (Artlip et al. 2014).

Horvath (2009) proposed a schematic model of how cold temperatures putatively mediate dormancy induction, suggesting that CBF transcription factors promote expression of *DAM* genes, possibly by chromatin remodeling (Horvath 2009). *DAM* genes are classified as belonging to the SVP/StMADS11 clade of MADS-box transcription factors, and due to protein sequence similarities, genes closely related to *DAM* are sometimes referred to as *SVP*-like genes. In *A. thaliana*, *SVP* is a *MADS-box* gene that regulates floral transition and contributes to the specification of floral meristems (Gregis et al. 2013). The *DAM* genes were first described in peach and presented distinct seasonal expression patterns (Bielenberg et al. 2008). From the six genes described, only *PpDAM5* and *PpDAM6* were regulated by cold exposure (Li et al. 2009). Moreover, the transcript accumulation pattern identified for these genes, e.g., induction during autumn and declining through the winter, suggests a growth repressing role (Li et al. 2009; Yamane et al. 2011). Additionally, it was recently shown that the silencing of *PpDAM6* is preceded by changes in the methylation status of H3K27 residues of histones bound to its chromatin (Leida et al. 2012), as well as occurs in the silencing of *FLC* and other genes that regulate vernalization

in *A. thaliana* (Angel et al. 2011). Although putative *DAM* orthologues were identified in pear (Ubi et al. 2010; Saito et al. 2013), apple (Falavigna et al. 2014), raspberry (Mazzitelli et al. 2007), kiwifruit (Wu et al. 2012), leafy spurge (Horvath et al. 2010) and apricot (Sasaki et al. 2011), a complete functional characterization of *DAM* genes remains to be reported.

Among efforts made to elucidate the involvement of *DAM* genes in bud dormancy and flowering, Horvath et al. (2010) reported that the overexpression of a leafy spurge *DAM* gene in *Arabidopsis* delayed flowering, as was also observed in *SVP* overexpressing lines (Gregis et al. 2013). Furthermore, Horvath et al. (2010) demonstrated that *DAM* genes are preferentially expressed in response to cold temperatures, causing a negative-regulation of *FT* or *FT*-like genes, leading to growth cessation and dormancy entrance. Interestingly, Sasaki et al. (2011) reported that overexpressing *PmDAM6* in poplar resulted in variable *FT* transcript levels, induction of growth cessation and precocious bud formation. On the other hand, Bai et al. (2013) found no correlation between the expression patterns of *DAM* and *FT* genes in pear, suggesting that both genes are not regulated in the Rosaceae family in the same manner as in leafy spurge. Finally, in the perennial kiwifruit, *SVP*-like genes were identified and functionally characterized in *Arabidopsis*. Out of four genes (*SVP1*, *SVP2*, *SVP3* and *SVP4*), only *SVP3* was able to rescue the flowering phenotype in *Arabidopsis svp* mutant lines (Wu et al. 2012). Distinct roles were therefore suggested for kiwifruit *SVP*-like genes in bud dormancy and flowering. Paradoxically, a report from the same authors showed that the ectopic expression of *SVP3* in kiwifruit and tobacco did not have any effect on growth and dormancy (Wu et al. 2014).

Several models have been devised for the regulation of dormancy induction and release. For example, Horvath (2009) proposed a model for bud dormancy induction where *DAM*, *FT* and a *FT*-like gene named CENTRORADIALIS (*CENL*) play key roles. The *DAM* genes would be induced after a short exposure to cold, probably through the action of CBF and chromatin remodeling mechanisms, as well as by the short-day photoperiod output from the circadian clock mediated by PHYA. Once induced, the DAM transcription factors would repress *FT/CENL*, causing growth cessation and dormancy induction. After long-term cold exposure, likely via chromatin modification, the down-regulation of *DAM* genes occurs leading to dormancy release. Similarly, Jiménez et al. (2010) proposed a simple conceptual model to explain the putative roles of DAM5/DAM6 in the endodormancy-to-ecodormancy transition. According to this model, the expression of *DAM5* and *DAM6* is triggered by short photoperiods. On the other hand, chilling exposure disrupts the circadian perception of photoperiodic stimuli, resulting in repression of *DAM5* and *DAM6*, and allowing the expression of the genes required for growth under permissive environmental conditions. Finally, Campoy et al. (2011) proposed a similar model integrating all this information combined with data generated studying dormancy in other species, such as chestnut and hybrid aspen.

An elegant mechanism to explain dormancy cycling was proposed by Rinne et al. (2001) based on low-temperature mediated enhancement of 1-3-ß-D-glucanases production. Removal of 1-3-ß-glucan from the plasmodesmata restores the symplasmic

communication network, leading to chilling-induced release from dormancy by the assumption of a proliferation-competent state. The same authors identified three groups of genes, members of *GLUCAN HYDROLASE 17* family (*GH17*), that are upregulated by chilling temperatures and GA biosynthesis in *Populus*. The group 1 *GH17* genes are transiently upregulated by short-term photoperiodic exposure in order to maintain the symplasmic paths to facilitate bud formation. On the other hand, group 2 and 3 *GH17* genes are upregulated by GA_3 and long-term chilling exposure, allowing callose removal and, thereby, enabling reopening of signaling conduits for *FT* transport to the apex. After sufficient chilling, growth-related genes are upregulated by elevated temperatures, mediated by GA_4, leading to bud burst (van der Schoot and Rinne 2011; Rinne et al. 2011). The models proposed for peach and poplar helped to better understand the dormancy processes in perennial trees; however they rely on advances made on species for which photoperiodic changes are the main inductor of bud dormancy (Horvath 2009; Campoy et al. 2011; van der Schoot and Rinne 2011; Rinne et al. 2011). Thus, the major findings related to dormancy progression in pipfruits are often neglected and therefore need to be better addressed.

Bud Dormancy in Pipfruits

Pipfruits differ from other plant models used to study bud dormancy, such as peach and poplar, at the physiological level because the most important environmental trigger for dormancy induction is low-temperature exposure (Heide and Prestrud 2005), instead of photoperiodic changes. Thus, it can be expected that different molecular pathways are being influenced during dormancy entrance in pipfruits. In this sense, several studies have been conducted to identify similarities as well as peculiarities of this process in apples and pears.

Pioneering work has been performed in apple exploring the contrasting phenotypes between 'Gala' and its spontaneous mutation 'Castel Gala'. This last cultivar requires only 50 % of the CR for dormancy release in comparison with the original cultivar, resulting in earlier bud break. Using suppression subtractive hybridization as a gene discovery tool and RT-qPCR for validation, Falavigna et al. (2014) identified 17 candidate genes, with transcripts coding for DAM, dehydrins, GAST1, LTI65, NAC, HTA8, HTA12 and RAP2.12-like proteins presenting major differences in gene expression between cultivars through the winter. One of the most noteworthy results was the transcriptional profile obtained for a *DAM*-like gene, whose expression was very similar to peach *PpDAM5* and *PpDAM6* genes (Li et al. 2009; Yamane et al. 2011). In an independent approach, Porto et al. (2015) carried out a transcriptomic assay aiming to analyze changes in apple gene (~ 57,000) expression in response to chilling accumulation in the field and under controlled conditions using a microarray chip. Cold exposure mainly repressed the expression of transcripts related to photosynthesis, whereas long-term cold exposure repressed flavonoid biosynthesis genes. These results indicate that photosynthesis and

auxin transport are major regulatory nodes of apple dormancy and unveil strong candidates for the control of bud dormancy. Genes related to the circadian clock, hormonal signaling, and regulation of growth and flower development were annotated, including the *MdFT1* gene. Interestingly, apple trees overexpressing *MdFT1* displayed early flowering despite a lack of any chilling exposure (Tränkner et al. 2010). Several studies overexpressing *FT* homologous genes in apple reported precocious flowering (Kotoda et al. 2010; Flachowsky et al. 2012; Wenzel et al. 2013), but the authors have not addressed its effects of dormancy process. These findings suggest the existence of common pathways (e.g., DAM family, *FT* homologs and hormone signaling) in the regulation of dormancy progression in apple in comparison with other better characterized species, such as peach and poplar. However, the identification of new pathways whose relationships to dormancy still need to be unveiled remains a possibility.

The availability of the pear genome sequence will likely become a very important tool to improve the genomics of many agronomic traits, including bud dormancy (Chagné et al. 2014; Wu et al. 2013). In fact, despite this advance, several efforts were performed trying to discover the molecular mechanisms underlying bud dormancy progression in pear. Two remarkable and independent pear transcriptomes were generated using RNA-seq to explore endo- and eco-dormant flower buds (Liu et al. 2012; Bai et al. 2013). Interestingly, both studies identified pathways already related to dormancy in other species, but also reported, for the first time, other dormancy-related pathways, such as endocytosis, glycerophospholipid metabolism, and biosynthesis of phenylpropanoids, stilbenoids, diarylheptanoids, gingerols and ether lipids. These data, along with those reported in apple (Falavigna et al. 2014; Porto et al. 2015), suggest that we are far from fully understanding bud dormancy in pipfruits and new research approaches must be explored.

Additionally, besides the whole RNA-seq data generated by Liu et al. (2012) and Bai et al. (2013), both authors presented transcript accumulation patterns for *DAM* genes and their results coincided with the first findings reported for this gene family in pear (Ubi et al. 2010; Saito et al. 2013). Two putative *DAM* genes were identified (namely *PpMADS13–1* and *PpMADS13–2*) and their expression pattern was analyzed by RT-qPCR during dormancy. They showed that both genes are gradually down-regulated concomitantly with endodormancy release (Ubi et al. 2010). After that, a third *DAM* gene was also isolated (*PpMADS13–3*), and its transcript levels showed a decrease near and after endodormancy release (Saito et al. 2013).

Two additional reports also investigated dormancy regulation in pear. Nishitami et al. (2012) identified two putatively novel dormancy-related transcription factors, NAC2 and PRR5, using a microarray chip to study the transition from endodormancy to ecodormancy in pear buds. Both genes displayed a sharp increase in the transcript accumulation levels during the end of endodormancy until ecodormancy. Likewise, Takemura et al. (2013) identified several genes that may play a role in regulating endodormancy release, highlighting the transcriptional profile obtained for clone 245 (*Auxin influx carrier component*), which was induced near and after bud break.

Another approach to investigate bud dormancy in pear was the characterization of carbohydrate metabolism. Marafon et al. (2011) demonstrated that the exposure of branches to cold temperatures affects starch and soluble sugar contents in wood and bud tissues of Japanese pears. Sufficient chilling supply during winter increased the activities of cell wall acid invertase and sucrose-6-phosphate synthase, yielding increased levels of reducing sugars and starch contents in bud tissues that are then used for budburst and blooming in spring (Marafon et al. 2011). Additionally, another study showed that endodormancy release occurred concomitantly with the accumulation of sorbitol in xylem sap, and the increase of sorbitol influx and catabolism in flower buds occurred only after bud break (Ito et al. 2012). Finally, trying to elucidate which physiological events were involved in the seasonal changes of carbohydrate dynamics during winter, the results found by Ito et al. (2013) suggest that carbohydrates in the shoot tissues may be converted to sorbitol and loaded into xylem sap. Therefore, sorbitol accumulation patterns could be synchronized with the progression of dormancy, whereas the total carbohydrate transported into shoots from other storage organs may be related to freezing tolerance acquisition rather than dormancy progression (Ito et al. 2013).

A groundbreaking discovery by Mason et al. (2014) uncovered fundamental roles of sugar signaling in bud dormancy. According to their report, lateral dormant buds under the effect of apical dominance, which is a form of paradormancy, resume growth upon receiving an extra amount of sugar supply. Sugar surplus in the phloem is a direct consequence of shoot decapitation, and this signal is much faster than auxin depletion across the stem. Lateral bud outgrowth induced by sugars is independent from auxin signaling, long regarded as the main regulator of apical dominance. This new and exciting evidence indicates that carbohydrate metabolism will probably have an increasing importance in studies involving bud dormancy progression in perennial species.

Concluding Remarks

Bud dormancy, especially dormancy release, remains one of the less understood processes in plant biology. This delay in relation to other well-characterized plant phenomena can be due to methodological issues inherent to the study of dormancy itself, as it is one of the most hermetic subjects at the experimental point of view. However, current approaches available in the fields of plant physiology and molecular biology may provide significant advances in the genetics and genomics of this trait. New technologies, such as high throughput data generation and functional analysis in heterologous systems, hold promise for unraveling the inner circuits of dormancy regulation. At the moment, quantitative genetics and comparative genomics seem to be the most fruitful paths toward the identification of components of dormancy regulation. Functional characterization of these components in their original species background is the next challenge, which can reveal how independently described nodes assemble into a full regulatory mechanism.

References

Alencar S, Silva-Junior O, Togawa R, Costa M, Revers L, Pappas G (2011) SNP discovery in apple cultivars using next generation sequencing. BMC Proc 5(Suppl 7):P42

Amasino RM, Michaels SD (2010) The timing of flowering. Plant Physiol 154:516–520

Angel A, Song J, Dean C, Howard M (2011) A Polycomb-based switch underlying quantitative epigenetic memory. Nature 476:105–108

Arora R, Rowland LJ, Tanino K (2003) Induction and release of bud dormancy in woody perennials: a science comes of age. Hortscience 38(5):911–921

Artlip TS, Wisniewski ME, Norelli JL (2014) Field evaluation of apple overexpressing a peach *CBF* gene confirms its effect on cold hardiness, dormancy, and growth. Environ Exp Bot 106:79–86

Bai S, Saito T, Sakamoto D, Ito A, Fujii H, Moriguchi T (2013) Transcriptome analysis of Japanese pear (*Pyrus pyrifolia* Nakai) flower buds transitioning through endodormancy. Plant Cell Physiol 54(7):1132–1151

Bielenberg DG, Wang Y, Li Z, Zhebentyayeva T, Fan S, Reighard GL et al (2008) Sequencing and annotation of the evergrowing locus in peach [*Prunus persica* (L.) Batsch] reveals a cluster of six MADS-box transcription factors as candidate genes for regulation of terminal bud formation. Tree Genet Genomes 4(3):495–507

Böhlenius H, Huang T, Charbonnel-Campaa L, Brunner AM, Jansson S, Strauss SH, Nilsson O (2006) CO/FT regulatory module controls timing of flowering and seasonal growth cessation in trees. Science 312:1040–1043

Bradshaw H, Stettler R (1995) Molecular genetics of growth and development in *Populus*. IV. Mapping QTLs with large effects on growth, form, and phenology traits in a forest tree. Genetics 139:963–973

Campoy JA, Ruiz D, Egea J (2011) Dormancy in temperate fruit trees in a global warming context: a review. Sci Hort 130:357–372

Celton J-M, Chagné D, Tustin SD, Terakami S, Nishitami C, Yamamoto T, Gardiner SE (2009) Update on comparative genome mapping between *Malus* and *Pyrus*. BMC Res Notes 2:182

Celton J-M, Martinez S, Jammes M-J, Bechti A, Salvi S, Legave J-M et al (2011) Deciphering the genetic determinism of bud phenology in apple progenies: a new insight into chilling and heat requirement effects on flowering dates and positional candidate genes. New Phytol 192:378–392

Chagné D, Crowhurst RN, Troggio M, Davey MW, Gilmore B, Lawley C et al (2012) Genome-wide SNP detection, validation, and development of an 8K SNP array for apple. PLoS One 7(2):e31745

Chagné D, Crowhurst RN, Pindo M, Thrimawithana A, Deng C, Ireland H et al (2014) The draft genome sequence of European Pear (*Pyrus communis* L. 'Bartlett'). PLoS One 9(4):e92644

Conner PJ, Brown SK, Weeden NF (1998) Molecular-marker analysis of quantitative traits for growth and development in juvenile apple trees. Theor Appl Genet 96:1027–1035

Dennis FG (1987) Two methods for studying rest: temperature alternation and genetic analysis. Hortscience 22:820–824

Doğramaci M, Horvath DP, Chao WS, Foley ME, Christoffers MJ, Anderson JV (2010) Low temperatures impact dormancy status, flowering competence, and transcript profiles in crown buds of leafy spurge. Plant Mol Biol 73:207–226

Falavigna VS, Porto DD, Buffon V, Margis-Pinheiro M, Pasquali G, Revers LF (2014) Differential transcriptional profiles of dormancy-related genes in apple buds. Plant Mol Biol Rep 32:796–813

Fan S, Bielenberg DG, Zhebentyayeva TN, Reighard GL, Okie WR, Holland D et al (2010) Mapping quantitative trait loci associated with chilling requirement, heat requirement and bloom date in peach (*Prunus persica*). New Phytol 185(4):917–930

FAO (2012) Food and Agriculture Organization of the United Nations. http://www.fao.org/home/en/. Accessed 13 May 2014

Faria DA, Mamani, EMC, Pappas GJ, Grattapaglia D (2011) Genotyping systems of *Eucalyptus* based on tetra-, penta-, and hexanucleotide repeat EST microsatellites and their use for individual fingerprinting and assignment tests. Tree Genet Genomes 7:63–77

Flachowsky H, Hanke M-V, Peil A, Strauss SH, Fladung M (2009) A review on transgenic approaches to accelerate breeding of woody plants. Plant Breed 128(3):217–226

Flachowsky H, Peil A, Hanke M, Tränkner C, Szankowski I et al (2012) Functional characterization of two antagonistic acting flowering genes in apple *Malus* x *domestica* Borkh. Acta Hort 929:351–356

Franklin KA (2009) Light and temperature signal crosstalk in plant development. Curr Opin Plant Biol 12(1):63–68

Frewen BE, Chen TH, Howe GT, Davis J, Rohde A, Boerjan W et al (2000) Quantitative trait loci and candidate gene mapping of bud set and bud flush in *Populus*. Genetics 154(2):837–845

Grattapaglia D, Kirst M (2008) Eucalyptus applied genomics: from gene sequences to breeding tools. New Phytol 179:911–929

Grattapaglia, D, Sederoff R (1994) Genetic linkage maps of *Eucalyptus grandis and Eucalyptus urophylla* using a pseudotestcross: mapping strategy and RAPD markers. *Genetics* 137:1121–1137

Gregis V, Andrés F, Sessa A, Guerra RF, Simonini S, Mateos JL et al (2013) Identification of pathways directly regulated by SHORT VEGETATIVE PHASE during vegetative and reproductive development in Arabidopsis. Genome Biol 14:R56

Hauagge R, Cummins JN (1991) Genetics of length of dormancy period in *Malus* vegetative buds. J Am Soc Hortic Sci 116(1):121–126

Heide OM, Prestrud AK (2005) Low temperature, but not photoperiod, controls growth cessation and dormancy induction and release in apple and pear. Tree Physiol 25:109–114

Helliwell CA, Wood CC, Robertson M, James Peacock W, Dennis ES (2006) The Arabidopsis FLC protein interacts directly in vivo with *SOC1* and *FT* chromatin and is part of a high-molecular-weight protein complex. Plant J 46:183–192

Horvath D (2009) Common mechanisms regulate flowering and dormancy. Plant Sci 177:523–531

Horvath DP, Anderson JV, Chao WS, Foley ME (2003) Knowing when to grow: signals regulating bud dormancy. Trends Plant Sci 8:534–540

Horvath DP, Sung S, Kim D, Chao W, Anderson J (2010) Characterization, expression and function of *DORMANCY ASSOCIATED MADS-BOX* genes from leafy spurge. Plant Mol Biol 73(1–2):169–179

Howe GT, Saruup P, Davies J, Chen THH (2000) Quantitative genetics of bud phenology, frost damage and winter survival in an F2 family of hybrid poplars. Theor Appl Genet 101:632–642

Hsu C, Adams JP, Kim H, No K, Ma C, Strauss SH et al (2011) *FLOWERING LOCUS T* duplication coordinates reproductive and vegetative growth in perennial poplar. Proc Natl Acad Sci USA 108(26):10756–10761

Iketani H, Abe K, Yamamoto T, Kotobuki K, Sato Y, Saito T et al (2001) Mapping of disease-related genes in Japanese pear using a molecular linkage map with RAPD markers. Breed Sci 51:179–184

Ito A, Sakamoto D, Moriguchi T (2012) Carbohydrate metabolism and its possible roles in endodormancy transition in Japanese pear. Sci Hortic 144:187–194

Ito A, Sugiura T, Sakamoto D, Moriguchi T (2013) Effects of dormancy progression and low-temperature response on changes in the sorbitol concentration in xylem sap of Japanese pear during winter season. Tree Physiol 33:398–408

Jackson JE (2003) Biology of apples and pears. Cambridge University Press, Cambridge

Jiménez S, Reighard GL, Bielenberg DG (2010) Gene expression of *DAM5* and *DAM6* is suppressed by chilling temperatures and inversely correlated with bud break rate. Plant Mol Biol 73:157–167

Kellerhals M (2009) Introduction to apple (*Malus* x *domestica*). In: Folta KM, Gardiner SE (eds) Genetics and genomics of Rosaceae. Springer, New York, p 73–84

Kirtman B, Power SB, Adedoyin JA, Boer GJ, Bojariu R, Camilloni I et al (2013) Near-term climate change: projections and predictability. In: Stocker TF, Qin D, Plattner G-K, Tignor M,

Allen SK, Boschung J et al (eds) Climate change 2013: the physical science basis. Contribution of working group I to the fifth assessment report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, p 953–1028

Korban SS, Tartarini S (2009) Apple structural genomics. In: Folta KM, Gardiner SE (eds) Genetics and genomics of Rosaceae. Springer, New York, p 85–119

Kotoda N, Hayashi H, Suzuki M, Igarashi M, Hatsuyama Y et al (2010) Molecular characterization of *FLOWERING LOCUS T*-like genes of apple (*Malus* x *domestica* Borkh.). Plant Cell Physiol (Apr) 51(4):561–575

Labuschagné IF, Louw JH, Schmidt K, Sadie A (2002) Genetic variation in chilling requirement in apple progeny. J Am Soc Hort Sci 127:663–672

Lang GA, Early JD, Martin GC, Darnell RL (1987) Endodormancy, paradormancy, and ecodormancy—physiological terminology and classification for dormancy research. Hortscience 22:371–377

Leida C, Conesa A, Llácer G, Badenes ML, Ríos G (2012) Histone modifications and expression of *DAM6* gene in peach are modulated during bud dormancy release in a cultivar dependent manner. New Phytol 193:67–80

Li Z, Reighard GL, Abbott AG, Bielenberg DG (2009) Dormancy-associated MADS genes from the EVG locus of peach [*Prunus persica* (L.) Batsch] have distinct seasonal and photoperiodic expression patterns. J Exp Bot 60(12):3521–3530

Liu G, Li W, Zheng P, Xu T, Chen L, Liu D et al (2012) Transcriptomic analysis of 'Suli' pear (*Pyrus pyrifolia* white pear group) buds during the dormancy by RNA-SEq. BMC Genomics 13:700

Mackay TFC (2001) The genetic architecture of quantitative traits. Annu Rev Genet 35:303–339

Mackay TFC, Stone EA, Ayroles JF (2009) The genetics of quantitative traits: challenges and prospects. Nat Rev Genet 10(8):565–577

Marafon AC, Citadin I, Amarante L, Herter FG, Hawerroth FJ (2011) Chilling privation during dormancy period and carbohydrate mobilization in Japanese pear trees. Sci Agric 68(4):462–468

Mason MG, Ross JJ, Babst BA, Wienclaw BN, Beveridge CA (2014) Sugar demand, not auxin, is the initial regulator of apical dominance. P Natl Acad Sci U S A 111(16):6092–6097

Mazzitelli L, Hancock RD, Haupt S, Walker PG, Pont SDA, McNicol J et al (2007) Co-ordinated gene expression during phases of dormancy release in raspberry (*Rubus idaeu*s L.) buds. J Exp Bot 58(5):1035–1045

Montanari S, Saeed M, Knäbel M, Kim Y, Troggio M, Malnoy M et al (2013) Identification of *Pyrus* single nucleotide polymorphisms (SNPs) and evaluation for genetic mapping in European pear and interspecific *Pyrus* hybrids. PLoS One 8(10):e77022

Myles S (2013) Improving fruit and wine: what does genomics have to offer? Trends Genet 29(4):190–196

Neale DB, Kremer A (2011) Forest tree genomics: growing resources and applications. Nat Rev Genet 12(2):111–122

Nishitami C, Saito T, Ubi BE, Shimuzi T, Itai A, Saito T et al (2012) Transcriptome analysis of *Pyrus pyrifolia* leaf buds during transition from endodormancy to ecodormancy. Sci Hortic 147:49–55

Olsen J, Junttila O, Nilsen J (1997) Ectopic expression of oat *phytochrome A* in hybrid aspen changes critical daylength for growth and prevents cold acclimatization. Plant J 12(6):1339–1350

Palmer J (2012) Apples and pears—Pipfruit in New Zealand. In: Te Ara—the Encyclopedia of New Zealand [updated 2002 Jul 13]. http://www.TeAra.govt.nz/en/apples-and-pears. Accessed 12 May 2014

Porto DD, Bruneau M, Perini P, Anzanello R, Renou J, dos Santos HP et al. (2015) Transcription profilling of the chilling requeriment for budbreak in apples: a putative role for FLC-like genes. J Exp Bot. doi:10.1093/jxb/erv061

Rinne PL, Kaikuranta PM, van der Schoot C (2001) The shoot apical meristem restores its symplasmic organization during chilling-induced release from dormancy. Plant J 26:249–264

Rinne PL, Welling A, Vahala J, Ripel L, Ruonala R, Kangasjärvi J, van der Schoot C (2011) Chilling of dormant buds hyperinduces *FLOWERING LOCUS T* and recruits GA-inducible 1,3-beta-glucanases to reopen signal conduits and release dormancy in *Populus*. Plant Cell 23:130–146

Rohde A, Bhalerao RP (2007) Plant dormancy in the perennial context. Trends Plant Sci 12:217–223

Rohde A, Prinsen E, De Rycke R, Engler G, Van Montagu M, Boerjan W (2002) *PtABI3* impinges on the growth and differentiation of embryonic leaves during bud set in poplar. Plant Cell 14:1885–1901

Saito T, Bai S, Ito A, Sakamoto D, Saito T, Ubi BE et al (2013) Expression and genomic structure of the *dormancy-associated MADS box* genes *MADS13* in Japanese pears (*Pyrus pyrifolia* Nakai) that differ in their chilling requirement for endodormancy release. Tree Physiol 33:654–667

Sasaki R, Yamane H, Ooka T, Jotatsu H, Kitamura Y, Akagi T et al (2011) Functional and expressional analyses of *PmDAM* genes associated with endodormancy in Japanese Apricot. Plant Physiol 157(1):485–497

Seo E, Lee H, Jeon J, Park H, Kim J, Noh YS, Lee I (2009) Crosstalk between cold response and flowering in Arabidopsis is mediated through the flowering-time gene *SOC1* and its upstream negative regulator *FLC*. Plant Cell 21:3185–3197

Srinivasan C, Dardick C, Callahan A, Scorza R (2012) Plum (*Prunus domestica*) trees transformed with poplar *FT1* result in altered architecture, dormancy requirement, and continuous flowering. PLoS One 7(7):e40715

Sun W, Zhang Y, Le W, Zhang H (2009) Construction of a genetic linkage map and QTL analysis for some leaf traits in pear (*Pyrus* L.). Front Agric China 3(1):67–74

Takemura Y, Kuroki K, Matsumoto K, Ban Y, Moriguchi T, Tamura F (2013) Identification and expression analysis of candidate genes related to endodormancy induction and breaking in *Pyrus pyrifolia*. Sci Hortic 155:65–71

Thomashow MF (2010) Molecular basis of plant cold acclimation: insights gained from studying the CBF cold response pathway. Plant Physiol 154:571–577

Tränkner C, Lehmann S, Hoenicka H, Hanke M, Fladung M et al (2010) Over-expression of an *FT*-homologous gene of apple induces early flowering in annual and perennial plants. Planta 232:1309–1324

Troggio M, Gleave A, Salvi S, Chagné D, Cestaro A, Kumar S et al (2012) Apple, from genome to breeding. Tree Genet Genomes 8(3):509–529

Troggio M, Surbanovski N, Bianco L, Moretto M, Giongo L, Banchi E et al (2013) Evaluation of SNP data from the *Malus* infinium array identifies challenges for genetic analysis of complex genomes of polyploid origin. PLoS One 8(6):e67407

Ubi B, Sakamoto D, Ban Y, Shimada Y, Ito A, Nakajima I et al (2010) Molecular cloning of dormancy-associated MADS-box gene homologs and their characterization during seasonal endodormancy transitional phases of Japanese Pear. J Am Soc Hort Sci 135(2):174–182

van der Schoot C Rinne PL (2011) Dormancy cycling at the shoot apical meristem: transitioning between self-organization and self-arrest. Plant Sci 180:120–131

van Dyk MM Soeker MK Labuschagne IF Rees DJG (2010) Identification of a major QTL for time of initial vegetative budbreak in apple (*Malus* x *domestica* Borkh.). Tree Genet Genomes 6(3):489–502

Velasco R, Zharkikh A, Affourtit J, Dhingra A, Cestaro A, Kalyanaraman A et al (2010) The genome of the domesticated apple (*Malus* x *domestica* Borkh.). Nat Genet 42:833–839

Wenzel S, Flachowsky H, Hanke M (2013) The Fast-track breeding approach can be improved by heat induced expression of the *FLOWERING LOCUS T* genes from poplar (*Populus trichocarpa*) in apple (*Malus* x *domestica* Borkh.). Plant Cell Tiss Org Cult 115:127–137

Wisniewski M, Norelli J, Bassett C, Artlip T, Macarisin D (2011) Ectopic expression of a novel peach (*Prunus persica*) CBF transcription factor in apple (*Malus* x *domestica*) results in short-day induced dormancy and increased cold hardiness. Planta 233:971–983

Wu RM, Walton EF, Richardson AC, Wood M, Hellens RP, Varkonyi-Gasic E (2012) Conservation and divergence of four kiwifruit *SVP*-like MADS-box genes suggest distinct roles in kiwifruit bud dormancy and flowering. J Exp Bot 63:797–807

Wu J, Wang Z, Shi Z, Zhang S, Ming R, Zhu S et al (2013) The genome of the pear (*Pyrus bretschneideri* Rehd.). Genome Res 23(2):396–408

Wu RM, Wang T, McGie T, Voogd C, Allan AC, Hellens RP et al (2014) Overexpression of the kiwifruit *SVP3* gene affects reproductive development and suppresses anthocyanin biosynthesis in petals, but has no effect on vegetative growth, dormancy, or flowering time. J Exp Bot. 65:4985–4995. doi:10.1093/jxb/eru264

Yamamoto T, Chevreau E (2009) Pear genomics. In: Folta KM, Gardiner SE (eds) Genetics and genomics of Rosaceae. Springer, New York, p 163–186

Yamamoto T, Kimura T, Shoda M, Imai T, Saito T, Sawamura Y et al (2002) Genetic linkage maps constructed by using an interspecific cross between Japanese and European pears. Theor Appl Genet 106:9–18

Yamane H, Ooka T, Jotatsu H, Sasaki R, Tao R (2011) Expression analysis of *PpDAM5* and *PpDAM6* during flower bud development in peach (*Prunus persica*). Sci Hortic 129:844–848

Zhebentyayeva TN, Fan S, Chandra A, Bielenberg DG, Reighard GL, Okie WR et al (2014) Dissection of chilling requirement and bloom date QTLs in peach using a whole genome sequencing of sibling trees from an F2 mapping population. Tree Genet Genomes 10(1):35–51

Chapter 6
Flower Bud Dormancy in Prunus Species

Erica Fadón, Maria Herrero and Javier Rodrigo

Introduction

In *Prunus* species, while flowering occurs in the spring, flower differentiation takes place at the end of the previous summer. These flower buds continue development until mid-autumn, when they generally enter a dormant stage (Perry 1971). The first step for dormancy establishment is growth cessation, even under growth conducive conditions. But also during dormancy, meristems acclimate to cold and remain unresponsive to growth signals (Cooke et al. 2012). To reestablish the ability to grow, flower buds need to pass a certain period of time under low temperatures, allowing growth to resume once environmental conditions are suitable (Perry 1971; Sedgley and Griffin 1989).

Establishment of dormancy in buds of temperate fruit trees is not just a survival strategy. Because cold is required for proper flowering, it is indeed one of the main drawbacks for the extension of temperate fruit trees to warmer latitudes (Atkinson et al. 2013). Moreover, cold requirements are genotype specific, independent of where the plant grows (Jansson and Douglas 2007), and is an essential criterion for selecting cultivars adapted to particular areas. Based on the agricultural importance of dormancy, the impact of predicted global warming in temperate climates (Hedhly et al. 2009) has renewed interest on the implications that increasing temperatures might have on bud dormancy and, thus, agriculture.

In spite of the relevance for adaptation of temperate woody perennials to different climatic conditions (Horvath et al. 2003), and the consistency of the dormancy process (Rohde and Bhalerao 2007), little information exists on what occurs in flower development during this time. Although information from different fields is

E. Fadón (✉) · M. Herrero
Estación Experimental Aula Dei, CSIC, Av. Montañana 1005, 50059 Zaragoza, Spain
email: ericafadon@eead.csic.es

E. Fadón · J. Rodrigo
Centro de Investigación y Tecnología Agroalimentaria (CITA), Av. Montañana nº 930, 50059 Zaragoza, Spain

© Springer International Publishing Switzerland 2015
J. V. Anderson (ed.), *Advances in Plant Dormancy,* DOI 10.1007/978-3-319-14451-1_6

accumulating, so far, a common mechanism has not been determined. Here we go through the information available on dormancy and flower development in temperate fruit trees, paying attention to flower development in the dormancy context, the ways to measure cold, and the information available on its genetic and physiological control.

Flower Bud Development

Flower induction, differentiation and development are conserved processes that have been extensively studied in model annual plants such as *Arabidopsis thaliana* (Smyth et al. 1990), tobacco (Koltunow et al. 1990), and tomato (Brukhin et al. 2003). In temperate woody plants, flower bud development has also been comprehensively studied in the model perennial species *Populus* (Bradshaw et al. 2000; Brunner and Nilsson 2004). In *Prunus* species, different studies have described early flower differentiation in flower buds containing several flowers such as sweet cherry (Guimond et al. 1998), sour cherry (Diaz et al. 1981), or plum (Ragland 1934a), and in flower buds containing a solitary flower as in peach (Chandler and Tufts 1933; Ragland 1934a, b; Dorsey 1935; Warriner et al. 1985; Luna et al. 1991), almond (Lamp et al. 2001), or apricot (Julian et al. 2010). However, during the development and life span of flowering, little is known about their adaptation to different environmental conditions (summer, autumn, winter, and finally spring).

The different descriptions for flower development in *Prunus* species share a common ground and follow a conserved pattern for flower differentiation. Before flower initiation, meristems are vegetative and produce bud scales. Flower initiation first becomes apparent at the shoot apex, with an increase in meristem size, the acquisition of a dome shape, and then followed by production of flower primordia (Diaz et al. 1981; Guimond et al. 1998; Lamp et al. 2001). In *Prunus* species, flower buds are initiated after fruit development, and flower development requires several months until blooming (Tukey 1989). Flowers are hermaphrodite, with four concentric whorls (sepals, petals, stamens, and carpel). Floral organogenesis proceeds in a centripetal way beginning with the initiation of the five sepals. Then petal primordia arise, alternating to the sepals within the calyx; and—within the corolla—multiple stamens are initiated. Finally, carpel initiation begins with emergence of a single primordium that expands along the apical flank as the carpel margins develop and finally close (Diaz et al. 1981; Guimond et al. 1998; Lamp et al. 2001). At late winter or early spring, depending on species, bud burst proceeds rapidly; bud scales separate and the sepals appear. Sepal expansion takes place concomitantly with the start of pistil elongation. Apparition of the petals is followed with the rapid elongation of the pistil (Julian et al. 2010). Finally, the flower opens, showing five sepals, five petals, numerous stamens, and one carpel (Sterling 1964).

Flower Bud Dormancy

The requirement of chilling accumulation during dormancy for adequate flower development was reported as early as the 19th century (Knight 1801). However, it was a century later when Coville (1920) demonstrated it experimentally, when for breeding purposes in blueberry (*Vaccinium corymbosum*), he could not get two reproductive cycles in the same year due to lack of chilling. Following his work, the basis for how dormancy affects trees was established. In temperate and cold climates, following the active growing season, flower buds of woody plants stop growing and enter a dormant state in autumn, which gets established prior to the exposure to low temperatures (Coville 1920). The state of bud dormancy deepens progressively (Rohde and Bhalerao 2007) until reaching a stage where the buds cannot grow, even under the most favorable conditions. Eventually, following an adequate exposure to chilling temperatures (Vegis 1964), such deep dormancy ends, and the buds are ready to burst.

Attempts to characterize the sequential phases of dormancy have resulted in more than 50 terms, most of them leading to confusion by the lack of physiological basis (Martin 1991). In order to solve this problem, the definition of dormancy has been simplified as "the absence of visible growth in any plant structure containing a meristem" (Lang et al. 1987) or "the inability to initiate growth from meristems, by elongation or cell division, under favorable conditions" (Rohde and Bhalerao 2007). Depending on the factors causing this state, three types of dormancy have been put forward: endodormancy, which is regulated by physiological factors inside the affected structure; paradormancy, regulated by physiological factors outside the affected structure; and ecodormancy, regulated by environmental factors (Lang et al. 1987).

Measuring Chilling Requirement in Fruit Trees

The importance of flower bud dormancy for insuring an adequate flowering response in temperate fruit trees (Perry 1971), along with the fact that cultivars have different chilling requirements (Jansson and Douglas 2007), has resulted in the development of different empirical models to estimate chilling requirements during dormancy. Chilling requirements of particular cultivars have been empirically calculated by estimating the end of endodormancy, when flower buds recover their capacity to grow. For this purpose, shoot cuttings, taken at different times during the winter, are placed under favorable growth conditions in controlled chambers; when a certain percentage of buds shows external phenological development (Weinberger 1950), or increase in weight (Brown and Kotob 1957), the chilling units up to cutting time are quantified in different ways based on temperature records.

A number of models have been developed to quantify the low temperature requirement. Early work is based on the records on the number of hours under 7.2ºC

(Chilling Hours), which are added throughout the estimated period of dormancy (Weinberger 1950). Subsequent models considered that not all temperatures affect the plant in the same way to release dormancy but that temperatures have a bell-shaped dependence up to the end of dormancy, and high temperatures have a negative effect on chilling accumulation (Erez and Lavee 1971). The "UTAH Model" proposes the sum of "chill-units," establishing a different chilling contribution for different temperature ranges. Thus, a negative value is given to warm temperatures and the temperatures below 1.4°C are not considered (Richardson et al. 1974). The "Dynamic Model" considers that the effect of chilling temperatures can be affected by warm temperatures, before chilling fulfillment, and proposes the use of "chill portions" (Fishman et al. 1987). The Dynamic Model appears as a better indicator of the response to chilling in the subtropics, while the UTAH Model better fits in cooler areas of temperate zones (Dennis 2003). These models are the most widely used for fruit trees, although there are many others (Bidabé 1965; Cesaraccio et al. 2004; Legave et al. 2008). A major difference between these models is the importance given to the sequence of temperatures during dormancy. According to the "Chilling Hours" and "Utah" models, the same temperature always has the same effect, regardless of when it occurs. However, in the "Dynamic Model," the same temperature at different times of the period can have different effects on chilling accumulation (Luedeling 2012).

In spite of their empiric nature, these models are useful to predict if a species or cultivar can adapt to a new geographical cultivation area. However, chilling requirements are cumbersome to calculate and have not been estimated across an adequate range of cultivars. Also, the available data are highly variable depending on the model used (Seeley et al. 1994), the heterogeneity at the time of the start and end-point of the period in which cold temperatures are considered (Dennis 2003), and the forcing condition used. Variability is also introduced in estimating the percentage of weight increase, or the phenological stage of bud development, which are considered as indicators for breaking dormancy (Julian et al. 2014).

These models were developed in an empirical way to fit what occurs in reality, and a proper biological indicator to calculate cold requirements would be most useful. However, this is hampered by the lack of knowledge on what happens inside the flower bud, and what are the mechanisms and the changes underpinning entering and breaking endodormancy.

Genetic Control of Dormancy

Studies focused on the genetic control of dormancy have proposed target genes for dormancy regulation that are also involved in flower differentiation and growth including *FLOWER LOCUS T* (*FT*) (Koornneef et al. 1991; Böhlenius et al. 2006; Hsu et al. 2011), *TERMINAL FLOWER 1* (*TFL1*)/*CENTRORADIALIS 1* (*CEN1*) (Bradley 1997; Mohamed et al. 2010; Mimida et al. 2011) or the *dormancy-associated MADS-box* (*DAM*) genes (Messenguy and Dubois 2003; Jiménez et al. 2010a, b).

Flower Regulation Genes

FT and CO are involved in the interaction between long-day photoperiod and the initiation of flowering of model annuals such as *Arabidopsis thaliana* (Koornneef et al. 1991). In aspen, a model woody perennial, the *CO-FT* signaling network under long-day photoperiod conditions displays an expression pattern very similar to that in *Arabidopsis*. But under a critical short-day photoperiod, *PtFT1* was reported to mediate vegetative growth cessation and bud set, regulating the entrance into dormancy (Böhlenius et al. 2006). Subsequent studies considered two similar poplar paralogs, *PtFT1* and *PtFT2*, to regulate these different functions. *PtFT1* was predominantly expressed in late winter, due to the effect of cold temperatures rather than photoperiod, and induced reproductive onset in undifferentiated meristems. However, *PtFT2* was only expressed under long-day photoperiods and warm temperatures and was involved in controlling vegetative growth (Hsu et al. 2011; Pin and Nilsson 2012). Further, down-regulation of *FT2* under short photoperiods coincides with growth cessation and bud set, which, as described in greater detail below, appears to involve interaction between DAMs and *FT* (see Cooke et al. 2012; Ríos et al. 2014; Sasaki et al. 2011).

TERMINAL FLOWER 1 (TFL1) is expressed in *Arabidopsis thaliana* during the vegetative phase, delaying the commitment to form an inflorescence (Bradley 1997). The relationship between the expressions of *FT* and *TFL1* regulates floral determination, and down-regulation of *TLF1* has been proposed to control vegetative growth and flower determination (Jaeger et al. 2013). Another closely related gene *CEN1* could also be involved. *CEN1* and *TFL1* belong to the same gene family and one or the other are usually present depending on the plant family. In *Populus*, *TFL1*-like genes do not appear to be present and flower onset and differentiation of shoot phenology is regulated by *PopCEN1/PopCEN2*, which seems to be important for dormancy: down-regulation of *PopCEN1* enables dormancy release, and as dormancy is released and growth resumes, up-regulation of *PopCEN1* promotes meristem indetermination (Mohamed et al. 2010). In apple, a *TFL1* homologue, *MdTFL1*, is down-regulated in the rib meristem zone during the determination of the flower bud. Subsequently, *APPLE FLORICAULA* (*AFL2a),* a *LEAFY* ortholog, and *Malus domestica APETALA 1* (*MdAP1)* are highly expressed in the floral meristem and in the developing floral organ primordial (Mimida et al. 2011).

An Evergreen Mutant and DAM Genes

In a peach *evergrowing* mutant able to continue growing under dormancy inducting conditions, a set of six *DAM* genes were proposed as candidates for controlling the non-dormant trait (Bielenberg et al. 2008). *DAM*s are members of the *MADS-box* genes and were derived from serial tandem duplications (Jiménez et al. 2009) and their expression is affected by short photoperiods (Li et al. 2009). *DAM1*, *DAM2,* and *DAM4* are related with seasonal cessation of elongation and terminal flower

bud formation late in summer. After that, expression of *DAM3*, *DAM5,* and *DAM6* seems to function in establishing and maintaining endodormancy. *DAM5* and *DAM6* are up-regulated with short-day photoperiods, reaching a peak with the chilling requirements fulfillment and then down-regulated with bud break and blooming (Li et al. 2009). The expression level of those genes, *DAM5* and *DAM6,* was proposed to predict the chilling time required to achieve bud break, due to the fact that these two factors are negatively and linearly related (Jiménez et al. 2010a). Thus, *DAM5* and *DAM6* could be promoting the transcription of negative regulators of bud development as *DAMs* have been identify in other species as transcription factors (Yamane et al. 2008, 2011; Esumi et al. 2010; Sasaki et al. 2011).

A body of evidence implicates epigenetic control of dormancy (Ríos et al. 2014). Several reports have suggested that dormancy and vernalization involve overlapping processes (Chourad 1960; Horvath 2009; Horvath et al. 2003), and the implications of epigenetics in vernalization have been put forward (Sung and Amasino 2005; Song et al. 2012; Ietswaart et al. 2012). Studies have indicated that histone modifications and DNA methylation might take place during various phases of the dormancy cycle (Horvath et al. 2003; Santamaría et al. 2011; Badenes et al. 2012; Vining et al. 2012; Lafon-Placette et al. 2013). While information is still fragmented, this is an active field where future contributions should lead to a better understanding of the physiological events impacting regulation of dormancy.

Integrating genetic studies associated with tree dormancy and vernalization established some common patterns. In *Arabidopsis*, FLOWERING LOCUS C (FLC), a member of the MADS-box family, regulates vernalization by repressing the transcription of *FT* (Pin and Nilsson 2012; Ríos et al. 2014). Similarly, in woody perennials evidence suggests that DAM-5 and-6, also members of a MADS-box family, are involved in repressing the expression of *FT2* under short-day condition (Sasaki et al. 2011). Furthermore, the similarity of these processes provides a framework for the epigenetic study of dormancy in woody perennials (see Pin and Nilsson 2012), as research on the epigenetic regulation of this mechanism is well advanced in *Arabidopsis* (Angel et al. 2011).

Physiology of Dormancy

While the genetic or epigenetic control of dormancy is far from clear in perennials, a different approach to understand dormancy has been to examine physiological changes. After two centuries of scientific interest in tree dormancy and its implications in fruit production, the physiological processes and the environmental factors that induce and break dormancy are not completely understood (Campoy et al. 2011; Luedeling 2012). However, during this time, there have been several attempts to identify biological markers related to the level of dormancy in different tissues of the tree. These have mainly been related to hormonal changes and to cell and organ isolation.

Hormonal Changes

Because plant hormones play an important role in plant growth and development and interesting interactions have been put forward in seed dormancy (Bewleyl 1997; Vanstraelen and Benková 2012), they have also been studied in relation with tree dormancy (Rinne et al. 2011; Atkinson et al. 2013). A classical theory for hormonal control of dormancy involved auxins, cytokinins, and abscisic acid (ABA) (Crabbé 1994). Gibberelins (GA) and ABA have been recognized as the key internal factors in seed dormancy (Vanstraelen and Benková 2012). At bud dormancy establishment, the action of low levels of gibberellins has been associated with growth cessation (Olsen et al. 1995; Eriksson and Moritz 2002), and alterations in ABA concentrations have been proposed to play a role in leaf drop in the autumn (Welling et al. 1997; Rinne et al. 2011). During bud dormancy, the establishment of cold acclimatization related to changes in the bud water status has also been proposed to impact freezing tolerance. This is supported by the high variations in dehydrins reported during dormancy (Faust et al. 1997; Rinne et al. 2010), since dehydrins are specific proteins that protect the cell against cellular dehydration (Faust et al. 1997; Arora et al. 2003). Although the mechanisms behind the hormone regulation are not well understood, hormones could act to regulate specific components of the cell cycle as a mechanism for regulating induction and breaking of dormancy (Horvath et al. 2003).

An indirect line of evidence for hormones impacting dormancy is evaluating the effect of external application of these growth regulators. For example, cytokinins have been used to overcome dormancy (Wang et al. 1991). Application of gibberellins to dormant buds can replace chilling to release dormancy, suggesting that chilling effects could be mediated by GA (Lang 1957). However, this approach is hampered by the fact that external application of growth regulators has different effects depending on the dormancy status. This could be due to a proposed superimposed process, which relates freezing resistance to membrane control, rendering meristems insensitive to the hormonal effects during dormancy (Faust et al. 1997).

Cell and Organ Isolation

Cell-to-cell communication status through plasmodesmata connections has been proposed to affect vegetative dormancy (Rinne et al. 2001, 2011). Detailed studies on the shoot apical meristems of *Populus* revealed changes in symplasmic domains that were dependent on the dormancy status and were highly influenced by temperatures (Rinne et al. 2001). Different internal stages of symplasmic communication have been characterized as online, offline and standby (Rinne et al. 2010). At the online stage, there is communication though plasmodesmata when the shoot apical meristems are proliferating and freeze sensitive. At the offline stage, the shoot apical meristem is dormant and cells are dehydrated, freezing-tolerant, and cell-to-cell communication is inhibited by callose containing sphincter complexes located at

the plasmodesmata and cell wall impregnations. Following chilling accumulation, plamodesmata communication is restored and changes in cell walls occur with lipids relocalization. These changes release the shoot bud meristem from dormancy, but this was termed the stand by status since bud growth is only resumed when suitable weather conditions occur with the accumulation of thermal units and water availability (Rinne et al. 2010), which finally results in bud burst. These changes have also been confirmed by the expression of GA-inducible 1,3-β-glucanases, which regulate the degradation of callose deposited at the plasmodesmata (Rinne et al. 2011; Paul et al. 2014). While all this work refers to vegetative buds it would be worthwhile to evaluate whether these on-off-on stages could also apply to dormancy in flower buds.

Interestingly, communication through the establishment of vascular connections has also been put forward in vegetative buds to accompany breaking of dormancy (Fonti et al. 2007; Begum et al. 2007, 2008). While the establishment of vascular connections has not been directly related with flower bud dormancy, vessel development has been associated with de-acclimation (Ashworth 1984) and frost sensitivity. During stamen development in apricots, one of the first changes following dormancy is the development of vascular connections, which was closely followed by the occurrence of meiosis (Julian et al. 2011). Later work on peach confirmed this finding showing the expression of sporopollenin genes (Ríos et al. 2013). After the completion of meiosis, anthers acquire a distinctive yellowish color that could be used as visual indicators for determining if flowers are dormant or not (Julian et al. 2014). While meiosis time is not concomitant with chilling fulfillment, it is genotype dependent and highly influenced by winter cold temperatures, being earlier in cold winters when chilling requirements are fulfilled early (Julian et al. 2014).

While further work is required, dormancy appears to be associated with isolation at the cell and organ level. But what triggers isolation and reestablishment of communication remains to be unveiled.

Perspectives

While there is a renewed interest to understand flower bud dormancy, and different approaches of investigation are being used, so far, information remains fragmented. Flower bud development has been characterized in temperate *Prunus* species (Chandler and Tufts 1933; Ragland 1934b; Dorsey 1935; Diaz et al. 1981; Warriner et al. 1985; Luna et al. 1991; Guimond et al. 1998; Lamp et al. 2001; Julian et al. 2010), but how this development relates to flower dormancy remains obscure. Likewise, while chilling requirements are known and are used to ascertain the possible adaptation of particular cultivars to particular areas (Dennis 2003), what lies behind these genetic differences remains elusive.

The search for the genetic control of dormancy is an active field and the involvement of different genes has been put forward. Some of these genes are known to be involved in flower development (Koornneef et al. 1991; Bradley 1997; Böhlenius

et al. 2006; Mohamed et al. 2010; Mimida et al. 2011; Hsu et al. 2011), while some are *MADS-box* genes (Jiménez et al. 2010a, b), and the interaction between them is starting to emerge. Our understanding of these interactions, as well as epigenetic regulation (Santamaría et al. 2011; Badenes et al. 2012; Ríos et al. 2014), will increase as research continues. The same applies to physiological studies, although recently the observation of cell isolation during dormancy (Rinne et al. 2001, 2011) provides a physical setting for dormancy. Thus, it appears that dormancy is accompanied by cell isolation both through plasmodesmata at the cell level and through vascular connections at the organ level (Begum et al. 2007; Julian et al. 2011). Determining how these complex genetic and physiological interactions occur will surely impact agriculture by providing a basis for extension of temperate fruit trees to new latitudes and providing clues for adaptation of temperate forests to global warming.

Acknowledgments This work was supported by Ministerio de Ciencia e Innovación (MICINN)-FEDER [AGL 12621-C02-01, AGL 2012-40239], INIA (RF2011-00029-CO03-01-02) and Gobierno de Aragón [group A43]. E.F. was supported by a doctoral fellowship [FPI BES-2010-037992] from MICINN.

References

Angel A, Song J, Dean C, Howard M (2011) A Polycomb-based switch underlying quantitative epigenetic memory. Nature 476:105–109

Arora R, Rowland LJ, Tanino K (2003) Induction and release of bud dormancy in woody perennials: a science comes of age. HortScience 38(5):911–921

Ashworth EN (1984) Xylem development in prunus flower buds and the relationship to deep supercooling. Plant Physiol 74(4):862–865

Atkinson CJ, Brennan RM, Jones HG (2013) Declining chilling and its impact on temperate perennial crops. Environ Exp Bot 91:48–62. (Elsevier B.V.)

Badenes ML, Rı G, Leida C, Conesa A, Lla G (2012) Histone modifications and expression of DAM6 gene in peach are modulated during bud dormancy release in a cultivar-dependent manner. New Phytol 6:67–80

Begum S, Nakaba S, Bayramzadeh V, Oribe Y, Kubo T, Funada R (2008) Temperature responses of cambial reactivation and xylem differentiation in hybrid poplar (*Populus sieboldii* x *P. grandidentata*) under natural conditions. Tree Physiol 28(12):1813–1819

Begum S, Nakaba S, Oribe Y, Kubo T, Funada R (2007). Induction of cambial reactivation by localized heating in a deciduous hardwood hybrid poplar (*Populus sieboldii* x P. *grandidentata*). Ann Bot 100(3): 439–447

Bewleyl JD (1997) Seed germination and dormancy. Plant Cell 9(7):1055–1066

Bidabé B (1965) Contrôle de l´époque de floraison du pommier par une nouvelle conception de l´action de températures. Comptes Rendus l'Academie d'Agriculture Fr 49:934–945

Bielenberg DG, Wang YE, Li Z, Zhebentyayeva T, Fan S, Reighard GL et al (2008) Sequencing and annotation of the evergrowing locus in peach [*Prunus persica* (L.) Batsch] reveals a cluster of six MADS-box transcription factors as candidate genes for regulation of terminal bud formation. Tree Genet Genomes 4(3):495–507

Böhlenius H, Huang T, Charbonnel-Campaa L, Brunner AM, Jansson S, Strauss SH et al (2006) CO/FT regulatory module controls timing of flowering and seasonal growth cessation in trees. Science 312(5776):1040–1043

Bradley D (1997) Inflorescence commitment and architecture in *Arabidopsis*. Science 275(5296):80–83
Bradshaw HD, Ceulemans R, Davis J, Stettler R (2000) Emergin model systems in plant biology: poplar (*Populus*) as a model forest tree. J Plant Growth Regul 19:306–313
Brown DS, Kotob FA (1957) Growth of flower buds of apricot, peach, and pear durin the rest period. Proc Am Soc Hortic Sci 69:158–164
Brukhin V, Hernould M, Gonzalez N, Chevalier C (2003) Flower development schedule in tomato *Lycopersicon esculentum* cv. sweet cherry. Sex Plant Rep 15:311–320
Brunner AM, Nilsson O (2004) Revisiting tree maturation and floral initiation in the poplar functional genomics era. New Phytol 12;164(1):43–51.
Campoy JA, Ruiz D, Egea J (2011) Dormancy in temperate fruit trees in a global warming context: a review. Sci Hortic 130(2):357–372. (Amsterdam, Elsevier B.V.)
Cesaraccio C, Spano D, Snyder RL, Duce P (2004) Chilling and forcing model to predict bud-burst of crop and forest species. Agric For Meteorol 126(1–2):1–13
Chandler W, Tufts WP (1933) Influence of the rest period on opening of buds of fruit trees in spring and on development of flower buds of peach trees. Proc Am Soc Hortic Sci 30:180–186
Chourad P (1960) Vernalization and its relations to dormancy. Annu Rev Plant Physiol 11:191–238
Cooke JEK, Eriksson ME, Junttila O (2012) The dynamic nature of bud dormancy in trees: environmental control and molecular mechanisms. Plant Cell Environ 35(10):1707–1728
Coville FV (1920) The influence of cold in stimulating the growth of plants. Proc Natl Acad Sci U S A 6(7):434–435
Crabbé J (1994). Dormancy. In: Arntzen CJ, Ritter EM (eds) Encyclopedia of Agricultural Science, Vol. 1. Academic Press, San Diego, pp 597–611
Dennis FG (2003) Problems in standardizing methods for evaluating the chilling requirements for the breaking of dormancy in buds of woody plants. HortScience 38(3):347–350
Diaz DH, Rasmussen HP, Dennis FG (1981) Scanning electron microscope examination of flower bud differentiation in sour cherry. J Am Soc Hortic Sci 4:513–515
Dorsey M (1935) Nodal development of the peach shoot as related to fruit bud formation. Proc Am Soc Hortic Sci 33:245–257
Erez A, Lavee S (1971) The effect of climatic conditions on dormancy development of peach buds. 1. Temperature. Proc Am Soc Hortic Sci 96:711
Eriksson ME, Moritz T (2002) Daylength and spatial expression of a gibberellin 20-oxidase isolated from hybrid aspen (*Populus tremula* L. x *P. tremuloides* Michx.). Planta 214(6):920–930
Esumi T, Kitamura Y, Hagihara C, Yamane H, Tao R (2010) Identification of a TFL1 ortholog in Japanese apricot (*Prunus mume* Sieb. et Zucc.). Sci Hortic 125(4):608–616. (Amsterdam, Elsevier B.V.)
Faust M, Erez A, Rowland LJ, Wang SY, Norman HA (1997) Bud dormancy in perennial fruit trees: physiological basis for dormancy induction, maintenance and release. HortScience 32:623–629
Fishman S, Erez A, Couvillon GA (1987) The temperature dependence of dormancy breaking in plants: mathematical analysis of a two-step model involving a cooperative transition. J Theor Biol 124:473–483
Fonti P, Solomonoff N, García-González I (2007) Earlywood vessels of Castanea sativa record temperature before their formation. New Phytol 173(3):562–570
Guimond CM, Andrews PK, Lang GA (1998) Scanning electron microscopy of floral initiation in sweet cherry. J Am Soc Hortic Sci 123:509–512
Hedhly A, Hormaza JI, Herrero M (2009) Global warming and sexual plant reproduction. Trends Plant Sci 14:30–36
Horvath DP (2009) Common mechanisms regulate flowering and dormancy. Plant Sci 177(6):523–531
Horvath DP, Anderson JV, Chao WS, Foley ME (2003) Knowing when to grow: signals regulating bud dormancy. Trends Plant Sci 8(11):534–540
Hsu C-Y, Adams JP, Kim H, No K, Ma C, Strauss SH et al (2011) FLOWERING LOCUS T duplication coordinates reproductive and vegetative growth in perennial poplar. Proc Natl Acad Sci U S A 108(26):10756–10761

Ietswaart R, Wu Z, Dean C (2012) Flowering time control: another window to the connection between antisense RNA and chromatin. Trends Genet 28(9):445–453. (Elsevier Ltd)
Jaeger KE, Pullen N, Lamzin S, Morris RJ, Wigge Pa (2013) Interlocking feedback loops govern the dynamic behavior of the floral transition in *Arabidopsis*. Plant Cell 25(3):820–833
Jansson S, Douglas CJ (2007) Populus: a model system for plant biology. Annu Rev Plant Biol 58:435–458
Jiménez S, Lawton-Rauh AL, Reighard GL, Abbott AG, Bielenberg DG (2009) Phylogenetic analysis and molecular evolution of the dormancy associated MADS-box genes from peach. BMC Plant Biol 9(1):81
Jiménez S, Li Z, Reighard GL, Bielenberg DG (2010a) Identification of genes associated with growth cessation and bud dormancy entrance using a dormancy-incapable tree mutant. BMC Plant Biol 10(1):25
Jiménez S, Reighard GL, Bielenberg DG (2010b) Gene expression of DAM5 and DAM6 is suppressed by chilling temperatures and inversely correlated with bud break rate. Plant Mol Biol 73(1–2):157–167
Julian C, Herrero M, Rodrigo J (2010) Flower bud differentiation and development in fruiting and non-fruiting shoots in relation to fruit set in apricot (*Prunus armeniaca* L.). Trees 24(5):833–841
Julian C, Rodrigo J, Herrero M (2011) Stamen development and winter dormancy in apricot (*Prunus armeniaca*). Ann Bot 108(4):617–625
Julian C, Herrero M, Rodrigo J (2014) Anther meiosis time is related to winter cold temperatures in apricot (*Prunus armeniaca* L.). Environ Exp Bot 100:20–25
Knight TA (1801) Account of some experiments on the ascent of the sap in trees. Philos Trans R Soc London 91:333–353
Koltunow AM, Truettner J, Cox KH, Wallroth M, Goldberg RB (1990) Different temporal and spatial gene expression patterns occur during anther development. Plant Cell 2(12):1201–1224
Koornneef M, Hanhart CJ, van der Veen JH (1991) A genetic and physiological analysis of late flowering mutants in *Arabidopsis thaliana*. Mol Gen Genet 229(1):57–66
Lafon-Placette C, Faivre-Rampant P, Delaunay A, Street N, Brignolas F, Maury S (2013) Methylome of DNase I sensitive chromatin in *Populus trichocarpa* shoot apical meristematic cells: a simplified approach revealing characteristics of gene-body DNA methylation in open chromatin state. New Phytol 197(2):416–430
Lamp BM, Connell JH, Duncan RA, Viveros M, Polito VS (2001) Almond flower development: floral initiation and organogenesis. J Am Soc Hortic Sci 126(6):689–696
Lang A (1957) The effect of gibberellin upon flower formation. Proc Natl Acad Sci U S A 43:709–711
Lang GA, Early JD, Martin GC, Darnell RL (1987) Endodormancy, paradormancy, and ecodormancy-physiological terminology and classification for dormancy research. HortScience 22(3):371–377
Legave JM, Farrera I, Almeras T, Calleja M (2008) Selecting models of apple flowering time and understanding how global warming has had an impact on this trait. J Hortic Sci Biotechnol 83:76–84
Li Z, Reighard GL, Abbott a.G, Bielenberg DG (2009) Dormancy-associated MADS genes from the EVG locus of peach [*Prunus persica* (L.) Batsch] have distinct seasonal and photoperiodic expression patterns. J Exp Bot 60(12):3521–3530
Luedeling E (2012) Climate change impacts on winter chill for temperate fruit and nut production: a review. Sci Hortic 144:218–29. (Amsterdam, Elsevier B.V.)
Luna V, Reinoso H, Lorenzo E, Bottini R, Abdala G (1991) Dormancy in peach (*Prunus persica* L) flower buds.2. Comparative morphology and phenology in floral and vegetative buds, and the effect of chilling and gibberellin-A3. Trees-Struct Funct 5:244–246
Martin GC (1991) Bud dormancy in deciduous fruit trees. In: Steward FC (ed) Plant physiol. A Treatise, vol X. Academic, San Diego, pp 183–225
Messenguy F, Dubois E (2003) Role of MADS box proteins and their cofactors in combinatorial control of gene expression and cell development. Gene 316:1–21

Mimida N, Ureshino A, Tanaka N, Shigeta N, Sato N, Moriya-Tanaka Y et al (2011) Expression patterns of several floral genes during flower initiation in the apical buds of apple (*Malus × domestica* Borkh.) revealed by in situ hybridization. Plant Cell Rep 30(8):1485–1492

Mohamed R, Wang C-T, Ma C, Shevchenko O, Dye SJ, Puzey JR et al (2010) *Populus* CEN/TFL1 regulates first onset of flowering, axillary meristem identity and dormancy release in *Populus*. Plant J 62(4):674–688

Olsen JE, Junttila O, Morizt T (1995) A localized decrease of GA in shoot tips of *Salix pentandra* seedling precedes cessation of shoot elongation under short photoperiod. Physiol Plant 95:627–632

Paul LK, Rinne PL, van der Schoot C (2014) Shoot meristems of deciduous woody perennials: self-organization and morphogenetic transitions. Curr Opin Plant Biol 17:86–95

Perry TO (1971) Dormancy of trees in winter. Science 171:29–36

Pin Pa., Nilsson O (2012) The multifaceted roles of FLOWERING LOCUS T in plant development. Plant Cell Environ 35(10):1742–1755

Ragland C (1934a) Fruit-bud differentiation in the sugar prune. Proc Am Soc Hortic Sci 32:50–51

Ragland C (1934b) The development of the peach fruit, with special reference to split-pit and gumming. Proc Am Soc Hortic Sci 31:1–21

Richardson EA, Seeley SD, Walker DR (1974) A model for estimating the completion of rest for "Redhaven" and "Elberta" peach trees. HortSci 9:331–332

Rinne PL, Kaikuranta PM, van der Schoot C (2001) The shoot apical meristem restores its symplasmic organization during chilling-induced release from dormancy. Plant J 26(3):249–264

Rinne PLH, Welling A, Schoot C Van Der (2010) Perennial life style of *Populus*: dormancy cycling and overwintering. In: Jansson S, Bhalerao R, Groover A (eds) Genetics and genomics of *Populus*. Springer, New York, pp 171–200

Rinne PLH, Welling A, Vahala J, Ripel L, Ruonala R, Kangasjarvi J et al (2011) Chilling of dormant buds hyperinduces FLOWERING LOCUS T and recruits GA-Inducible 1,3- -Glucanases to reopen signal conduits and release dormancy in *Populus*. Plant Cell 23(1):130–146

Ríos G, Tadeo FR, Leida C, Badenes ML (2013) Prediction of components of the sporopollenin synthesis pathway in peach by genomic and expression analyses. BMC Genomics 14:40

Ríos G, Leida C, Conejero A, Badenes ML (2014) Epigenetic regulation of bud dormancy events in perennial plants. Front Plant Sci 5:247

Rohde A, Bhalerao RP (2007) Plant dormancy in the perennial context. Trends Plant Sci 12(5):217–223

Santamaría ME, Rodríguez R, Cañal MJ, Toorop PE (2011) Transcriptome analysis of chestnut (*Castanea sativa*) tree buds suggests a putative role for epigenetic control of bud dormancy. Ann Bot 108(3):485–498

Sasaki R, Yamane H, Ooka T, Jotatsu H, Kitamura Y, Akagi T et al (2011) Functional and expressional analyses of PmDAM genes associated with endodormancy in Japanese Apricot. Plant Physiol 157(1):485–497

Sedgley M, Griffin AR (1989) Sexual reproduction of tree crops. Academic, London, p 392

Seeley PSD, Seeley SD, Dennis FG, Lang GA (1994) Workshop Papers and Authors Dormancy—The Black Box. 29(11):1994

Smyth DR, Bowman JL, Meyerowitz EM (1990) Early flower development in *Arabidopsis*. Plant Cell 2:755–767

Song J, Angel A, Howard M, Dean C (2012) Vernalization-a cold-induced epigenetic switch. J Cell Sci 125:3723–3731

Sterling C (1964) Comparative morphology of carpel in Rosaceae. 1. Prunoideae-*Prunus*. Am J Bot 51(1):36–44

Sung S, Amasino RM (2005) Remembering winter: toward a molecular understanding of vernalization. Annu Rev Plant Biol 56(1):491–508

Tukey (1989) Growth factors and plant regulants in the manipulation of plant development and cropping in tree fruits. In: Wright CJ (ed) Manipulation of fruiting. pp 343–361

Vanstraelen M, Benková E (2012) Hormonal interactions in the regulation of plant development. Annu Rev Cell Dev Biol 28:463–487

Vegis A (1964) Dormancy in higher plants. Annu Rev Plant Physiol 15:185–224
Vining KJ, Pomraning KR, Wilhelm LJ, Priest HD, Pellegrini M, Mockler TC et al (2012) Dynamic DNA cytosine methylation in the *Populus trichocarpa* genome: tissue-level variation and relationship to gene expression. BMC Genomics 13(1):27
Wang SY, Jiao HJ, Faust M (1991) Changes in metabolic enzyme activities during thidiazuron-induced lateral budbreak of apple. HortScience 26(2):171–173
Warriner CL, Johnson JL, Smith MW (1985) Comparison of the initiation and development of Redhaven peach flowers in standard and meadow orchard trees. J Am Soc Hortic Sci 110(3):379–383
Weinberger JH (1950) Chilling requirements of peach varieties. Proc Am Soc Hortic Sci 56:122–128
Welling A, Kaikuranta P, Rinne P (1997) Photoperiodic induction of dormancy and freezing tolerance in Betula pubescens. Involvement of ABA and dehydrins. Physiol Plant 100(1):119–125
Yamane H, Kashiwa Y, Ooka T, Tao R, Yonemori K (2008) Suppression subtractive hybridization and differential screening reveals endodormancy-associated expression of an SVP/AGL24-type MADS-box gene in lateral vegetative buds of Japanese apricot. J Am Soc Hortic Sci 133(5):708–16
Yamane H, Ooka T, Jotatsu H, Hosaka Y, Sasaki R, Tao R (2011) Expressional regulation of PpDAM5 and PpDAM6, peach (*Prunus persica*) dormancy-associated MADS-box genes, by low temperature and dormancy-breaking reagent treatment. J Exp Bot 62(10):3481–3488

Chapter 7
Dormancy-Associated *MADS-BOX* Genes: A Review

David P. Horvath

Dormancy-associated MADS-BOX (*DAM*) genes encode transcription factors that were first implicated to play a role in dormancy following studies (see below) of a mutation in peach (*Prunus persica*) originally named *evergreen* (Rodriguez et al. 1994) and later *evergrowing* (Wang et al. 2002). Plants carrying this mutation have a reduced requirement for long-term cold normally needed to break endodormancy in lateral buds and fail to form terminal buds under otherwise endodormancy inducing conditions. This mutation was caused by a large deletion on linkage group 1 of peach (Bielenberg et al. 2008). Upon delineation of this deletion and subsequent sequencing of the deleted region in wild-type peach, it was noted that 6 of the 11 genes that were affected by the deletion were members of the StMADS11 family of transcription factors (Bielenberg et al. 2008). Nearly simultaneously with the research effort on peach, two transcriptomics studies conducted with another perennial crop, raspberry (*Rubus idaeus*) (Mazzitelli et al. 2007), and an herbaceous perennial weed, leafy spurge (*Euphorbia esula*) (Horvath et al. 2008), identified genes with similarity to StMADS11-like transcription factors that were preferentially expressed during endodormancy induction and subsequently repressed following endodormancy release. Combined, these results provided enough expression and functional evidence to hypothesize that these transcription factors might play a role in endodormancy establishment and maintenance. Subsequent communication between these three groups led to the naming of this family of transcription factors genes as *DAM* genes (Bielenberg et al. 2008).

Numerous *DAM-like* genes can be found in most dicot plant species, but only a few have been shown to have expression patterns consistent with a role in dormancy. In poplar (*Populus trichocarpa*), a phylogenetic analysis of *DAM*-like genes, including the *DAM* genes identified from peach, suggested that *PtMADS7*- (as denoted by Leseberg et al. 2006, also designated as Potri.005G150500, Populus trichocarpa v3.0, DOE-JGI, http://www.phytozome.net/poplar) and *PtMADS21*

D. P. Horvath (✉)
Sunflower and Plant Biology Research Unit, USDA-Agricultural Research Service,
1605 Albrecht Blvd., Fargo, ND, 58102-2765, USA
e-mail: david.horvath@ars.usda.gov

© Springer International Publishing Switzerland 2015

J. V. Anderson (ed.), *Advances in Plant Dormancy,* DOI 10.1007/978-3-319-14451-1_7

likely shared a common ancestor with the six *DAM* genes of peach (Jiménez et al. 2009). However, expression analysis suggested that most *DAM*-like genes of poplar were not induced by dormancy inducing conditions (Chen 2008). Rather, a small cluster of *DAM*-like genes on chromosome 7 (P*tMAS27*- Potri.007G115200, *PtMADS28*- Potri.007G115100, and *PtMADS29*- Potri.007G115000) of poplar were all induced by short photoperiod and one (*PtMADS27*) also by cold (Chen 2008). More recently, a microarray analysis of poplar dormancy transitions demonstrated limited induction for any of these *DAM*-like genes during endodormancy or ecodormancy (Howe et al. 2014). Interestingly though, one gene (Potri.001G328400) appears to encode a shortened version of a *DAM*-like transcript, and it was up-regulated only during paradormancy—much like the truncated splice variant of the leafy spurge *DAM* gene (Horvath et al. 2010). Of the six *DAM*-like genes in peach, *DAM3*, *DAM5* and *DAM6* were all preferentially expressed during the dormant phase (Li et al. 2009). However, only *DAM5* and *DAM6* were differentially expressed between two peach cultivars with known variation in winter chill requirements for transition from endodormancy to ecodormancy (Li et al. 2009). In addition to the *DAM* genes from raspberry and leafy spurge, *StMADS16* from potato (*Solanum tuberosum*), which has significant similarity to *DAM* genes from other species (Horvath et al. 2008), was also shown to be differentially expressed in endodormant potato buds (Campbell et al. 2008). Differential expression of *DAM*-like genes has also been associated with dormancy processes in kiwifruit (*Actinidia chinensis*) (Wu et al. 2012), pear (*Pyrus communis*) (Ubi et al. 2010), and Japanese apricot (*Prunus mume*) (Sasaki et al. 2011). Although most of these sequences have been identified in rosaceous plant species, the identification of the *StMADS16* gene that was differentially expressed during potato dormancy transitions suggest that *DAM* genes are likely also present and functional in the *Asteridae* as well (Campbell et al. 2008). Additionally, a *DAM*-like gene recently cloned from tea (*Camellia sinensis*), another member of the *Asteridae*, is not only up-regulated during endodormancy, but also appears to regulate bud phenology in poplar when overexpressed (Hao et al. unpublished observations).

Genetic Associations of DAM Genes with Dormancy

Further evidence that *DAM* genes play a significant role in dormancy comes from genetic studies that identified quantitative trait loci (QTLs) that affect bud dormancy. For example, Fan et al. (2010) identified 4 QTLs for chilling requirement from an initial cross of two peach varieties ('Contender' and 'Fla.92–2C') that differed in their chilling requirements (the time at chilling temperatures needed to shift from endodormancy to ecodormancy) with 378 F2 progeny. The QTL on chromosome 1 that explained over 40% of the variation overlapped with the *EVG* locus. Likewise, a QTL for chilling requirement was found on a syntenic region in the almond (*Prunus amygdalus*) genome (Sánchez-Pérez et al. 2012). These same

populations (Fan et al. 2010) were used to identify QTLs for chilling requirements in an F2 population of peach, and these QTLs were mapped and associated with sequence variation to identify candidate genes (Zhebentyayeva et al. 2014). This study strongly implicated *PpeDAM5* and *PpeDAM6* as candidate genes underlying the QTL on chromosome 1. These observations, along with the correlation between accumulation of *DAM* transcripts in high and low chilling requiring cultivars described above, provides strong evidence that genetic variation in *DAM* genes in the *Prunus* genus likely contributes to chilling requirements for endodormancy release. However, this may not be the case for all species as a large genome wide association study among various population of poplar from Europe identified six QTLs associated with endodormancy induction and bud set (Rohde et al. 2011), but none overlapped with the locations of known *DAM*-like genes. This study specifically focused on endodormancy induction in poplar rather than release, which might account for the lack of an observed association. It might also be possible that natural selection in poplar resulted in altered alleles for genes upstream or downstream of *DAM* in the dormancy regulatory process rather than in the DAM gene itself.

Structural Characteristics of DAM Genes

DAM genes are members of the type II (MIKC^c) subfamily of MADS-box transcription factors since they contain the MADS-box (M), intervening (I-), keratin-like (K-), and C-terminal (C-) domain with the super-scripted (c) standing for "classic" as opposed to a related group that contains a longer I region and are denoted as MIKC* (Becker and Theissen 2003). The DNA binding domain (MADS-box) is encoded in the first exon and is highly conserved. This exon is generally followed by a long multi-thousand base intron. In most *DAM* genes, the remainder of the transcription factor is encoded by five to eight additional exons. This 3′ region of these MADS-box transcription factors is likely involved in regulating protein–protein interactions (as most such *MADS*-box proteins can form functional dimers), transcription factor activity, and regulation of target gene expression. Additionally, at least in a *DAM* gene from leafy spurge and *MAD16* from potato, there are two additional non-coding exons in the 5′ untranslated region (UTR) of the gene (Horvath et al. 2010).

There is evidence for splice variants that are preferentially expressed during different dormancy states (Horvath et al. 2013). For example, a truncated splice variant of the leafy spurge *DAM* transcript preferentially accumulates during endodormancy (Horvath et al. 2008, 2013). This splice variant contains only the exon encoding the DNA binding MADS-box portion of the protein (coding exon 1) and a short exon located in what is normally the first coding region intron of the longer, more canonical *DAM* transcripts. However, the longer splice variant accumulates less than the short variant during endodormancy but is highly expressed throughout ecodormancy. Some additional differential splicing was observed in two exons contained within non-coding 5′UTR of *DAM* in response to seasonal differences (Horvath

et al. 2008). Generally, four to six splice variants are noted among the transcripts of the three *DAM* genes in poplar, but no studies on the specificity to dormancy states for the expression of these variants have been conducted.

DAM genes are often found in tandemly duplicated copies at various loci. In peach, six tandemly duplicated *DAM* genes are found on chromosome 1 of peach (Bielenberg et al. 2008). Similar numbers and arrangements of *DAM* genes have been found in Japanese apricots (*P. mume*) (Sasaki et al. 2011). In poplar, *DAM*-like genes are scattered in small clusters of two or three genes (Horvath et al. 2008). In contrast, three different BAC clones have been isolated that contain copies of very similar *DAM* genes that differ only in the organization of flanking genes and repetitive elements (Horvath unpublished observations). However, even though other genes are present on the bacterial artificial chromosome (BAC) clones, only a single *DAM* gene is present, suggesting perhaps that these genes are not arranged in tandem repeats in leafy spurge (Horvath et al. 2013).

DAM Gene Function

Members of the *DAM*-like gene family, namely *AGAMOUS-LIKE 24* (*AGL24*) and *SHORT VEGETATIVE PHASE* (*SVP*), had been well characterized in the model annual species arabidopsis (*Arabidopsis thaliana*) and are known to have roles in promoting or inhibiting flowering respectively (Gregis et al. 2006). Previous work in poplar indicated that *FLOWERING LOCUS T 2* (*FT2*), when overexpressed, could prevent seasonal growth cessation and delayed induction of endodormancy (Bohlenius et al. 2006, Hsu et al. 2011). *FT* is known to be negatively regulated by SVP in arabidopsis (Jang et al. 2009). These two observations led to the hypothesis that *DAM* genes may partially regulate endodormancy by inhibiting the expression of *FT* (Horvath et al. 2008). Some evidence supporting this hypothesis was demonstrated in arabidopsis by overexpressing *DAM1* of leafy spurge (Horvath et al. 2010). These transgenic arabidopsis had delayed flowering and reduced *FT* accumulation compared to non-transgenic controls. Likewise, overexpression of several *DAM*-like genes from kiwifruit in arabidopsis also delayed flowering (Wu et al. 2012). Further, preliminary data indicate that an antibody specific to leafy spurge DAM1 can precipitate specific regions of the promoter from leafy spurge *FT* (Hao et al. unpublished observations). Intriguingly, the portion of the *FT* promoter proposed to be bound by leafy spurge DAM1 contains a sequence that is similar to a CArG box, a known binding motif for MADS box transcription factors (Riechmann et al. 1969). A very similar sequence is also conserved in the promoter of *FT2* from poplar, which is specifically associated with growth cessation and endodormancy induction (Hsu et al. 2011). Moreover, when peach *DAM6* was overexpressed in poplar, the most affected transgenic lines showed a significant decrease in *FT2* transcript accumulation (Sasaki et al. 2011).

SUPPRESSOR OF OVEREXPRESSION OF CO 1 (*SOC1*) is another gene involved in regulating flowering in arabidopsis (Moon et al. 2003). However, *SOC1* expression is also correlated with endodormancy release in apricot (*P. armeniaca*) (Trainin et al. 2013). The authors of this study point out that *DAM* genes share significant similarities to AGL24, which is a known positive regulator of *SOC1* in arabidopsis (Lee and Lee 2010). However *SOC1*, like *FT*, had reduced transcript accumulation during endodormancy in poplar (Ruttink et al. 2007). This would be an unexpected observation if some poplar *DAM-like* genes were acting analogously to *AGL24* and positively regulating *SOC1*. However, it is noted that transcripts from a *SOC1*-like gene had increased accumulation in leafy spurge ($p < 0.07$) during endodormancy (Horvath et al. 2008) and in tree peony (Zhang et al. 2014). Thus, the functional nature of poplar *DAM*-like genes is unclear.

DAM Gene Regulation

DAM genes are often induced by low temperatures and variations in photoperiods depending on the species. *DAM* genes also appear to be up-regulated by conditions that induce endodormancy. In peach, *DAM5* and *DAM6* are up-regulated by short photoperiod (Li et al. 2009). Likewise, the pear *DAM* genes are up-regulated primarily by cold temperatures (Saito et al. 2013). In leafy spurge, expression of *DAM* was greatest under long photoperiod with cold mornings, nights and evening (Horvath et al. 2010). The promoters of leafy spurge *DAM* genes were compared to the promoters of their most similar genes in poplar and peach (Table 7.1). Among the conserved (and potentially significant) promoter elements present in both leafy spurge, poplar, and peach were the evening element (AAATATCT) (Harmer et al. 2000). A similar sequence (AAATATCA) is present in the peach *DAM6* promoter (Table 7.1). This element serves as the binding site for LATE ELONGATED HYPOCOTYL (LHY) and CIRCADIAN CLOCK ASSOCIATED 1 (CCA1), which are key regulators of the circadian clock machinery (Alabadi et al. 2001). Likewise, one or more C-REPEAT/DRE BINDING FACTOR (CBF) binding sites (CCGAC) (Baker et al. 1994) can be found in the promoters of *DAM* genes from leafy spurge, peach, and one (*MADS27*) of the most similar poplar *DAM*-like genes (Table 7.1). These CBF binding sites are suspected of playing a role in *DAM* gene expression in leafy spurge (Horvath et al. 2010, 2013). More recently, direct binding of a CBF binding factor to the promoter of a pear *DAM* gene has been confirmed by chromatin immunoprecipitation assays (ChIP) (Saito et al. 2014). Additionally, other conserved elements in both leafy spurge and poplar *DAM* gene promoters such as a long element with the consensus sequence TYTTGCTKGCTATRRRAWWCTTYTTYTT have been observed. There is also a similar sequence (TTTTGCTTTGCTAT) in the peach *DAM* promoters (Table 7.1). Combined, these conserved elements strongly support a role in circadian and CBF-regulated expression of *DAM*

Table 7.1 Promoter elements from two leafy spurge *DAM* genes (BAC2 and BAC3) and three related poplar genes (MADS27–29) and two related peach genes (DAM5–6)

Species	Gene	CBF binding site	Bases(-) from TATA box	Evening element	Bases(-) from TATA box	Conserved unknown function	Bases(-) from TATA box
Leafy spurge	DAM1 (BAC2)	CCGAC, CCGAC	1261, 2158	AAAATATCT	632	TTGCTTGGCTAT	543
Leafy spurge	DAM1 (BAC3)	CCGAC	2330	AAAATATCT	632	TTGCTTGGCTAT	543
Poplar	MADS27 (Potri.007G115200)	CCGAC	2705	AAAATATCT	2602	TTGCTTGGCTAT	1386
Poplar	MADS28 (Potri.007G115100)	CCGAC	NA	AAAATATGT, AAAATAACT	237, 2688	TTGCTTGGCTAT	1172
Poplar	MADS29 (Potri.007G115000)	CCGAC	NA	AAAATATCT	1758	TTGCTGGGCTAT	768
Peach	DAM5 (ppa010822m.g)	CCGAC, GGCTG, CCGAC	197, 1268, 1402	TAGATATTT	177	TTGCTTTGCTATT	1356
Peach	DAM6 (ppa010714m.g)	CCGAC, CCGAC, CCGAC	247, 311, 1597	AAAATATCA	608	TTGCTTGGGTTAT	470

genes and also intimate the presence of another transcription factor or factors that interact with the unknown but conserved element.

DAM genes have been likened to the *FLOWERING LOCUS C* (*FLC*) gene of arabidopsis given its expression patterns and role in controlling *FT* expression. *FLC* is regulated by several transcription factors including CBF (Seo et al. 2009), LHY and CCA1 (Fujiwara et al. 2008). *FLC* is also regulated at the level of chromatin (He and Amasino 2005), and the ability of specific transcription factors that might induce the expression of this gene is limited by chromatin remodeling. As chromatin remodeling is also implicated in bud dormancy processes (Horvath et al. 2003, Rohde and Bhalerao 2007), it was logical to examine the role of chromatin regulation of *DAM* gene expression. ChIP assays confirmed regulation by two different systems. During endodormancy transition, leafy spurge *DAM* genes transition from an open chromatin state with lower levels of tri methylation of lysine 4 of histone 3 (H3K4triMe) and during transition to ecodormancy higher levels of closed chromatin were observed with enhanced tri methylation of lysine of 27 (H3K27triMe) (Horvath et al. 2010; Leida et al. 2012).

Conclusions and Future Prospectus

There is reasonable evidence to suggest *DAM* genes play a key role in regulating the dormancy responses in buds of many perennials by binding to and regulating specific genes involved in establishing and maintaining the endodormant and ecodormant state. There is also good evidence that *DAM* genes are regulated by circadian signals, CBF-like transcription factors, and chromatin modifications as well as by other currently uncharacterized transcription factors. Additional studies are needed to further define the signaling factors responsible for regulating the expression of *DAM* genes. Also, because DAMs contain domains associated with protein–protein interactions, and because many MADS-box proteins are known to form functional multimers with other MADs-box proteins (de Folter et al. 2005), it is highly likely that DAM proteins associate with other proteins to regulate gene expression. It is also highly likely that DAM proteins, interacting with various other MADS proteins, may have multiple different targets that assist in dormancy maintenance and release. Consequently, there is a need for a more general assessment of genes bound by DAM and to look for additional proteins that might interact with and modify the function of DAM transcription factors.

Manipulating this class of transcription factors either through conventional breeding or through genetic engineering could lead to more stable production in perennial crops and other horticulturally important perennial species, and thus protect these important plants from the expected climate change associated with global warming. Additionally, these genes could serve as potential targets for impeding the spread of perennial invasive species such as leafy spurge. Thus, further studies of these important transcription factors are warranted.

References

Alabadi D, Oyama T, Yanovsky MJ, Harmon FG, Mas P, Kay SA (2001) Reciprocal regulation between TOC1 and LHY/CCA1 within the *Arabidopsis* circadian clock. Science 293:880–883

Baker SS, Wilhelm KS, Thomashow MF (1994) The 5′-region of *Arabidopsis thaliana COR15a* has cis-acting elements that confer cold-, drought- and ABA-regulated gene expression. Plant Mol Biol 24:701–713

Becker A, Theissen G. (2003) The major clades of MADS-box genes and their role in the development and evolution of flowering plants. Mol Phylogenet Evol 29:464–489

Bielenberg DG, Wang Y, Li Z, Zhebentyayeva T, Fan S, Reighard GL, Scorza R, Abbott AG (2008) Sequencing and annotation of the *evergrowing* locus in peach [*Prunus persica* (L.) Batsch] reveals a cluster of six MADS-box transcription factors as candidate genes for regulation of terminal bud formation. Tree Genet Genomes 4:495–507

Bohlenius H, Huang T, Charbonnel-Campaa L, Brunner AM, Jansson S, Strauss SH, Nilsson O (2006) CO/FT regulatory module controls timing of flowering and seasonal growth cessation in trees. Science 312:1040–1043

Campbell MA, Segear E, Beers L, Knauber D, Suttle J (2008) Dormancy in potato tuber meristems: chemically induced cessation in dormancy matches the natural process based on transcript profiles. Funct Integr Genomics 8:317–328

Chen K-Y (2008) Type II MADS-box genes associated with poplar apical bud development and dormancy. Thesis, Department of Plant Sciences and Landscape Architecture. University of Maryland

de Folter S, Immink RGH, Kieffer M, Parenicova L, Henz SR, Weigel D, Busscher M, Kooiker M, Colombo L, Kater MM et al (2005) Comprehensive interaction map of the Arabidopsis MADS box transcription factors. Plant Cell 17:1424–1433

Fan S, Bielenberg DG, Zhebentyayeva TN, Reighard GL, Okie WR, Holland D, Abbott AG (2010) Mapping quantitative trait loci associated with chilling requirement, heat requirement and bloom date in peach (*Prunus persica*). New Phytol 185:917–930

Fujiwara S, Oda A, Yoshida R et al (2008) Circadian clock proteins LHY and CCA1 regulate SVP protein accumulation to control flowering in *Arabidopsis*. Plant Cell 20:2960–2971

Gregis V, Sessa A, Colombo L, Kater MM (2006) AGL24, SHORT VEGETATIVE PHASE, and APETALA1 redundantly control AGAMOUS during early stages of flower development in *Arabidopsis*. Plant Cell 18:1373–1382

Harmer SL, Hogenesch JB, Straume M, Chang HS, Han B, Zhu T, Wang X, Kreps JA, Kay SA (2000) Orchestrated transcription of key pathways in *Arabidopsis* by the circadian clock. Science 290:2110–2113

He Y, Amasino RM (2005) Role of chromatin modification in flowering-time control Trends Plant Sci 10:30–35

Horvath DP, Anderson JV, Chao WS, Foley ME (2003) Knowing when to grow: signals regulating bud dormancy. Trends Plant Sci 8:534–540

Horvath DP, Chao WS, Suttle JC, Thimmapuram J, Anderson JV (2008) Transcriptome analysis identifies novel responses and potential regulatory genes involved in seasonal dormancy transitions of leafy spurge (*Euphorbia esula* L.). BMC Genomics 9:536

Horvath DP, Sung S, Kim D, Chao WS, Anderson JV (2010) Characterization, expression and function of DORMANCY-ASSOCIATED MADS-BOX genes from leafy spurge. Plant Mol Biol 73:169–179

Horvath DP, Kudrna D, Talag J, Anderson JV, Chao WS, Wing RA, Foley ME, Doğramacı M (2013) BAC library development, and clone characterization for dormancy-responsive DREB4A, DAM, and FT from leafy spurge (*Euphorbia esula* L.) identifies differential splicing and conserved promoter motifs. Weed Sci 61:303–309

Howe GT, Horvath DP, Dharmawardhana P, Priest HD, Mockler TC, Strauss SH (2014) Transcriptome changes associated with vegetative bud dormancy in *Populus*: chromatin modifications, transcription factors, phytohormone signaling, miRNAs, and DNA sequence motifs. Front Plant Sci (Submitted Aug 31, 2014)

Hsu C-Y, Adams JP, Kim H et al (2011) *FLOWERING LOCUS T* duplication coordinates reproductive and vegetative growth in perennial poplar. Proc Natl Acad Sci U S A 108:10756–10761

Jang S, Torti S, Coupland G (2009) Genetic and spatial interactions between FT, TSF and SVP during the early stages of floral induction in *Arabidopsis*. Plant J 60:614–625

Jiménez S, Lawton-Rauh AL, Reighard GL, Abbott AG, Bielenberg DG (2009) Phylogenetic analysis and molecular evolution of the dormancy associated MADS-box genes from peach. BMC Plant Biol 9:81

Lee J, Lee I (2010) Regulation and function of SOC1, a flowering pathway integrator. J Exp Bot 61:2247–2254

Leida C, Conesa A, Llacer G, Badenes ML, Rios G (2012) Histone modifications and expression of DAM6 gene in peach are modulated during bud dormancy release in a cultivar-dependent manner. New Phytol 193:67–80

Leseberg CH, Li A, Kang H, Duvall M, Mao L (2006) Genome-wide analysis of the MADS-box gene family in *Populus trichocarpa*. Gene 378:84–94

Li Z, Reighard GL, Abbott AG, Bielenberg DG (2009) Dormancy associated MADS genes from the EVG locus of peach [*Prunus persica* (L.) Batsch] have distinct seasonal and photoperiodic expression patterns. J Exp Bot 60:3521–3530

Mazzitelli L, Hancock RD, Haupt S, Walker PG, Pont SDA, McNicol J, Cardle L, Morris J, Viola R, Brennan R et al (2007) Co-ordinated gene expression during phases of dormancy release in raspberry (*Rubus idaeus* L.) buds. J Exp Bot 58:1035–1045

Moon J, Suh SS, Lee H, Choi KR, Hong CB, Paek NC, Kim SG, Lee I (2003). The SOC1 MADS-box gene integrates vernalization and gibberellin signals for flowering in *Arabidopsis*. Plant J 35:613–623

Riechmann JL, Wang M, Meyerowitz EM (1996) DNA-binding properties of *Arabidopsis* MADS domain homeotic proteins APETALA1 APETALA3, PISTILLATA and AGAMOUS. Nucleic Acids Res 24:3134–3141

Rodriguez J, Sherman WB, Scorza R, Wisniewski M, Okie WR (1994) Evergreen peach, its inheritance and dormant behavior. J Am Soc Hortic Sci 119:789–792

Rohde A, Bhalerao RP (2007). Plant dormancy in the perennial context. Trends Plant Sci 12:217–223

Rohde A, Storme V, Jorge V, Gaudet M, Vitacolonna N, Fabbrini F, Ruttink T, Zaina G, Marron N, Dillen S, Steenackers M, Sabatti M, Morgante M, Boerjan W, Bastien C (2011) Bud set in poplar—genetic dissection of a complex trait in natural and hybrid populations. New Phytol 189:106–121

Ruttink T, Arend M, Morreel K, Storme V, Rombauts S, Fromm J, Bhalerao R, Boerjan W, Rohde A (2007) A molecular timetable for apical bud formation and dormancy in poplar. Plant Cell 19:2370–2390

Saito T, Bai S, Ito A, Imai T, Nakajima I, Moriguchi T (2013) Regulatory mechanism of DORMANCY ASSOCIATED MADS-BOX gene in Japanese pear. In, Abstract and Programme Book, 5th International Plant Dormancy Symposium 2013, Auckland, New Zealand, p 44

Saito T, Bai S, Imia T, Ito A, Nakajima I, Moriguchi T (2014) Histone modification and signalling cascade of the dormancy-associated MADS-box gene, PpMADS13-1, in Japanese pear (*Pyrus pyrifolia*) during endodormancy. Plant, Cell & Environment (Article first published online: 15 DEC 2014) DOI: 10.1111/pce.12469

Sánchez-Pérez R, Dicenta F, Martínez-Gómez P (2012) Inheritance of chilling and heat requirements for flowering in almond and QTL analysis. Tree Genet Genomes 8:379–389

Sasaki R, Yamane H, Ooka T, Jotatsu H, Kitamura Y, Akagi T, Tao R (2011) Functional and expressional analyses of PmDAM genes associated with endodormancy in Japanese apricot (*Prunus mume*). Plant Physiol 157:485–497

Seo E, Lee H, Jeon J, Park H, Kim J, Noh YS, Lee I (2009). Crosstalk between cold response and flowering in *Arabidopsis* is mediated through the flowering-time gene SOC1 and its upstream negative regulator FLC. Plant Cell 21:3185–3197

Trainin T, Bar-Ya'akov I, Holland D (2013). ParSOC1, a MADS-box gene closely related to *Arabidopsis* AGL20/SOC1, is expressed in apricot leaves in a diurnal manner and is linked with chilling requirements for dormancy break. Tree Genet Genomes 9:753–766

Ubi BE, Sakamoto D, Ban Y, Shimada T, Ito A, Nakajima I, Takemura Y, Tamura F, Saito T, Moriguchi T (2010) Molecular cloning of dormancy-associated MADS-box gene homologs and their characterization during seasonal endodormancy transitional phases of Japanese pear. J Am Soc Hortic Sci 135:174–182

Wang Y, Georgi L, Reighard GL, Scorza R, Abbott, AG (2002) Genetic mapping of the evergrowing gene in peach [*Prunus persica* (L.) Batsch]. J Hered 93:352–358

Wu R-M, Walton EF, Richardson AC, Wood M, Hellens RP, Varkonyi-Gasic E (2012) Conservation and divergence of four kiwifruit SVP-like MADS-box genes suggest distinct roles in kiwifruit bud dormancy and flowering. J Exp Bot 63:797–807

Zhebentyayeva TN, Fan S, Chandra A, Bielenberg DG, Reighard GL, Okie WR, Abbott AG (2014) Dissection of chilling requirement and bloom date QTLs in peach using a whole genome sequencing of sibling trees from an F2 mapping population. Tree Genet Genomes 10:35–51

Chapter 8
Functional Characterization of Japanese Apricot (*Prunus mume*) *DORMANCY-ASSOCIATED MADS-box1* (*PmDAM1*), a Paralog of *PmDAM6*, Using *Populus* Transformants

Hisayo Yamane and Ryutaro Tao

Introduction

The genetic and molecular regulation of bud dormancy in woody perennials has been extensively studied in the model woody plant, poplar (*Populus* spp.), and much knowledge has been gained, as reviewed by Cooke et al. (2012), Rinne et al. (2010), and Rohde and Bhalerao (2007). Additionally, characterization of molecular networks regulating dormancy in various woody species, including horticulturally important fruit tree species, has been carried out by omics studies that use the target plants themselves (as reviewed by Yamane 2014). Through genetic and molecular studies, genes similar to the *StMADS11* clade MADS-box genes of *Arabidopsis*, such as *SHORT VEGETATIVE PHASE* (*SVP*) and *AGAMOUS-LIKE24* (*AGL24*), were identified as candidates for dormancy regulation in peach (Bielenberg et al. 2008) and Japanese apricot (Yamane et al. 2008). In the Japanese apricot genome, six tandemly arrayed *PmDAM* genes (*PmDAM1*–*PmDAM6*) have been identified (Sasaki et al. 2011; Zhang et al. 2012). A seasonal expression analysis using reverse transcription quantitative polymerase chain reaction (RT-qPCR) assays of *PmDAM* genes (Sasaki et al. 2011), genome-wide transcriptomic analysis using the Japanese apricot EST dormant bud database (http://bioinf.mind.meiji.ac.jp/JADB/) (Habu et al. 2012), and 60-K microarray analysis (Habu et al. 2014) demonstrated that *PmDAM* genes were preferentially expressed in dormant buds and down-regulated during dormancy release of lateral vegetative buds. Moreover, RT-qPCR and microarray analyses revealed that all six *PmDAM* genes were down-regulated following prolonged artificial cold exposure. In peach, *DAM6* expression was negatively correlated with the time required for terminal bud break (Jimenéz et al. 2010), and this negative correlation was also reported for lateral vegetative (Yamane et al. 2011a) and flower (Yamane et al. 2011b, c) buds. Down-regulation

H. Yamane (✉) · R. Tao
Laboratory of Pomology, Graduate School of Agriculture,
Kyoto University, Kyoto, Japan
email: hyamane@kais.kyoto-u.ac.jp

© Springer International Publishing Switzerland 2015

J. V. Anderson (ed.), *Advances in Plant Dormancy,* DOI 10.1007/978-3-319-14451-1_8

of the *SVP*-like gene during dormancy release has been reported in other perennial fruits, such as raspberry (*Rubus idaeus* L.) (Mazzitelli et al. 2007). In Japanese pear, the expression of the *DAM*-like gene *MADS13* was up-regulated toward dormancy establishment and down-regulated toward dormancy release (Saito et al. 2013). These reports collectively suggested that *DAMs* were promising candidate genes for bud dormancy regulation.

Presently, *PmDAM6* is the only Rosaceae *DAM* gene that has been functionally characterized using a transgenic study (Sasaki et al. 2011). Overexpressing *35S:PmDAM6* in transgenic poplars demonstrated their growth inhibitory functions, which included growth cessation and terminal bud set under environmental conditions that promote shoot tip growth in wild-type (WT) poplars. Seasonal expression patterns of *PmDAM*s in leaves and vegetative buds were roughly classified into two patterns. *PmDAM1–PmDAM3* showed expression peaks in summer when shoot growth cessation of long branches was observed, while *PmDAM4–PmDAM6* showed peaks in autumn when leaf senescence was observed and buds were in deep dormancy. *PmDAM*s, including *PmDAM6*, may have functional redundancy owing to the high amino acid sequence identity of the MADS-box domain (approximately 80–90 %). Because no transgenic studies have been conducted using a *PmDAM* other than *PmDAM6*, in this study, we conducted functional studies of *PmDAM1* using poplar transformants. Based on phenotypic observations, the biological role of *PmDAM1* and its involvement in dormancy regulation is discussed.

Materials and Methods

Microarray Analysis

Monitoring of bud transcriptional activity during seasonal bud development was previously performed using a custom microarray (Habu et al. 2014) constructed from Japanese apricot bud ESTs (Habu et al. 2012). Gene expressions of lateral vegetative buds collected from June to March (each from one biological with three technical replicates) (Habu et al. 2014) were used. A principal component analysis (PCA) using the entire transcriptome represented on the array was performed by a Subio software platform (Subio Inc., Kagoshima, Japan) to verify correlations among seasonal bud samples and to visualize the similarity of gene expression levels among bud samples.

Poplar Transformation

To construct the binary vector *p35S:PmDAM1*, *PmDAM1* cDNA (DDBJ Acc. no. AB576350) of *Prunus mume* ‘Nanko’ was ligated into the T-DNA region of the pGWB2 binary vector (Nakagawa et al. 2007) using the Gateway cloning system with LR Clonase II (Life Technologies, Carlsbad, CA). The *p35S:PmDAM1* vector was introduced into *Agrobacterium tumefaciens* strain EHA105 and used

to transform hybrid poplar (*Populus tremula* × *P. tremuloides*; clone T89) for the constitutive expression of *PmDAM1* under the control of the cauliflower mosaic virus 35S promoter. Five independently transformed lines were obtained. Relative expression intensities of *PmDAM1* among these transformants were compared by RT-qPCR as described in Sasaki et al. (2011). *PtACTIN* was used as a reference gene for the normalization of cDNA amounts as described in Sasaki et al. (2011). RT-qPCR was performed with cDNAs synthesized from total RNAs extracted from the leaves of each transgenic plant using the RNeasy Plant Mini Kit (Qiagen). PCR conditions were as follows: 45 cycles at 95°C for 10 s, 57 °C for 10 s, and 72 °C for 16 s, with an initial denaturation at 95 °C for 5 min. For *PtACTIN*, a dissociation curve analysis was performed to confirm that the fluorescence was only derived from gene-specific amplification. Transgenic shoots were rooted and acclimatized as described in Sasaki et al. (2011).

Phenotypic Observation of Poplar Transformants

Experiment 1

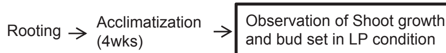
The experimental design to observe dormancy phenotypes, such as bud set and bud outgrowth, is shown in Fig. 8.1. Four different transgenic lines and WT plants (5–6 plants per line) were grown in a growth chamber [16/8 h of light/dark, 23 °C; long photoperiod (LP) condition] for 9 weeks after acclimatization in 2012. Shoot lengths and bud set percentages were recorded weekly (Experiment 1-a; Fig. 8.1). Plants were then transferred to a growth chamber [8/16 h of light/dark; short photoperiod (SP) condition] at 15 °C and grown for 8 weeks. Plants were decapitated (upper half of shoot was removed) and defoliated and grown for 4 weeks in 5 °C in the dark. Then, plants were grown in the LP condition for 4 weeks, followed by the SP condition for 12 weeks. Again, plants were decapitated and defoliated and transferred to the LP condition, and the bud flushing percentage was recorded weekly for 4 weeks (Experiment 1-b; Fig. 8.1). After growing for several months under the LP conditions, all plants were decapitated on November 25, 2013, and stored at 5 °C in the dark for 3 months. On February 21, 2014, plants (5 plants per line) were transferred to a greenhouse without additional heating and grown until April 2014. Bud flushing was observed weekly in April (Experiment 1-c; Fig. 8.1).

Experiment 2

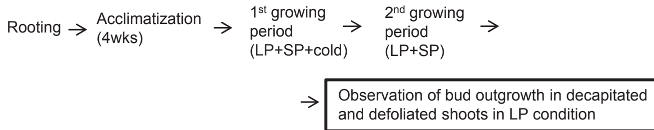
In 2013, three different transgenic lines and WT plants (5–8 plants per line) were grown under SP conditions for 12 weeks after acclimatization. Then, plants were transferred to a cold room (5 °C in the dark). After 6 weeks, plants were transferred to LP conditions, and the terminal bud flushing percentage was observed weekly for 6 weeks (Fig. 8.1).

Experiment 1

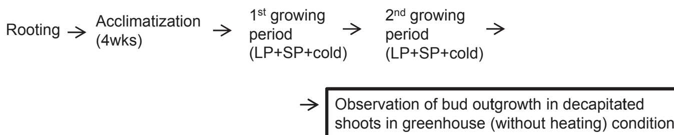
1-a (Shoot growth and bud setting phenotype) (Fig. 8.5)



1-b (Bud outgrowth phenotype prior to cold) (Fig. 8.6)



1-c (Bud outgrowth phenotype in semi-field after cold) (Fig. 8.7)



Experiment 2

(Bud outgrowth phenotype in forcing condition after cold)

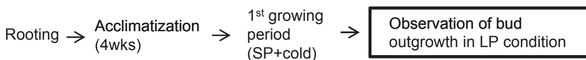


Fig. 8.1 Schematic of the experimental designs used to assess the dormancy phenotypes of transgenic poplars expressing Japanese apricot (*Prunus mume*) *DORMANCY-ASSOCIATED MADS-box1* (*PmDAM1*). In Experiment 1, three different dormancy phenotypes were observed (1-a, 1-b, and 1-c). After the plants rooted, they were planted in plastic pots covered with plastic bags and grown in 16-h light, at 23 °C for 4 weeks of acclimatization. At 4 weeks, the plastic bags were removed and the plants were transplanted into larger pots and then used for the experiments. The details of LP, SP, and cold treatment conditions were described in the text

Results and Discussion

PCA was performed based on the entire transcriptome represented on a microarray (58,627 probes) among ten different bud samples from June to March, and the results are shown in Fig. 8.2. The first two principal components (PC1 and PC2) explained 71.3 % of the total variability in gene expression (51.0 and 20.3 %, respectively). The PCA plot suggested that buds sampled from June to October were similar. Additionally, there was a high level of similarity among buds sampled from November to January. Habu et al. (2014) performed a cluster analysis based on the changes in expression levels of differentially expressed genes after prolonged chilling (2345 up-regulated and 1059 down-regulated), and this analysis also divided monthly buds into the same three sets as shown in Fig. 8.2. Buds collected in June were included in the same clade as buds sampled in September and October, when they were in the deep dormant period. Because June buds can open when they are grown as one-node cuttings, as opposed to September and October buds, it was assumed that June buds differed from September and October buds qualitatively.

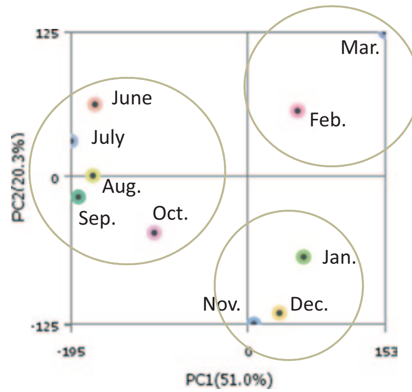


Fig. 8.2 Principal component analysis (PCA) based on the entire transcriptome (58,267 probes) represented on a Japanese apricot (*Prunus mume*) custom microarray constructed by Habu et al. (2014). The *x*-axis represents PC1, and the *y*-axis represents PC2, which explained 51.0 and 20.3 % of the total variability in gene expression, respectively. Ten vegetative bud samples were plotted monthly, from June to March (Habu et al. 2014)

However, the PCA suggested that buds collected from June to October had similar gene expression patterns. These results may reflect that the buds from June to October have similar physiological states, namely nongrowing, but dormancy may be affected through similar or different mechanisms.

A seasonal expression analysis of *PmDAM* genes from lateral vegetative buds located in the middle portions of long branches was performed (Sasaki et al. 2011). Seasonal expression patterns of *PmDAM*s in leaves and vegetative buds were roughly classified into two patterns. *PmDAM1–PmDAM3* showed expression peaks in the summer when shoot growth cessation on long branches was observed, while *PmDAM4–PmDAM6* showed peaks in autumn when leaf senescence was observed and buds were in deep dormancy (Fig. 8.3; Sasaki et al. 2011; Yamane 2014). Functional studies of *PmDAM6* were conducted using poplar transformants, and its inhibitory effects on apical shoot growth have already been demonstrated (Sasaki et al. 2011). However, no transgenic studies had been conducted on a *PmDAM* other than *PmDAM6*. Because *PmDAM1–PmDAM3* showed seasonal expression patterns different from *PmDAM6* (Sasaki et al. 2011), and *PmDAM1* showed higher expression levels in vegetative buds than *PmDAM2* and *PmDAM3* (data not shown), we chose *PmDAM1* as the target gene for further functional characterization.

The five independent lines of *35S:PmDAM1* poplars obtained had distinct expression intensities of *PmDAM1*, as shown in Fig. 8.4. Compared with the highest expressing line, DAM1–12, transcript levels of DAM1–8 and DAM1–9 were 1/5 and 1/10, respectively, whereas those of DAM1–33 and DAM1–49 were less than 1/10. The *35S:PmDAM1* lines grew at similar rates to WT plants under LP conditions (Fig. 8.5a). This is inconsistent with the observed growth patterns of *35S:PmDAM6* poplars, which exhibited inhibited shoot growth (Sasaki et al. 2011; Yamane 2014). Although early bud set was observed in all four transgenic lines (Fig. 8.5b), the

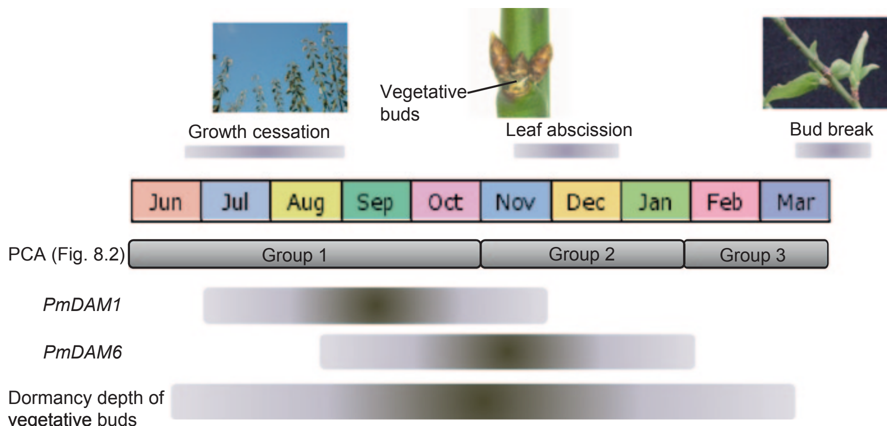


Fig. 8.3 The seasonal timing of growth cessation, leaf abscission, bud development, and bud break in Japanese apricot 'Nanko' grown in Kyoto, Japan. A principal component analysis (PCA) (Fig. 8.2) of microarray studies (Habu et al. 2014) divided the monthly bud samples into three groups, group 1 (June to October), group 2 (November to January), and group 3 (February and March). The seasonal expression patterns of *PmDAM1* and *PmDAM6*, and the depth of seasonal dormancy for vegetative buds are shown. The transcription levels of these genes are indicated by shading, *faint* (low) to *dark* (high) and the depth of seasonal dormancy are indicated, *faint* (non-deep) to *dark* (deep)

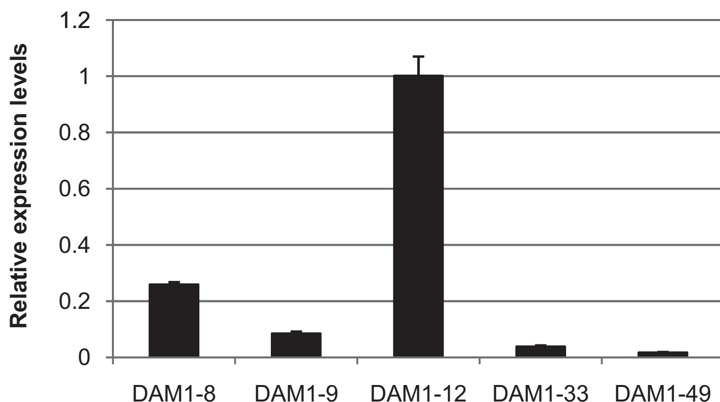


Fig. 8.4 Relative expression intensities of five different transgenic poplars (*35S:PmDAM1,* lines, No. 8, 9, 12, 33, and 49) expressing the Japanese apricot (*Prunus mume*) *DORMANCY-ASSOCIATED MADS-box 1* (*PmDAM1*). Gene expression levels were measured by RT-qPCR using *PmDAM1*-specific primers and a TaqMan probe (Sasaki et al. 2011). *PtACTIN* (Sasaki et al. 2011) was used as the reference gene

reopening of terminal buds was occasionally observed in some *35S:PmDAM1* lines (Fig. 8.5c). These results suggested that *PmDAM1* could not interfere with early shoot growth but could inhibit shoot growth during growth cessation. Additionally, PmDAM1 could act not just as a growth inhibitor but could promote apical growth when early bud set was induced. Thus, the reopening of terminal buds observed

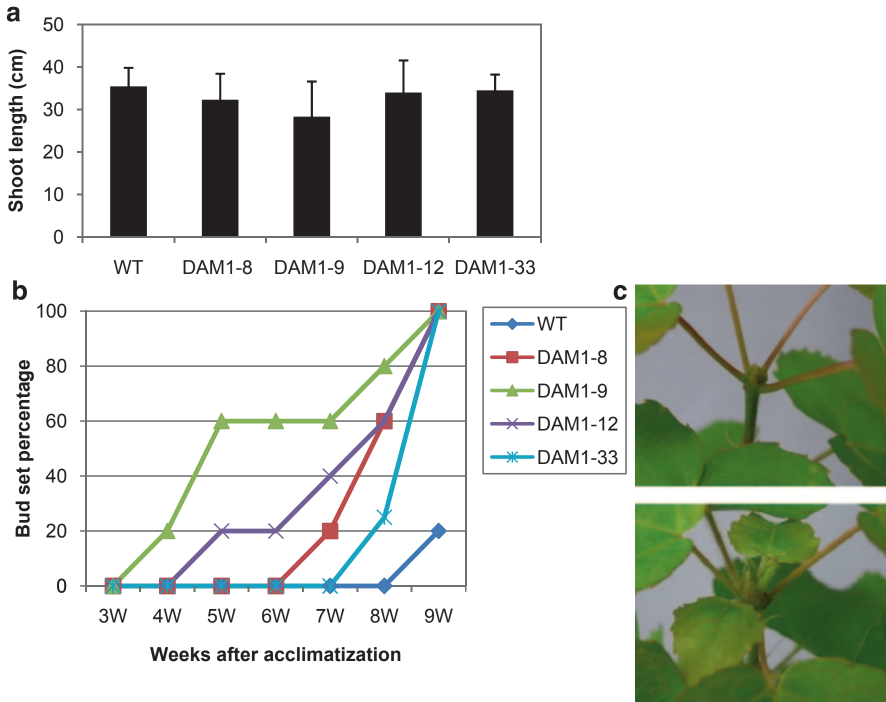


Fig. 8.5 The Japanese apricot (*Prunus mume*) *DORMANCY-ASSOCIATED MADS-box 1* (*PmDAM1*) showed growth inhibitory effects in poplar. **a** Shoot length of wild-type (WT) and four different *35S:PmDAM1* lines at 9 weeks after acclimatization (5 to 6 plants per line). **b** Bud set was induced in *35S:PmDAM1* poplars and bud set percentages of WT and four *35S:PmDAM1* lines are shown. **c** Although bud set was observed earlier in *35S:PmDAM1* (*upper panel*), the reopening of terminal buds (*lower panel*) was observed soon after bud set in some plants

soon after bud set suggested that PmDAM1 could both promote and inhibit apical growth depending on the growing conditions. Because the growth inhibitory effect of *PmDAM1* was obvious only when growth-promoting factors were limited, it may be that PmDAM1 acted weakly, relative to PmDAM6, even though *PmDAM1* and *PmDAM6* had inhibitory effects on the apical growth of poplar (Sasaki et al. 2011).

Transgenic plants grown under SP conditions set terminal buds at the same time (data not shown). Plants were decapitated and defoliated, and exposed to growth-promoting conditions prior to the cold treatment (Experiment 1-b; Fig. 8.1). Although WT and DAM1–9 plants showed bud burst and flushing within 3 weeks, more than 60% of the DAM1–12 plants did not show bud flushing (Fig. 8.6) even after 4 weeks. This could be explained by the hypothesis that the high expression level of *PmDAM1* in DAM1–12 inhibited the resumption of growth in decapitated and defoliated shoots.

A 50-day period of chilling exposure was sufficient to fulfill chilling requirements and induced dormancy release in WT and *35S:PmDAM6* poplar (Yamane, H. unpublished data). In this study, the terminal bud flushing rates of WT and *35S:PmDAM1* under LP conditions, after SP-induced terminal bud set

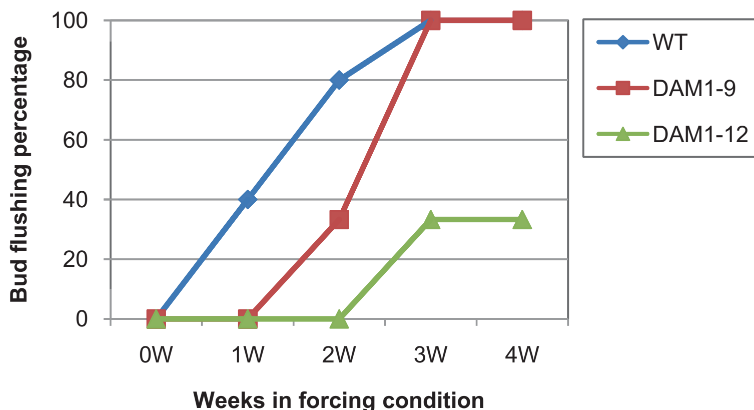


Fig. 8.6 After induction of bud set by short photoperiod (SP) conditions (8/16 h of light/dark), decapitation and defoliation of shoots induced lateral bud outgrowths in transgenic poplars expressing Japanese apricot (*Prunus mume*) *DORMANCY-ASSOCIATED MADS-box 1* (*PmDAM1*). Resumption of bud outgrowth was delayed in the *35S:PmDAM1* poplars

followed by 6 weeks of cold exposure (Experiment 2; Fig. 8.1), were compared. The first bud burst was observed in lateral buds, preceding terminal buds in some plants, and the timing of first bud burst in any bud was similar between WT and *35S:PmDAM1* (data not shown). Terminal bud flushing occurred earlier in WT and DAM1–8, followed by DAM1–12 and then DAM1–9. These results suggested that *PmDAM1* did not significantly affect dormancy release or subsequent bud flushing. However, when WT and *35S:PmDAM1* plants were grown from February to April in semi-field conditions, after sufficient cold exposure (3 months) (Experiment 1-c; Fig. 8.1), one *35S:PmDAM1* line, DAM1–12, showed delayed growth resumption, even though the bud flushing rates of the other *35S:PmDAM1* lines were similar to those of WT (Fig. 8.7). The observation that increased expression levels of *PmDAM1* in DAM1–12, over that in DAM1–8 or 1–9, resulted in a delayed bud flushing phenotype is consistent with the hypothesis that *PmDAM1* expression caused delayed bud flushing. Taken together, dormancy release and bud outgrowth were not affected by *PmDAM1* when plants were grown under controlled forcing conditions, although bud outgrowth was delayed by *PmDAM1* expression when plants were grown in semi-field conditions. Thus, further studies are required to determine whether *PmDAM1* plays a role in dormancy maintenance and inhibited dormancy release in poplar.

In summary, these preliminary observations are consistent with the hypothesis that, as is the case with *PmDAM6*, *PmDAM1* enhanced the rate of terminal bud formation in poplar. However, these results require replication to verify their consistency. In addition, as suggested by Yamane (2014), the effects of *PmDAM1* (this study) and *PmDAM6* (Sasaki et al. 2011) on dormancy maintenance were ambiguous. Because bud dormancy is a quantitative polygenic trait in perennial woody plants (Arora et al. 2003; Rohde et al. 2011; Tzonev and Erez 2003), it could be considered that the overexpression of singular *PmDAM*s could not

Fig. 8.7 Transgenic poplars expressing Japanese apricot (*Prunus mume*) *DORMANCY-ASSOCIATED MADS-box 1* (*PmDAM1*) showed growth inhibitory effects during the transition from dormancy release to bud outgrowth. **a** After short photoperiod (SP) conditions (8/16 h of light/dark) and cold treatment that induced dormancy release, the percentage of bud outgrowth from decapitated shoots was observed on April 2014 in a greenhouse without supplemental heating. **b** Photograph taken April 15, 2014

maintain the dormant state even though PmDAMs themselves function in dormancy maintenance in intact plants. Alternatively, factors other than PmDAMs may play significant roles in dormancy maintenance. This study suggests that, in addition to *PmDAM6*, *PmDAM1* may participate in bud dormancy regulation by modifying apical growth. *PmDAM1* was up-regulated during summer when shoot growth cessation was observed and then down-regulated long before the dormancy release of Japanese apricot (Sasaki et al. 2011). If this seasonal expression pattern is taken into account, then *PmDAM1* may enhance dormancy induction during summer and inhibit bud outgrowth when trees are defoliated accidentally during summer and autumn. Further studies will be required to evaluate this possibility.

References

Arora R, Rowland LJ, Tanino K (2003) Induction and release of bud dormancy in woody perennials: a science comes of age. HortScience 38(5):911–921

Bielenberg DG, Li Z, Zhebentyayeva T, Fan S, Reighard GL, Scorza R, Abbott AG (2008) Sequencing and annotation of the evergrowing locus in peach [*Prunus persica* (L.) Batsch] reveals a cluster of six MADS-box transcription factors as candidate genes for regulation of terminal bud formation. Tree Genet Gen 4(3):495–507

Cooke JK, Eriksson ME, Junttila O (2012) The dynamic nature of bud dormancy in trees: environmental control and molecular mechanisms. Plant Cell Environ 35(10):1707–1728

Habu T, Yamane H, Igarashi K, Hamada K, Yano K, Tao R (2012) 454-pyrosequencing of the transcriptome in leaf and flower buds of Japanese apricot (*Prunus mume* Sieb. et Zucc.) at different dormant stages. J Jpn Soc Hortic Sci 81(3):239–250

Habu T, Yamane H, Sasaki R, Yano K, Fujii H, Shimizu T et al (2014) Custom microarray analysis for transcript profiling of dormant vegetative buds of Japanese apricot during prolonged chilling exposure. J Jpn Soc Hortic Sci 83(1):1–16

Jimenéz SG, Reighard L, Bielenberg DG (2010) Gene expression of *DAM5* and *DAM6* is suppressed by chilling temperatures and inversely correlated with bud break rate. Plant Mol Biol 73(1–2):157–167

Mazzitelli L, Hancock RD, Haupt S, Walker PG, Pont SDA, McNicol J et al (2007) Co-ordinated gene expression during phases of dormancy release in raspberry (*Rubus idaeus* L.) buds. J Exp Bot 58(5):1035–1045

Nakagawa T, Kurose T, Hino T, Tanaka K, Kawamukai M, Niwa Y et al (2007) Development of series of gateway binary vectors, pGWBs, for realizing efficient construction of fusion genes for plant transformation. J Biosci Bioeng 104(1):34–41

Rinne PLH, Welling A, van der Schoot C (2010) Perennial life style of *Populus*: dormancy cycling and overwintering. In: Jansson S, Bhalerao R, Groover A (eds) Genetics and genomics of *Populus*. Springer, New York, pp 171–200

Rohde A, Bhalerao RP (2007) Plant dormancy in the perennial context. Trends Plant Sci 12(5): 217–223

Rohde A, Storme V, Jorge V, Gaudet M, Vitacolonna N, Fabbrini F et al (2011) Bud set in poplar—genetic dissection of a complex trait in natural and hybrid populations. New Phytol 189(1):106–121

Saito T, Bai S, Ito A, Sakamoto D, Saito T, Ubi BE et al (2013) Expression and genomic structure of the *dormancy-associated MADS box* genes MADS13 in Japanese pears (*Pyrus pyrifolia* Nakai) that differ in their chilling requirement for endodormancy release. Tree Physiol 33(6):654–667

Sasaki R, Yamane H, Ooka T, Jotatsu H, Kitamura Y, Akagi T et al (2011) Functional and expressional analyses of *PmDAM* genes associated with endodormancy in Japanese apricot (*Prunus mume*). Plant Physiol 157(1):485–497

Tzonev R, Erez A (2003) Inheritance of chilling requirement for dormancy completion in apricot vegetative buds. Acta Hortic 622:429–436

Yamane H (2014) Regulation of bud dormancy and bud break in Japanese apricot (*Prunus mume* Siebold & Zucc.) and peach [*Prunus persica* (L.) Batsch]: a summary of recent studies. J Jpn Soc Hortic Sci 83:187–202

Yamane H, Kashiwa Y, Ooka T, Tao R, Yonemori K (2008) Suppression subtractive hybridization and differential screening reveals endodormancy-associated expression of an *SVP/AGL24*-type MADS-box gene in lateral vegetative buds of Japanese apricot. J Am Soc Hortic Sci 133(5):708–716

Yamane H, Ooka T, Jotatsu H, Hosaka Y, Sasaki R, Tao R (2011a) Expressional regulation of *PpDAM5* and *PpDAM6*, peach (*Prunus persica*) dormancy-associated MADS-box genes, by low temperature and dormancy-breaking reagent treatment. J Exp Bot 62(10):3481–3488

Yamane H, Ooka T, Jotatsu H, Sasaki R, Tao R (2011b) Expression analysis of *PpDAM5* and *PpDAM6* during flower bud development in peach (*Prunus persica*). Sci Hortic 129(4): 844–848

Yamane H, Tao R, Ooka T, Jotatsu H, Sasaki R, Yonemori K (2011c) Comparative analyses of dormancy-associated MADS-box genes, *PpDAM5* and *PpDAM6*, in low- and high-chill peaches (*Prunus persica* L.). J Jpn Soc Hortic Sci 80(3):276–283

Zhang Q, Chen W, Sun L, Zhao F, Huang B, Yang W et al (2012) The genome of *Prunus mume*. Nat Commun 3:1318

Chapter 9
The Genetic Components Involved in Sensing Chilling Requirements in Apricot

Taly Trainin, Irit Bar-Ya'akov and Doron Holland

Abbreviations

DAM	dormancy-associated MADS-box
QTL	quantitative trait loci
SOC1	Suppressor Of CONSTANS
LG	linkage group
SSR	single sequence repeat
GDR	Genome Database for Rosaceae

Introduction

In geographical regions with relatively hot winter climates, chilling is a limiting factor in cultivating many species of deciduous fruit trees due to its requirement for proper dormancy induction processes, for normal flower development, and for dormancy break (Erez and Couvillon 1986; Erez and Lavee 1971; Erez et al. 1979). All of these factors are crucial for the ability to commercially produce good yielding, high-quality fruit. Most fruit trees of the Rosaceae family are particularly prone to damage caused by the lack of chilling. Among fruit trees of the Rosaceae family, apricot is considered one of the most sensitive species to the lack of chilling (Trainin et al. 2013). Yet, despite their high chilling requirements, certain local apricot species and some that originated from North Africa are much less sensitive to chilling and are able to break dormancy and produce high yield, even under conditions where winter temperatures were mild (Olukolu et al. 2009; Trainin et al. 2013). Such accessions were crossed with European and American cultivars that have high chilling requirements, which produced populations that segregated for

D. Holland (✉) · T. Trainin · I. Bar-Ya'akov
Department of Fruit Tree Sciences, Newe Ya'ar Research Center, Agricultural Research Organization, P.O. Box 1021, 30095 Ramat Yishay, Israel
email: vhhollan@volcani.agri.gov.il

© Springer International Publishing Switzerland 2015

J. V. Anderson (ed.), *Advances in Plant Dormancy,* DOI 10.1007/978-3-319-14451-1_9

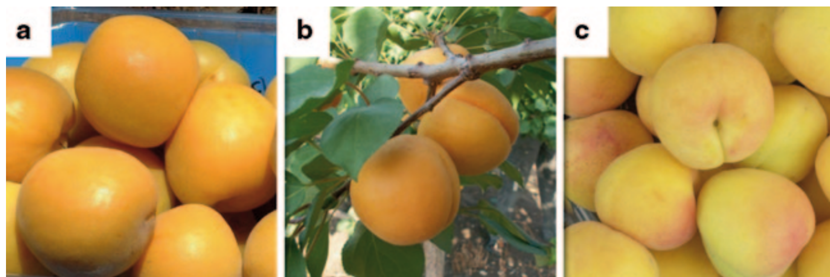


Fig. 9.1 Newly bred apricot cultivars with low chilling requirements especially suited for growth in regions with warm winter, Newe Ya'ar Research Center, Israel. **a** 'Tarog', **b** 'Daniel' **c** 'Nitzan'

chilling requirements in the early F1 cross (Olukolu et al. 2009). Moreover, it was possible to breed new apricot cultivars that are especially suitable for commercial growth in geographical regions with warm (i.e., less than 300 chilling hours below 7 ºC) winter conditions (Fig. 9.1, Fig. 9.2). The early segregation of our breeding population at the F1 stage enabled us to map, for the first time, quantitative trait loci (QTLs) for chilling requirements in apricot and later on in peach (Fan et al. 2010; Olukolu et al. 2009).

Studies on apricot and peach revealed that a major QTL is present in linkage group 1 (LG1) (Fan et al. 2010; Olukolu et al. 2009). The QTL site in LG1 of peach overlaps the peach *ever-growing* locus (*evg*) where a deletion of 6 tandem repeats of *dormancy-associated MADS-Box* (*DAM)* genes was previously reported to be the site of the *evg* mutation (Bielenberg et al. 2004, 2008; Fan et al. 2010; Rodriguez et al. 1994). Since one of the most prominent phenotypes of the *evg* mutation is its inability to enter dormancy, the overlap of the major chilling requirement QTL with that of the *evg* locus strengthened the assumption that *DAM* genes control chilling requirements in apricot and peach. Further studies specifically pointed to *DAM5* and *DAM6* as the most relevant genes for chilling requirements in peach (Jiménez et al. 2010a, 2010b; Li et al. 2009; Yamane et al. 2011), *Prunus mume* (Sasaki et al. 2011), and apricot (Olukolu et al. 2009). Further studies provided evidence for epigenetic control associated with dormancy break in response to chilling exposure in peach (Leida et al. 2012a, 2012b; Rios et al. 2014). In these studies, a decrease in H3 acetylation and increase in H3K27 trimethylation in the chromatin of the ATG region of *DAM6* were revealed in two different peach cultivars. Apart from the peach and apricot *DAM* genes, no other specific genes that are involved in dormancy break were reported for fruit trees of the Rosaceae family.

Interestingly, in addition to the major QTL found on LG1, an additional QTL that attributed about 20 % of the chilling requirement phenotype was found on LG2 of apricot (Olukolu et al. 2009). Recent studies show that certain alleles of a *ParSOC1* gene present in peach and apricot map to LG2 and are associated with chilling requirements in a collection of apricot accessions in the Newe Ya'ar Research Center in Israel (Trainin et al. 2013). This work summarizes the available data on the *ParSOC1* gene in apricot, discusses its possible role, and shows that polymorphic

Fig. 9.2 Apricot grown in the Arava desert in “Yair” Experimental Station, Israel. Trees are able to break dormancy, flower, and set fruit when exposed to less than 100 chilling hours. Bud break, flowering, and fruit precede regular growth conditions by about one month in the Newe Ya’ar Research Center, north of Israel. **a** Full leaf coverage in end of April. **b** Flowering in the beginning of March. **c** Fruit color break in mid-April. Trees were grown in a screen house

SOC1-like alleles are also found in apple (*M. domestica*), another member of the Rosaceae fruit trees family.

Methods

Most of the data are based on methods described in Trainin et al. 2013. Phylogenetic analysis was performed using the Phylogeny.fr software (Dereeper et al. 2008, 2010). For the genomic structure of the Apple *MdSOC1* gene, the published coding sequence (GenBank Accession No. GU983664.1) was aligned with the published genomic sequences: contig MDC019611.360 for chromosome 2 and contigs MDC004726.279 and MDC18698.258 for chromosome 7 (GDR database; Jung et al. 2013) using NCBI BLAST (bl2seq).

Results and Discussion

SOC1-like Genes in Apricot and Apple

Rosaceae *SOC1-like* genes, which are highly homologous to the *Arabidopsis SOC1* gene, were found in the genomes of peach (*P. persica*), apricot (*P. armeniaca*), and apple (*M. domestica*). The phylogenetic tree of various deduced proteins of *SOC1-like* genes indicates that Rosaceae SOC1-like proteins belong to a clade, which is distinct from the DAM proteins' clade that are more similar to *Arabidopsis* AGL24 protein (Fig. 9.3). The Rosaceae *SOC1* genes are grouped in the same cluster in the phylogenetic tree. The prunus *SOC1* genes, including the *ParSOC1* from apricot and *PpSOC1* from peach, form a subcluster inside the Rosaceae group. While peach and apricot contain only one copy of the *SOC1-like* gene in their genome, there are two *SOC1-like* genes in apple: GenBank Accession No. GU983664.1 on chromosome 2—chr2:21,522,765..21,572,764 and on chromosome 7—chr7:12,080,883..12,090,958 spanning contigs MDC004726.279 and MDC18698.258 [The Genome Database for Rosaceae (GDR), Jung et al. 2013]. Although these two genes encode for an identical protein, their DNA sequence is different. Of particular interest is the 5′ transcribed portion of the Rosaceae *SOC1-like* genes that contains a single sequence repeat (SSR) based on reiterations of a $(CT)_n$ motif (Fig. 9.4a). The presence of the $(CT)_n$ motif in apple as well as in peach and apricot indicates that this motif was present before the evolutionary split between the *Malus* and *Prunus* group in the Rosaceae family.

ParSOC1 Alleles are Highly Polymorphic Among Different Apricot Accessions

We genotyped the DNA from 48 different apricot accessions, which represent the most divergent accessions in the apricot collection. Using a specific set of primers flanking the SSR sequence in the 5′ region of the *ParSOC1* gene allowed us to identify sequence variations for 13 different *ParSOC1* alleles among apricots. Most of the accessions were heterozygous for the *ParSOC1* allele (Fig. 9.4b; Trainin et al. 2013). The differences reside in the length of the $(CT)_n$ motif, which ranged between a few to several dozen nucleotides. Because the $(CT)_n$ motif is in the transcribed region of the gene, but in the 5′ untranslated region (UTR), it might be involved in control of mRNA stability. Apart from the $(CT)_n$ motif, the rest of the gene and the coding sequence itself is identical in all apricots analyzed so far. Moreover, high conservation among apricot, peach, and apple genes is observed including the position and length of the introns (Fig. 9.4a), suggesting an important role for the Rosaceae *SOC1-like* gene.

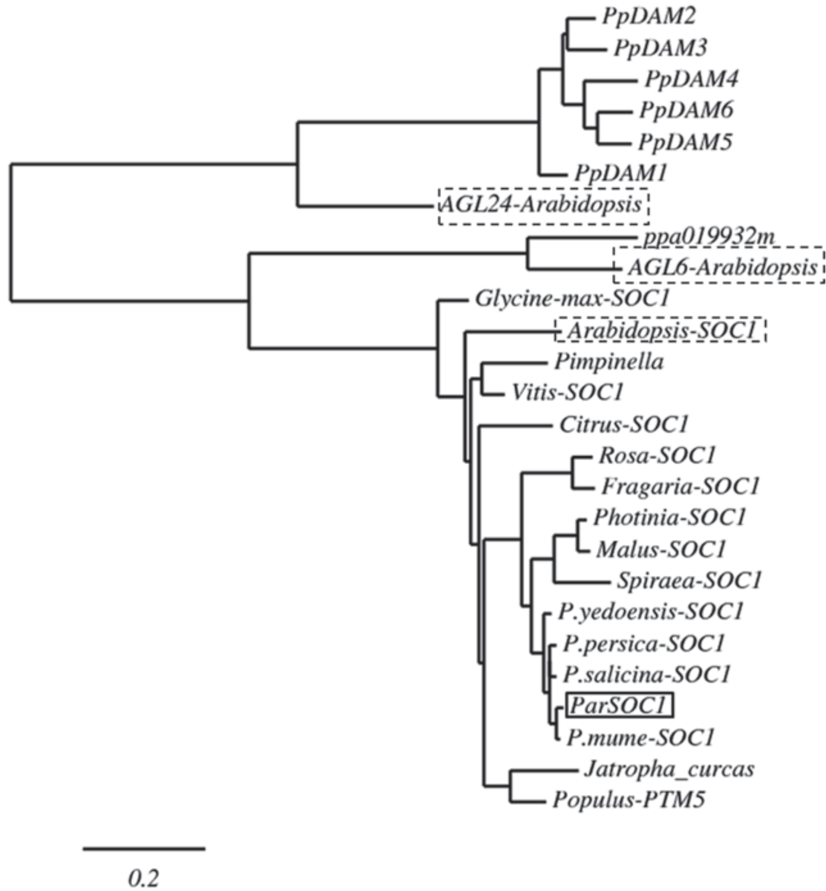


Fig. 9.3 Phylogenetic tree showing clustering of ParSOC1 and its homologues with respect to other known AGAMOUS-like proteins (AGL24, AGL6). *Arabidopsis* proteins are highlighted by dashed-lined boxes. ParSOC1 is marked by a solid-lined box. Accession numbers of SOC1-like proteins shown in the alignment: *ParSOC1* (bankit1155742, FJ472817), *Prunus mume* SOC1-like protein (AEO20229.1), *Prunus yedoensis* SOC1-like protein (AEO20233.1), *Spiraea cantoniensis* SOC1-like protein (AEO20234.1), *Fragaria vesca* SOC1 (ACR24128.1), Rosa hybrid cultivar SOC1-like protein (AEO20230.1), *Photinia serratifolia* SOC1-like protein (AEO20232.1), *Malus x domestica* SOC1-like (BAI49494.1), *Vitis vinifera* SOC1 (ABF56527.1), *Glycine max* SOC1 (NP_001236377.1), *Citrus sinensis* SOC1-like protein (ABS84659.1), *Populus tremuloides* MADS-box protein PTM5 (AF377868), *Arabidopsis thaliana SOC1* (NP_182090.1), *Pimpinella brachycarpa* transcription activator (AAC33475.1), *Jatropha curcas* (JHL05D22.4). Peach *PpSOC1* (*PpSOC1* is positioned on the peach genome map on scaffold 2: 17,024,317…17,029,252), *Prunus salicina* SOC1-like gene (JX524767)). GenBank accession numbers for other proteins: peach PpDAM1 (ABJ96361), PpDAM2 (ABJ96370), PpDAM3 (ABJ96371), PpDAM4 (ABJ96365), pDAM5 (ABJ96366), PpDAM6 (ABJ96367), *Arabidopsis* AGL24 (AEE84922.1), *Arabidopsis* AGL6 (NP_182089.1). Peach-annotated AGL6: (ppa019932m; http://www.rosaceae.org/peach/genome/)

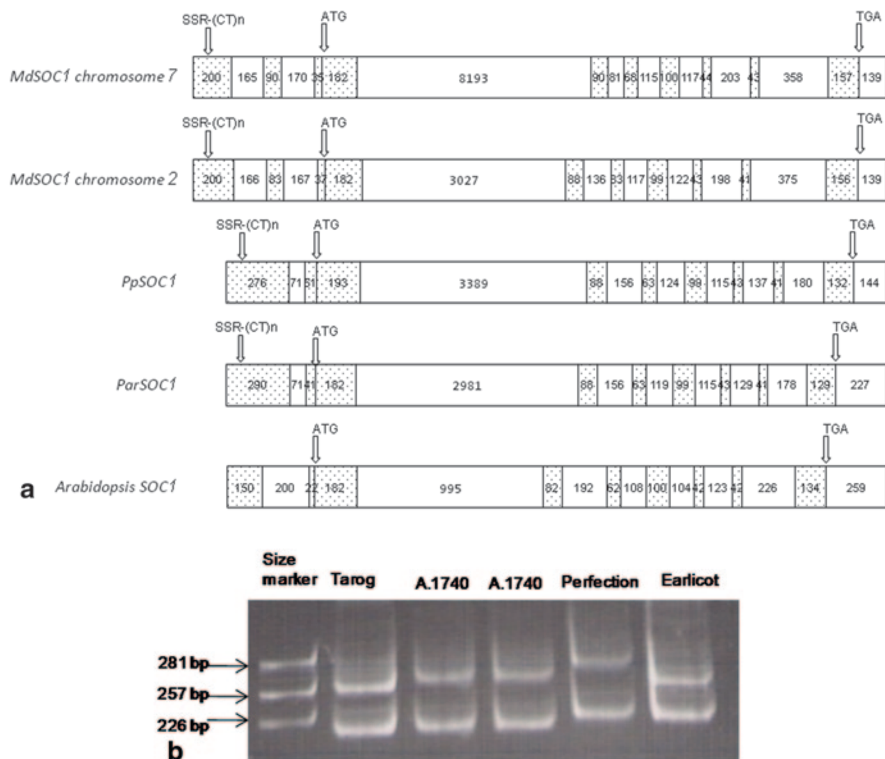


Fig. 9.4 **a** Genomic structure of apricot, peach, and apple *SOC1* genes. Schematic presentation of intron/exon structure of the Rosaceae *SOC1* genes compared to *Arabidopsis SOC1* gene (based on GenBank Accession No. AC003680 and Johansen et al. 2002). *Dotted boxes* represent transcribed sequences (exons and 5' or 3' UTR), and *white boxes* represent introns. Numbers in boxes represent the length in bp. SSR region (CT)n, ATG translation initiation site, and TGA translation stop site are marked by *arrows*. The scheme is based on the following sequences: apricot *ParSOC1*—GenBank Accession Nos. FJ472817, JX546224: peach *PpSOC1* putative gene positioned on the peach genome v1.0 on scaffold 2: 17,024,317…17,029,252 (http://www.phytozome.org/peach); apple *MdSOC1*—the structure is based on alignment (using BLAST (bl2seq)) of the coding sequence (GenBank Accession No. GU983664.1) with the published genomic sequences: contig MDC019611.360 for chromosome 2 and contigs MDC004726.279 and MDC18698.258 for chromosome 7 (GDR database; Jung et al. 2013). **b** Allelic composition of *ParSOC1* in different apricot cultivars and their corresponding sizes. A 6 % mini-acrylamide gel showing transcription of the various alleles in leaves (from Trainin et al. 2013)

Associating Chilling Requirements with the *ParSOC1* Gene

The presence of the *ParSOC1* gene in a region that was mapped as a QTL for chilling requirements in the apricot genome (Olukolu et al. 2009; Trainin et al. 2013) and the known involvement of the *Arabidopsis* SOC1 protein in integrating vernalization and photoperiod signals suggest that *ParSOC1* might be involved in regulating chilling requirements in apricot and perhaps other fruit trees of the Rosaceae family. To elucidate the role of *SOC1-like* genes in Rosaceae, the

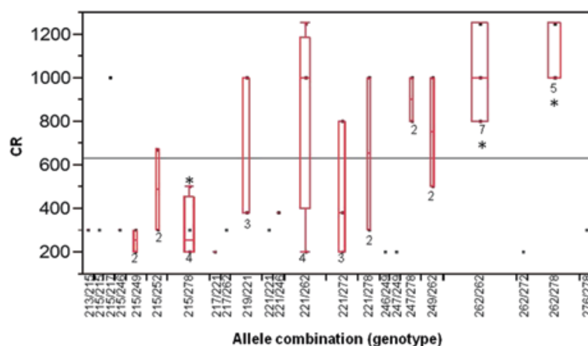


Fig. 9.5 Association of vegetative chilling requirements with the various *ParSOC1* genotypes found in the apricot collection. The box plots show that allele combinations 262/262 or 262/278 are associated with high chilling requirements, while allele combination 215/278 is associated with low chilling requirements. *Asterisks* indicate significantly different allele combinations determined according to nonparametric comparisons for each pair using the Wilcoxon method. The allele combination 215/278 is significantly different from the allele combination 262/262 ($p<0.01$) and from 262/278 ($p<0.05$). Box width is proportional to the number of cultivars carrying a specific allele combination (the number of cultivars carrying a specific allele combination is written below the box). Other allele combinations have only one representative (*small squares*). The Wilcoxon/Kruskal–Wallis one-way test and chi-square approximation (*rank sums*) were used for the analysis of association of specific alleles with chilling requirements using the JMP statistical analysis program. Box plot was generated by the JMP software (from Trainin et al. 2013)

high polymorphism of *ParSOC1* was utilized to check whether certain alleles of *ParSOC1* are associated with chilling requirements. For this purpose, the chilling requirements of 48 apricot accessions were determined as described in Olukolu et al. 2009 and Trainin et al. 2013. In these studies, only chilling requirements for vegetative bud break were determined. The chilling requirements among the analyzed accessions ranged between 200 and 1250 chilling hours as described in Trainin et al. 2013 and presented in Fig. 9.5. Of particular interest is the 262 allele, which is present in 42 % of the accessions and is highly associated with high chilling requirements (p value = 0.0001). The 215 allele that is present within 25 % of the analyzed accessions is associated with low chilling requirements (p value = 0.005). These results support the assumption that *ParSOC1* or a gene in its close proximity is involved in regulating chilling requirements in apricot cultivars, as determined under the conditions in Newe Ya'ar.

Possible Physiological Role of *ParSOC1*

Perhaps a clue to the functional role of SOC1 may be derived from the mode of *ParSOC1* expression in apricot. *ParSOC1* is expressed in almost all of the apricot tissues analyzed so far, including the roots and the flowers but not in developing and ripened fruit. When transformed to *Arabidopsis* under the control of the 35S

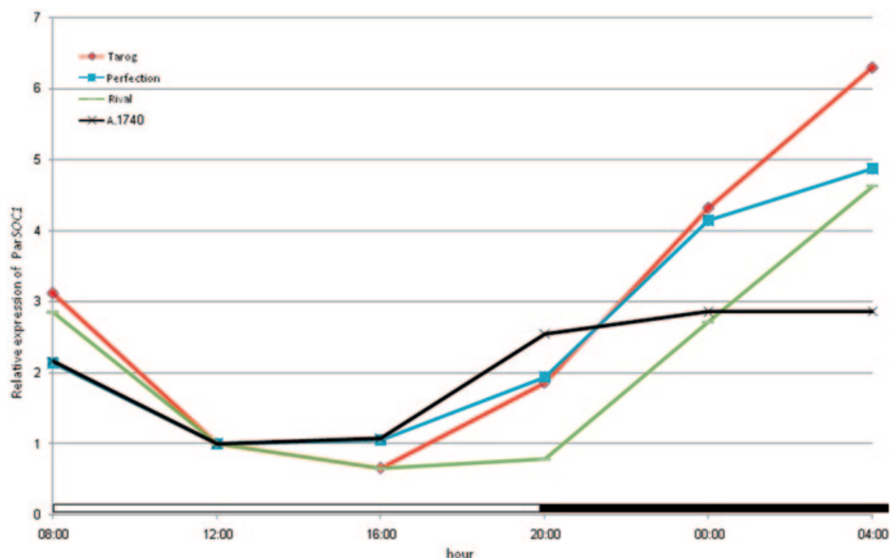


Fig. 9.6 Diurnal expression of *ParSOC1* in leaves of 4 apricot cultivars (mid-June 2006). Northern analysis. Expression levels were normalized against 18S ribosomal RNA and expressed as a fraction of the expression at 12:00. *Open boxes* on x-axis represent daylight, and *black boxes* represent darkness. Sunrise, 5:32; sunset, 19:50 (adapted from Trainin et al. 2013)

promoter, no significant influence on flowering time was observed. *ParSOC1* is expressed in apricot leaves in a diurnal manner and reaches its peak of expression toward the end of the night (Fig. 9.6). This gene was also expressed in dormant apricot buds, and repression of steady-state levels was observed upon exposure of dormant buds to 6 ºC for 200 h (data not shown). The data suggest that expression of *ParSOC1* is sensitive to environmental conditions such as chilling but is also under the control of an internal clock.

In *Arabidopsis*, the *soc1* mutation alters the circadian clock (Salathia et al. 2006). Perhaps *ParSOC1* is part of a mechanism that responds to the environment by modulating the internal clock of the plant. The absence of *ParSOC1* expression in the fruit may indicate that it interferes with fruit or seed development. Homologues of *SOC1* were found in strawberry (Mouhu et al. 2013; Lei et al. 2013). In transgenic strawberry plants where *SOC1* gene expression was silenced, runner formation was not normal. This phenotype was suppressed upon application of GA (Mouhu et al. 2013). Based on these data and expression data on GA biosynthetic genes, it was suggested by the authors that SOC1 could be involved in the control of GA biosynthesis. As suggested for strawberry, *ParSOC1* could be involved in regulating gibberellic acid biosynthesis in apricot. It is not known, however, whether gibberellic acid is involved in regulating chilling requirements in Rosaceae fruit trees. The function of ParSOC1 might involve interactions with other MADS-box proteins such as the proteins encoded by the *DAM (AGL24-like*) genes. This assumption is based on the fact that in *Arabidopsis*, the proteins encoded by *AGL24* and *SOC1*

were shown to interact and regulate each other (Liu et al. 2008). In addition, the strawberry SOC1 can interact with *Arabidopsis* AGL24 in yeast 2 hybrid and BiFC assays (Lei et al. 2013).

One of the questions that remain unanswered is whether chilling requirements in other fruit trees of the Rosaceae such as apples and pears are also regulated by genes similar to *ParSOC1*. Although major QTLs for chilling requirements were reported for these tree species, the genes involved were not yet identified. The possible involvement of *SOC1* in apple chilling requirements is now under investigation.

References

Bielenberg DG, Wang Y, Fan S, Reighard GL, Scorza R, Abbott AG (2004) A deletion affecting several gene candidates is present in the evergrowing peach mutant. J Hered 95:436–444

Bielenberg DG, Wang Y, Li Z, Zhebentyayeva T, Fan S, Reighard GL, Scorza R, Abbott AG (2008) Sequencing and annotation of the evergrowing locus in peach [prunus persica (L.) Batsch] reveals a cluster of six MADS-box transcription factors as candidate genes for regulation of terminal bud formation. Tree Genet Genomes 4(3):495–507

Dereeper A, Guignon V, Blanc G, Audic S, Buffet S, Chevenet F, Dufayard JF, Guindon S, Lefort V, Lescot M, Claverie JM, Gascuel O (2008) Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Res 36(Suppl 2):W465–W469

Dereeper A, Audic S, Claverie JM, Blanc G (2010) BLAST-EXPLORER helps you building datasets for phylogenetic analysis. BMC Evol Biol 10:8

Erez A, Couvillon GA (1986) A characterization of the influence of moderate temperatures on the rest completion in the peach. J Amer Soc Hort Sci 112:677–680

Erez A, Lavee S (1971) The effect of climatic conditions on dormancy development of peach buds. I. Temperature. J Amer Soc Hort Sci 96:711–714

Erez A, Couvillon GA, Hendershott CH (1979) Quantitative chilling enhancement and negation in peach buds by temperatures in a daily cycle. J Amer Soc Hort Sci 104:536–540

Fan S, Bielenberg DG, Zhebentyayeva TN, Reighard GL, Okie WR, Holland D, Abbott AG. (2010) Mapping quantitative trait loci associated with chilling requirement, heat requirement and bloom date in peach (*Prunus persica*). New Phytol 185:917–930

Jiménez S, Reighard GL, Bielenberg DG (2010a) Identification of genes associated with growth cessation and bud dormancy entrance using a dormancy-incapable tree mutant. BMC Plant Biol 10:25. doi:10.1186/1471–2229-10–25

Jiménez S, Reighard GL, Bielenberg DG (2010b) Gene expression of DAM5 and DAM6 is suppressed by chilling temperatures and inversely correlated with bud break rate. Plant Mol Biol (2010) 73:157–167 doi:10.1007/s11103–010-9608–5

Johansen B, Pederson LB, Skipper M, Frederiksen S (2002) MADSbox gene evolution—structure and transcription patterns. Mol Phylogenet Evol 23:458–480

Jung S, Ficklin S, Lee T Cheng CH, Blenda A, Zheng P, Yu J, Bombarely A, Cho I, Ru S, Evans K, Peace C, Abbott AG, Mueller LA, Olmstead MA, Main D (2013). The genome database for rosaceae (GDR): year 10 update. Nucl Acids Res. doi:10.1093/nar/gkt1012

Lei HJ, Yuan HZ, Liu Y, Guo XW, Liao X, Liu LL, Wang Q, Li TH (2013) Identification and characterization of FaSOC1, a homolog of suppressor of overexpression of constans1 from strawberry. Gene 531:158–67. doi:10.1016/j.gene.2013.09.036

Leida C, Conesa A, Llácer G, Badenes ML, Ríos G (2012a) Histone modifications and expression of DAM6 gene in peach are modulated during bud dormancy release in a cultivar-dependent manner. New Phytol 193:67–80. doi:10.1111/j.1469–8137.2011.03863.x

Leida C, Conejero A, Arbona V, Gómez-Cadenas A, Llácer G, Badenes ML (2012b) Chilling-dependent release of seed and bud dormancy in peach associates to common changes in gene expression. PLoS One 7(5):e35777

Li Z, Reighard GL, Abbott AG, Bielenberg DG (2009) Dormancy- associated MADS genes from the EVG locus of peach [Prunus persica (L.) Batsch] have distinct seasonal and photoperiodic expression patterns. J Exp Bot 60:3521–3530

Liu C, Chen H, Er HL, Soo HM, Kumar PP, Han JH, Liou YC, Yu H (2008) Direct interaction of AGL24 and SOC1 integrates flowering signals in Arabidopsis. Development 135:1481–1491

Mouhu K, Kurokura T, Koskela EA, Albert VA, Elomaa P, Hytönen T (2013) The Fragaria vesca homolog of suppressor of overexpression of constans1 represses flowering and promotes vegetative growth. Plant Cell 25:3296–3310

Olukolu BA, Trainin T, Fan S, Kole C, Bielenberg DG, Reighard GL, Abbott AG, Holland D (2009) Genetic linkage mapping for molecular dissection of chilling requirement and budbreak in apricot (*Prunus armeniaca* L). Genome 52:819–828

Rios G, Leida C, Conejero A, Badenes ML (2014) Epigenetic regulation of bud dormancy events in perennial plants. Front Plant Sci 3:5:247

Rodriguez A, Sherman WB, Scorza R, Wisniewski M, Okie WR (1994) 'Evergreen' peach, its inheritance and dormant behavior. J Am Soc Hort Sci 119(4):789–792

Salathia N, Davis SJ, Lynn JR, Michaels SD, Amasino RM, Millar AJ (2006) FLOWERING LOCUS C- dependent and independent regulation of the circadian clock by the autonomous and vernalization pathways. BMC plant biol 6:10

Sasaki R, Yamane H, Ooka T, Jotatsu H, Kitamura Y, Akagi T, Tao R (2011) Functional and expressional analyses of *PmDAM* genes associated with endodormancy in Japanese apricot. Plant Physiol 157:485–497

Trainin T, Bar-Ya'akov I, Holland D (2013) *ParSOC1*, a MADS-box gene closely related to Arabidopsis AGL20/SOC1, is expressed in apricot leaves in a diurnal manner and is linked with chilling requirements for dormancy break. Tree Genet Genomes 9:1–14

Yamane H, Ooka T, Jotatsu H, Hosaka Y, Sasaki R, Tao R (2011) Expressional regulation of PpDAM5 and PpDAM6, peach (Prunus persica) dormancy-associated MADS-box genes, by low temperature and dormancy-breaking reagent treatment. J Exp Bot 62:3481–3488

Chapter 10
Prolonged Exposure of Grapevine Buds to Low Temperatures Increases Dormancy, Cold Hardiness, and the Expression of *Vvα-AMYs* Genes

Francisco J. Pérez and Sebastián Rubio

Introduction

It is well known that temperate fruit trees require exposure to low temperatures during the winter to uniformly sprout in the spring. This chilling requirement (CR) is an important agronomic trait that is species dependent and genetically determined (Fans et al. 2010). A failure to achieve the CR in warm winter regions results in poor and uneven bud break (Erez 1995; Topp et al. 2008). Thus, the CR limits the geographical locations for growing temperate fruit trees (Sherman and Beckman 2003). Despite the importance of this agronomic trait, little is known about the mechanism by which low temperatures promote uniform bud break in temperate fruit trees. Because low temperatures have the dual effect of favoring endodormancy (ED) release and increasing cold hardiness (CH) in temperate fruit trees, it seems reasonable that insufficient chilling during the winter could extend the period of ED or impair the development of CH. One or both of these effects could be responsible for the irregular pattern of bud break observed in response to warm winter conditions.

Bud ED, or winter recess, in woody perennials is a physiological stage that enables plants to survive long periods of adverse conditions. It is characterized by growth cessation, arrested cell division, and reduced metabolic and respiratory activity (Arora et al. 2003). At the most fundamental level, bud ED can be considered the inability of meristems to resume growth under favorable conditions (Rohde and Bhalerao 2007). In grapevines (*Vitis vinifera* L), as in other temperate fruit trees, the depth of bud ED is measured as the delay in sprouting of single-bud cuttings under forced conditions. The time required to reach 50 % of bud break (BR_{50}) under forced conditions (Pérez et al. 2007) is a widely used parameter for monitoring depth of bud ED.

In temperate regions, perennial species are able to increase their CH in response to low, non-freezing temperatures. This process is called cold acclimation which

F. J. Pérez (✉) · S. Rubio
Facultad de Ciencias, Laboratorio de Bioquímica Vegetal, Universidad de Chile,
Casilla 653, Santiago, Chile
e-mail: frperez@uchile.cl

© Springer International Publishing Switzerland 2015

J. V. Anderson (ed.), *Advances in Plant Dormancy,* DOI 10.1007/978-3-319-14451-1_10

involves the coordinated expression of thousands of genes to induce physiological and biochemical changes in the buds (Sakai and Larcher 1987). In grapevines, as in other perennial species, cold acclimation is accompanied by a decrease in the freezing point of intracellular water, a phenomenon known as supercooling (Gusta and Wisniewski 2013). Differential thermal analysis (DTA) has been widely used to measure the exotherms of deep supercooled buds. Two exotherms are generally observed in the cold-acclimated buds, a high-temperature exotherm (HTE) and a low-temperature exotherm (LTE); these correspond to the heat released during the freezing points of extracellular and intracellular water (Burke et al. 1976). In grapevine buds, lethal tissue damage takes place at temperatures below LTE (Pierquet and Stushnoff 1980; Mills et al. 2006; Ferguson et al. 2011). Such observations indicate that LTE can be considered a measure of the degree of CH. To gain further insights into mechanisms underlying the effects of low temperatures on uniform bud break, we analyzed the evolution of BR_{50} and LTE parameters during the annual cycle of grapevine buds, determined the effect of low temperatures on these two parameters, and quantified starch content and expression of α-amylase genes (*Vvα-AMYs*) in single-bud cuttings.

Materials and Methods

Plant Material

Plant material was collected from 8-year-old (*Vitis vinifera* L cv. Thompson seedless) vines growing in the experimental station of the Chilean National Institute of Agriculture Research (INIA), located in Santiago (33°34′ S latitude). For the forced bud break assay, canes were randomly collected every 2–3 weeks between December 11 and mid-August, and for exotherm determinations, buds were collected weekly between April 22 and August 27. Canes were cut at both ends, leaving the central section with 10–12 buds for the analyses.

Temperature Measurements

Temperature data records were obtained from the local weather station of the National Institute of Agricultural Research (INIA, La Platina), which is located in the same location as the vineyards. Daily mean temperatures were used as inputs in the discrete dynamic model for estimating CH (Ferguson et al. 2011).

Bud Dormancy Status

On each collection date, 40 single-bud cuttings (10–12 cm length) were mounted on a polypropylene sheet and floated in tap water in a plastic container. The cuttings

were then transferred to a growth chamber set at 23 ± 2 °C with a 16-h photoperiod (forcing conditions). Bud break was assayed every 5 d for a period of 30 d. The appearance of visible green tissue at the tip of the bud was indicative of bud break. The depth of bud dormancy was determined using the BR_{50}, which is an estimate of the mean time required to reach 50 % bud break under forced conditions (Pérez et al. 2007).

LTE Measurements

Dormant basal buds were collected weekly from field-grown grapevines from April 22 to August 27 of 2012 and 2013. Single buds were placed directly on each thermoelectric module (TEM) of Kryoscan, a device used to perform DTA (Badelescu and Ernst 2006), and 16 buds were analyzed for each collection date. Exotherms were identified using a plot of the temperature output (x-axis) versus the TEM output (mV) with Sigma Plot 10 software.

Starch Determinations

The starch content of buds was determined after ethanol extraction of the soluble sugars by acid extraction using the anthrone reagent (Hansen and Moller 1975)

RNA Purification and cDNA Synthesis

Total RNA was isolated and purified from grapevine buds (0.5–0.7 g^{-1} fr.wt) using a modified method of Chang et al. (1993), as described in Noriega et al. (2007). DNA was removed by treatment with RNAase-free DNAase (1 U/µg) (Invitrogen, CA, USA) at 37 °C for 30 min. First-strand cDNA was synthesized from 5 µg of purified RNA with 1 µL oligo$(dT)_{12–18}$ (0.5 µg × μL^{-1}) as primer, 1 µL dNTP mix (10 mM), and Superscript ® II RT (Invitrogen, USA).

Real-time Quantitative PCR

Real-time quantitative PCR (RT-qPCR) was performed using an Eco Real-Time PCR system (Illumina, Inc. SD, USA) using KAPA SYBR Fast Master mix (KK4602) and KAPA Taq DNA Polymerase (Kapa Biosystems, USA). Primers suitable for the amplification of 100–150-bp products for each gene under study were designed using the PRIMER3 software (Rozen and Skaletsky, 2000) (Table 10.1). The amplification of cDNA was performed under the following conditions: denaturation at 94 °C for 2 min and 40 cycles at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s. Two biological replicates with three technical repetitions were performed for

Table 10.1 Primers used for real-time quantitative RT-qPCR experiments

VvNCDE1	GSVIVTO1038080001	5′ TTTGTGCAC-GACGAGAAGAC 3′	5′ AGGGAACTCGT-GAGGGAAGT 3′
Vvα-AMY1	GSVIVT01031746001	5′ ACTCTGCAAC-ACTGGCCTTT 3′	5′ CCTCTTTCAGAC-CCCACTCA 3′
Vvα-AMY2	GSVIVT01020069001	5′ TGAAGC-GAACTGAAGTG-GTG 3′	5′ AGAAACACCCCAATG-CAGAA 3′
Vvα-AMY3	GSVIVT01032922001	5′ GCCATTTC-CACGAGATA-AGC 3′	5′ CGGAGGCCAAAAT-CATAGAA 3′
Vvα-AMY4	GSVIVT01008714001	5′ TGGCACAG-GACAACTTTCAG 3′	5′ TGAATGTGACAGCCCTT-GAA 3′

each treatment. The induction or repression of transcription was calculated by the ΔΔCq method (Livak and Schmittgen, 2001) using *VvUBIQUITIN* as the reference gene. *VvUBIQUITIN* was selected as a reference gene because the transcript level was stable across the treatments.

Results and Discussion

The Development of CH and ED are Sequential Events in Grapevine Buds

In most perennial plants from temperate regions, ED and CH are overlapping processes (Rohde and Bhalerao 2007). In *V. vinifera*, ED and CH are sequential events; the buds become endodormant in the late summer (Kühn et al. 2009) and develop CH in the late autumn (Fig. 10.1a). Moreover, these two processes are triggered by different environmental cues. The short-day (SD) photoperiod of late summer induces ED (Kühn et al. 2009; Pérez et al. 2009; Grant et al. 2013), whereas low temperatures in autumn induce CH. Salzmann et al. (1996) demonstrated that SD photoperiod at warm temperatures results in the development of ED but not CH in grapevine buds. Such data indicate that decreasing temperatures are necessary to induce CH. Although ED precedes CH in grapevine buds, it is not known whether the entrance of buds into ED is required for the further development of CH. A superimposition of dormancy and LTE curves (Fig. 10.1a) revealed that LTE values began to decrease in late April; at this time, buds were fully endodormant and daily mean temperatures started to drop below 14 °C (Fig. 10.1b). However, these results do not indicate whether a relationship between CH and ED exists, since a decrease in temperatures could randomly coincide with ED.

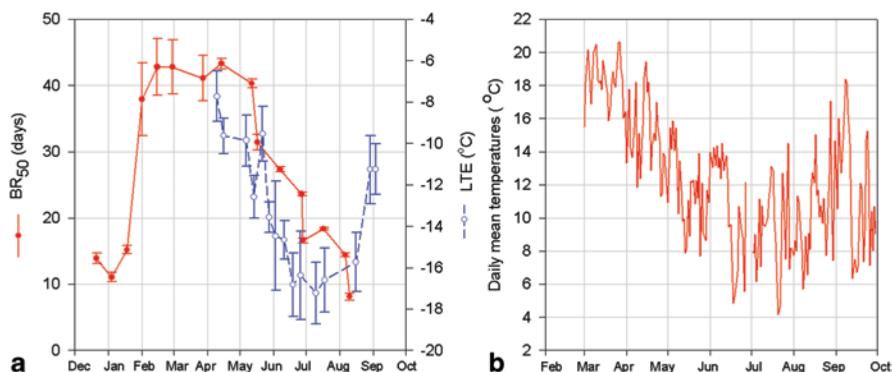


Fig. 10.1 Comparison of daily mean temperature, endodormancy, and cold hardiness in grapevine buds. **a** The development of endodormancy (ED) and cold hardiness (CH) in grapevine buds during the year 2013 in Santiago, Chile. **b** Daily mean temperatures in Santiago, Chile, during the year 2013. The depth of ED was determined as described previously (Pérez et al. 2007). CH was determined by measuring the low-temperature exotherm (LTE) using Peltier modules

To analyze the effects of temperatures on dormant and non-dormant buds, single-bud cuttings were collected on December 16 (non-dormant) and on June 11 (dormant). Cuttings were exposed to low (5 °C) and ambient (14 °C) temperatures, and exotherms were measured over time. In dormant buds, two exotherms were detected. The LTE responded to temperature, decreasing at low temperatures and increasing at ambient temperature (Fig. 10.2a). In non-dormant buds, only one broad large peak was detected that indicated the overlap of HTE and LTE. This single peak was observed at −7 ± 1 °C and did not vary with temperatures (Fig. 10.2b). The results shown in Fig. 10.2a indicate that dormant buds can be cold-acclimated or deacclimated depending on whether they were exposed to low or ambient temperatures. Conversely, non-dormant buds were not cold-acclimated when they were exposed to low temperatures (Fig. 10.2b). Interestingly, the ectopic expression of genes that impair the entrance of buds into ED, such as *PHYTOCHROME A* in hybrid aspen (Olsen et al. 1997) and *FLOWERING LOCUS T* in apples (Tränker et al. 2010) and in plum (Srinivasan et al. 2012), prevents cold acclimation. This suggests that the entrance of buds into ED is necessary for the further development of CH.

Low Temperatures Trigger ED Depth in Grapevines, but not ED Release

Single-bud cuttings were collected on April 26 (fully ED) and on June 26 (ED release). Cuttings chilled (5 °C) for 6 weeks had a delayed bud-break response under forced conditions (23 ± 1 °C, 16-h light) compared to non-cooled (14 °C) buds (Fig. 10.3). It is generally believed that low temperatures favor the release of grapevine buds from ED (Dokoozlian et al. 1995; Mathiason et al. 2009; Ben-Mohamed et al. 2010).

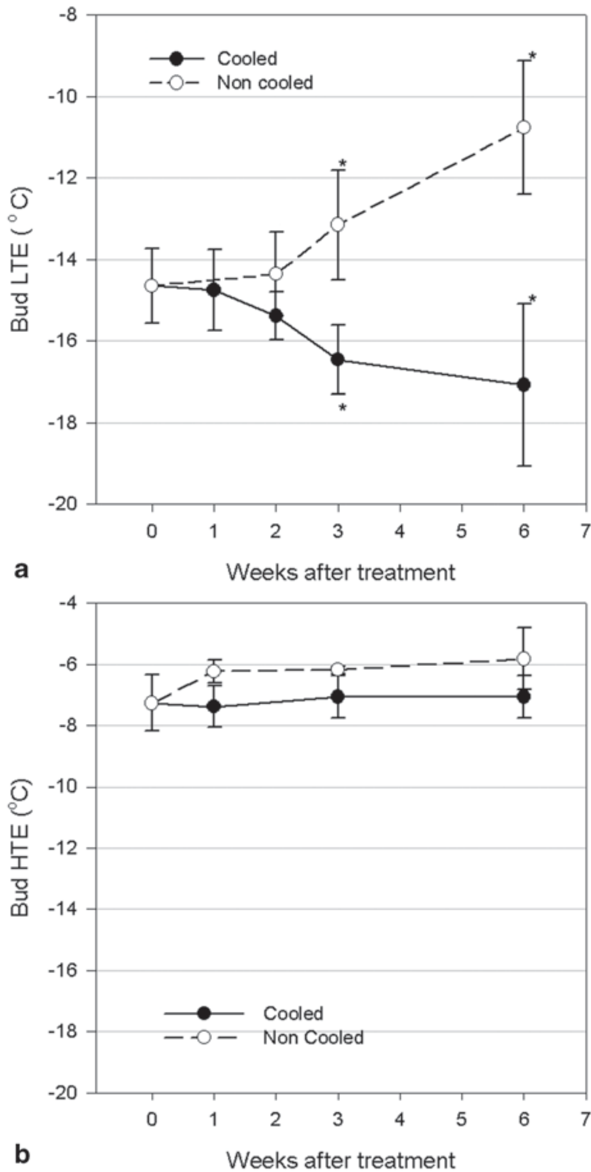


Fig. 10.2 **a** Cold acclimation and deacclimation of dormant grapevine buds exposed to low (5 °C for 6 weeks) and ambient temperatures (14 °C for 6 weeks). Canes showing partial cold acclimation were collected on June 11 after being exposed to 200 chilling hours (CH) in the field. Cold acclimation was determined by measuring weekly the low-temperature exotherm (LTE) in single buds. Values correspond to the average of 12 single buds, *bars* represent standard deviation, and (*) indicates significant differences at $p < 0.05$. **b** Low (5 °C for 6 weeks) and ambient (14 °C for 6 weeks) temperature effects on the high-temperature exotherm (HTE) in non-dormant buds. Canes were collected on December 16 at the paradormancy (PD) stage. Values correspond to the average of 12 single buds, and *bars* represent standard deviation

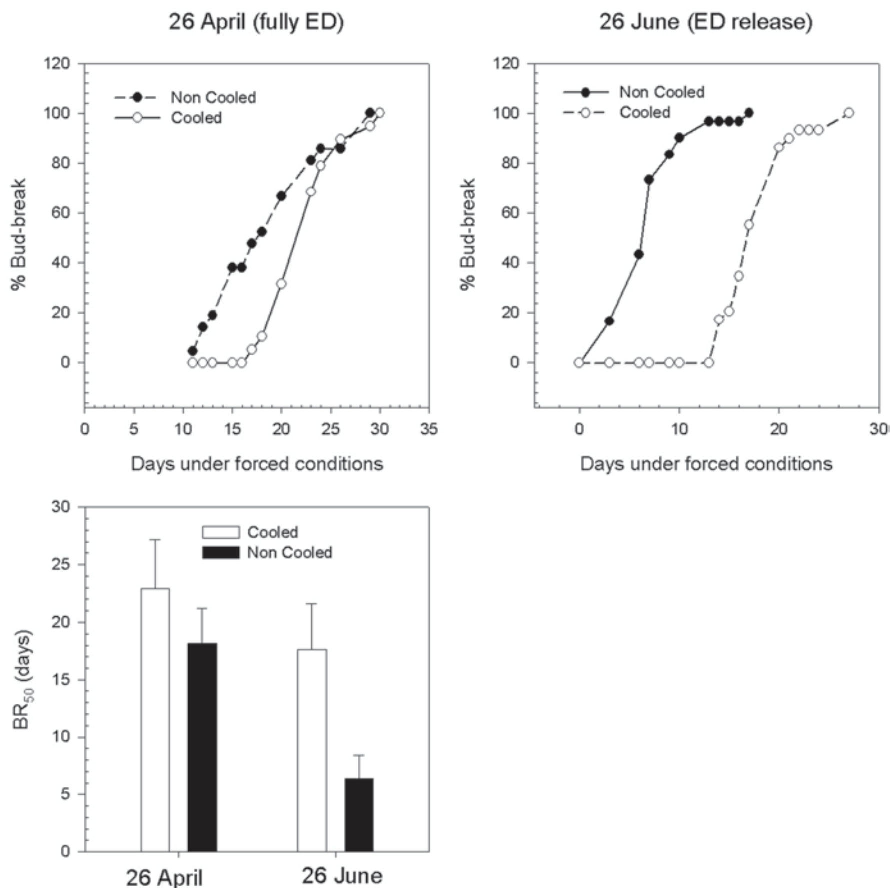


Fig. 10.3 Low temperatures increased the depth of endodormancy (ED) in single-bud cuttings of grapevines. Cooled (5 °C for 6 weeks) and non-cooled (14 °C for 6 weeks) single-bud cuttings were assayed for bud break under forced conditions (23 ± 1 °C, 16-h light), and the percentage of bud break and the BR_{50} were determined. Analyses were carried out with buds collected on April 26 (fully endodormancy) and on June 26 (ED release). BR_{50} values and standard error were calculated by probit analysis (Minitab statistical software)

Our results indicate the opposite that depth of ED rather than release of ED is increased by low temperatures. In previous studies, the lack of non-cooled samples as controls prevented drawing conclusions regarding the effect of time on bud-break response. In our study, the use of non-cooled samples as controls and their comparison with cooled samples eliminated time as a variable. Similar results were previously obtained by studying dormancy curves in two regions of Chile with different amounts of cold accumulation during the winter season (Pérez et al. 2007). In the above study, increased BR_{50} values were obtained in the region of Santiago, which accumulates 60 chilling portions (CP) during the winter season compared to

the region of Coquimbo, which accumulates only 20 CP (Pérez et al. 2008) and has decreased BR_{50} values. These results indicate that an increase in cold accumulation is correlated with deeper ED. The SD photoperiod is the environmental factor that induces ED in grapevine buds (Kühn et al. 2009; Grant et al. 2013), and low temperatures synergize its effect. As a deeper ED is achieved, the bud-break response becomes more homogeneous. Therefore, in grapevines, CR fulfillment may be correlated with a certain degree of ED depth.

CH in Grapevine Buds is a Reversible Phenomenon Dependent on Temperature and Stage of Dormancy

As mentioned above, the acquisition of CH in grapevine buds is triggered by low temperatures. However, CH develops only in buds that have reached the ED stage. Therefore, induction of grapevine bud ED by SD photoperiod is crucial for the further development of CH by low temperatures. In the absence of low temperatures, dormant buds did not develop CH; this has been demonstrated by Salzman et al. (1996). In contrast with grapevines, the development of CH (temperatures of −20 °C) in juvenile trees such as birch is first initiated by exposure to a SD photoperiod. A second and deeper level of CH (freezing resistances down to −70 °C) requires cold exposure (Rinne et al. 2001; Welling et al. 2002). In tropical regions, where the photoperiod remains constant over the year, the vine remains active and does not enter into recess or ED (Possingham 1992). The sprouting of buds under such conditions is poor, and irregular and chemicals are often required to overcome these limitations (Possingham 1994). Thus, under tropical conditions, buds do not achieve ED or cold-acclimate and the lack of development in one or both of these processes could account for the poor and irregular sprouting.

Low Temperatures Stimulate Starch Breakdown and Activation of **Vvα-AMYs** *in Grapevine Buds*

Starch accumulates in grapevine buds during the ED period. In Santiago, Chile buds enter into ED at the end of January (Kühn et al. 2009); at this time, the starch level was 20.8 ± 2.6 mg × g^{-1} fr.wt. On April 26 (fully ED), the starch level increased to 30.5 ± 3 mg × g^{-1} fr.wt and continued to increase until the onset of low temperatures. A concentration of 60 ± 5 mg × g^{-1} fr.wt. was reached in June. In buds collected on April 26 (fully ED) and exposed to low temperatures, the starch content was reduced significantly compared to non-cooled buds (Fig. 10.4). After 3 weeks of cold exposure, approximately 18.6 mg × g^{-1} fr.wt of starch was consumed in cooled buds, whereas only 4.12 mg × g^{-1} fr.wt was consumed in non-cooled buds. In buds collected on June 26 (ED release), the content of starch was also reduced by low temperatures. After 3 weeks of cold exposure, 23.7 mg × g^{-1} fr.wt of starch was con-

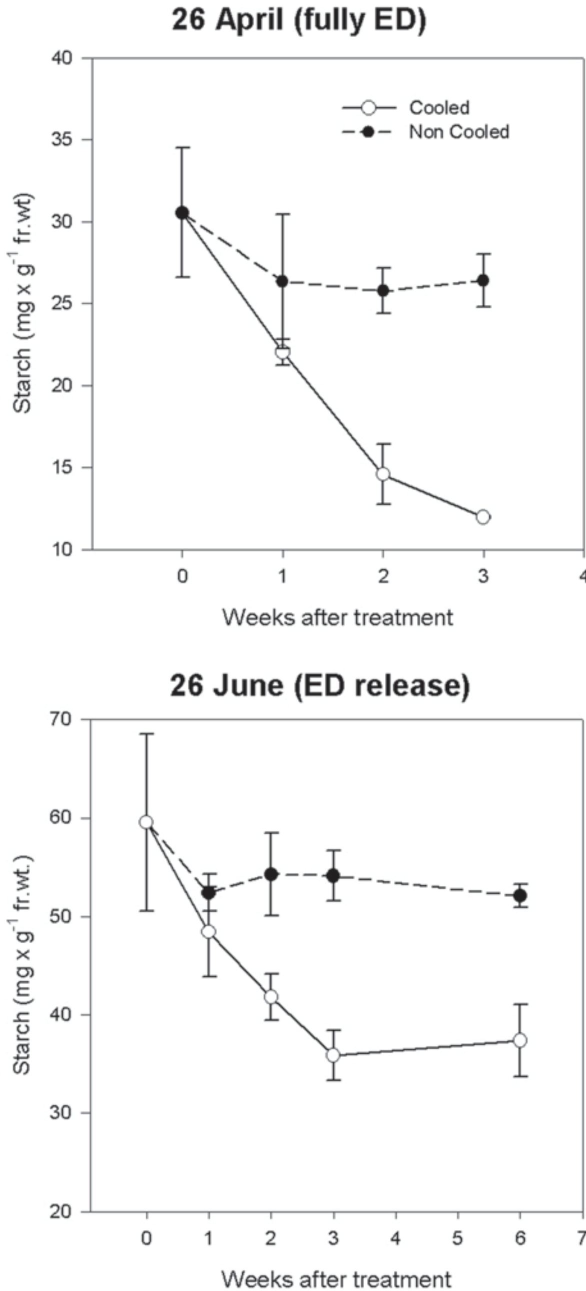


Fig. 10.4 Low temperatures induced starch hydrolysis in grapevine buds. Buds were collected on April 26 (fully endodormant) and exposed to low (5 °C for 3 weeks) and ambient temperatures (14 °C for 3 weeks). Buds collected on June 26 (ED release) were exposed to low (5 °C for 6 weeks) and ambient temperatures (14 °C for 6 weeks). Values are the average of three replicates, and *bars* correspond to standard deviations

sumed in cooled buds, whereas in non-cooled buds, only 5.4 mg × g^{-1} fr.wt of starch was consumed (Fig. 10.4).

An increase in starch-degrading enzymes with a subsequent accumulation of soluble sugars was found in *Arabidopsis* during a period of cold acclimation (Maruyama et al. 2009). In potato tubers, it was reported that cold-induced sweetening (CIS) is fueled by starch-derived hexoses; gene expression and the enzymatic activity of these starch-degrading enzymes are induced by low temperatures (Bagnaresi et al. 2008). Likewise, in crown buds of leafy spurge, an herbaceous perennial, an inverse relationship developed between starch and soluble sugar content during the autumn-induced shift from para- to ED and soluble sugar continued to increase through the cold-induced transition to ecodormancy (Anderson et al. 2005). In grapevine buds collected at the fully ED stage (April 26) and the ED-release stage (June26), the expression of *Vvα-AMY* genes was up-regulated after prolonged exposure to low temperatures (Figs. 10.5, 10.6). Of the four *Vvα-AMY* genes that are expressed in grapevine buds, *Vvα-AMY2* was the most up-regulated by low temperatures (Figs. 10.5, 10.6).

Interestingly, it has been reported that in *Vitis amurensis*, *Vvα-AMY2* is strongly up-regulated after 8 h of cold exposure. In *V. vinifera* cv Muscat of Hamburg, this gene is not up-regulated. *Vitis amurensis* is a wild grapevine species with remarkable cold tolerance, exceeding that of *V. vinifera* (Zhang et al. 2013; Xin et al. 2013). After such a short period of cold exposure, it seems reasonable that cold-resistant genes such as *Vvα-AMY2* express earlier and at greater levels in vines most resistant to cold (Xin et al. 2013). The plant hormone gibberellic acid (GA_3), which is a common inducer of amylase genes in cereals (Bethke et al. 1997), did not induce *Vvα-AMY1* and *Vvα-AMY2* (Rubio et al. 2014), which were the most highly induced by low temperatures (Figs. 10.5, 10.6). Because the dormancy-breaking compound hydrogen cyanamide (H_2CN_2) induced the expression of *Vvα-AMY1* and *Vvα-AMY2* (Rubio et al. 2014), these two genes could be involved in the bud-break response in grapevines. Based on these results, it can be concluded that the induction of *Vvα-AMY* genes by cold winter temperatures is an important factor in the degradation of starch and the subsequent accumulation of sugars. Sugar accumulation prior to bud break can be of great importance for sprouting, as sprouting is a process with a large energy requirement. In areas that lack cold winters, a negative effect on bud sprouting could be due to a lower accumulation of sugars in the bud prior to bud break.

ABA Mediates the Effect of Low Temperatures on Increasing CH and ED Depth

The phytohormone abscisic acid (ABA) is considered a central regulator of abiotic stress resistance in plants. ABA coordinates a complex regulatory network enabling plants to cope with decreases in water availability (Cutler et al. 2010; Kim et al. 2010). ABA content increases significantly in plants under drought or salinity stress conditions. Such an increase stimulates stomatal closure, changes in gene

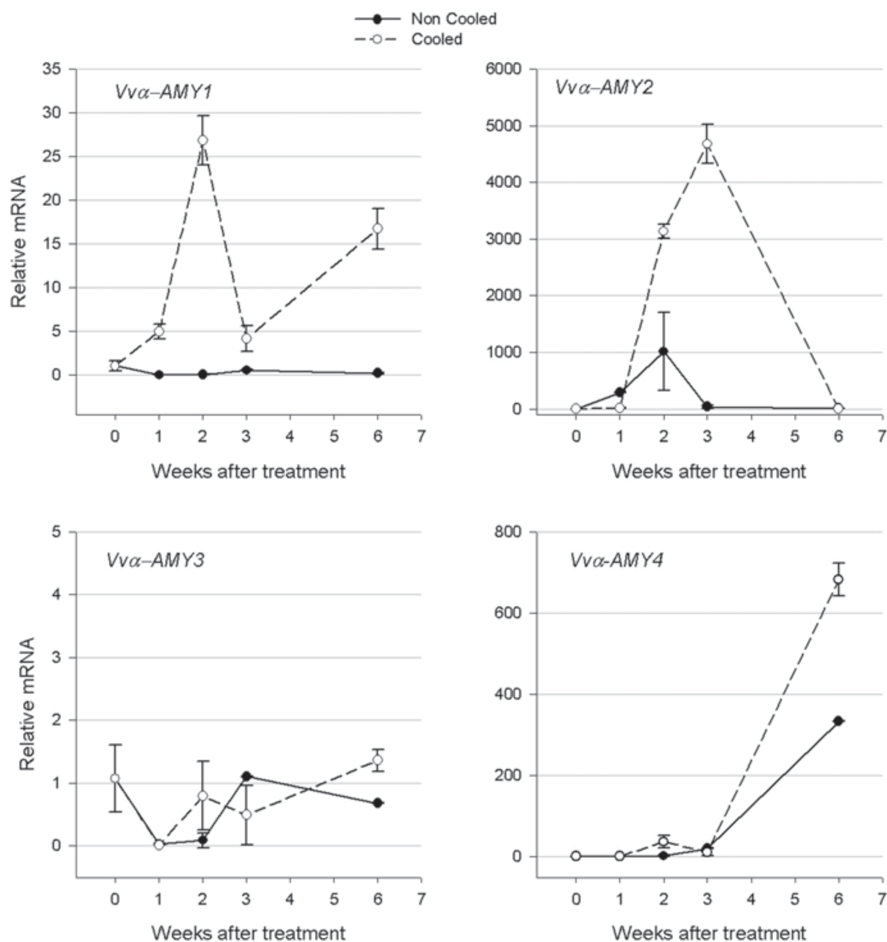


Fig. 10.5 The expression of *Vvα-AMY1* and *Vvα-AMY2* was up-regulated after the prolonged exposure of grapevine buds to low temperatures. Buds were collected on April 26 (fully endodormant) and exposed to low (5 °C for 6 weeks) and ambient (14 °C for 6 weeks) temperatures. Gene expression analysis was performed by RT-qPCR using *VvUBIQUITIN* as a reference gene. Values are the average of three biological replicates, and *bars* correspond to standard deviations

expression, and the accumulation of osmo-compatible solutes, thus improving the plant's ability to cope with stress conditions (Seki et al. 2007; Cutler et al. 2010; Kim et al. 2010). ABA also plays important roles during plant development, as it is involved in embryo and seed development, the promotion of seed dormancy (Finkelstein et al. 2008), and the acquisition of CH. In *V. vinifera* cv Cabernet franc grapevines, the exogenous applications of ABA at different developmental stages corresponding to *veraison* (the onset of ripening) and 20, 30, 40, and 55 days

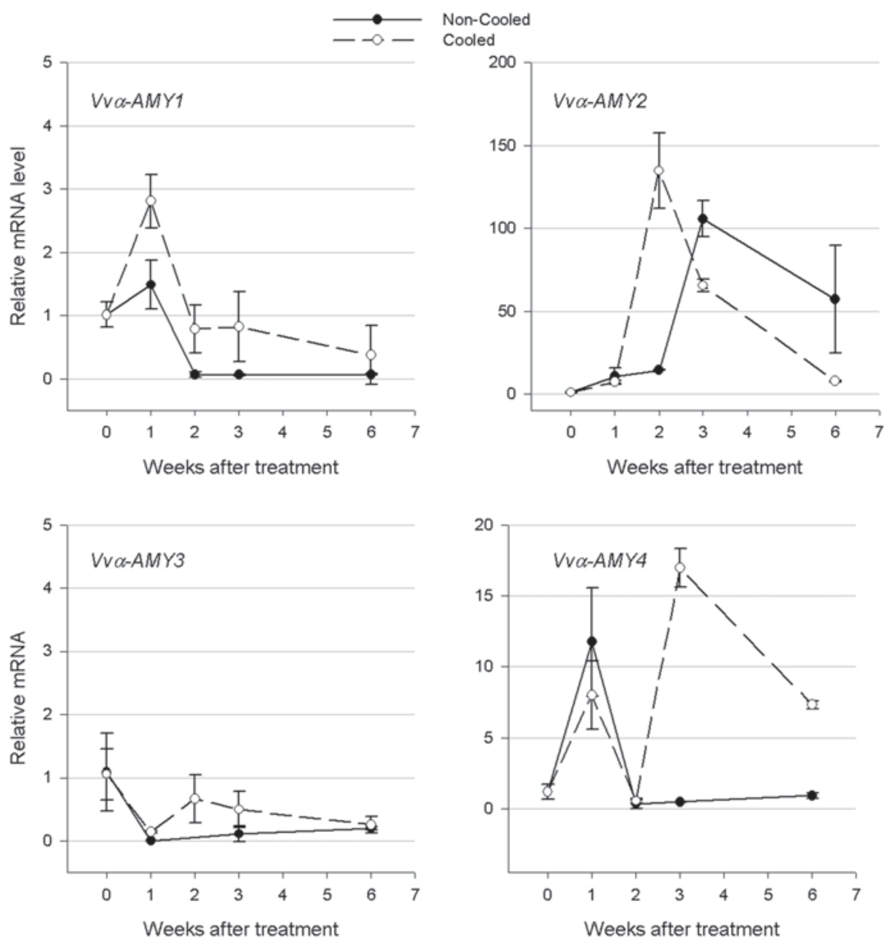


Fig. 10.6 The expression of *Vvα-AMY1*, *Vvα-AMY2*, and *Vvα-AMY4* was up-regulated after the prolonged exposure of grapevine buds to low temperatures. Buds were collected on June 26 (endodormant release) and exposed to low (5 °C for 6 weeks) and ambient (14 °C for 6 weeks) temperatures. Gene expression analysis was performed by RT-qPCR using *VvUBIQUITIN* as a reference gene. Values are the average of three biological replicates, and *bars* correspond to standard deviations

post-veraison and at concentrations ranging between 1.5 and 2.5 mM enhance dormancy and increase CH (Zhang and Dami 2012). In this study, the applications of 0.1 mM ABA to single-bud cuttings of *V. vinifera* cv Thompson seedless collected on April 22 (fully ED) increased CH compared to non-treated cuttings; significant differences were observed after 3 weeks of cold treatment (Fig. 10.7a). The gene *VvNCED1*, which encodes for a key enzyme in the ABA biosynthetic pathway, was also up-regulated by 3 weeks of cold exposure (Fig. 10.7b). This result suggests that

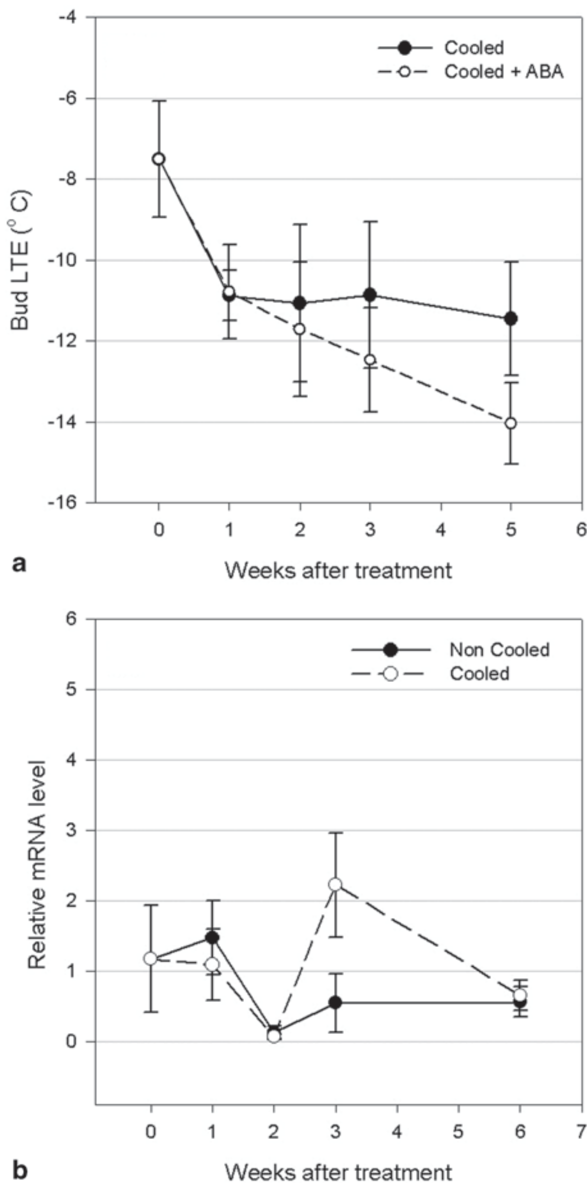


Fig. 10.7 Abscisic acid (ABA) synergizes the effect of low temperatures to increase cold hardiness (CH). **a** Single-bud cuttings collected on April 22 (fully endodormant) were separated in two groups: one group was treated with 0.1 mM ABA, and the other group was treated with water as a control. Both groups were placed in the refrigerator for 5 weeks, and ten samples were retired weekly for LTE measurements. Values are the average of ten buds, and *bars* correspond to standard deviations. **b** Gene expression analysis of *VvNCED1* in grapevine buds exposed to low (5 °C for 6 weeks) and room (14 °C for 6 weeks) temperatures. Analysis was performed by RT-qPCR using *VvUBIQUITIN* as a reference gene in buds collected on June 26 and exposed to low (5 °C for 6 weeks) and ambient (14 °C for 6 weeks) temperatures. Values are the average of three biological replicates, and *bars* correspond to standard deviations

the observed increases in CH and ED depth, as a result of low temperatures, could involve ABA biosynthesis and signaling mechanisms.

Conclusions

In conclusion, we report that ED and CH are dynamic physiological states in sequentially passing grapevine buds. Both states are induced by different environmental signals; ED is induced by SD photoperiod, while CH is induced by low temperatures. Only dormant buds develop CH, indicating that buds need to reach a state of ED before low temperatures can induce CH. Low temperatures increased ED depth, CH, and starch breakdown in grapevine buds, suggesting that the stimulation of any of these factors, or all three, would be necessary for buds to sprout evenly in the spring. Finally, we conclude that the effect of low temperatures on homogeneous bud sprouting could involve ABA biosynthesis and signaling.

Acknowledgements Financial support for Fondecyt project 1140318 is gratefully acknowledged.

References

Anderson JV, Gesch RW, Jia Y, Chao WS, Horvath D (2005) Seasonal shifts in dormancy status, carbohydrate metabolism, and related gene expression in crown buds of leafy spurge. Plant Cell Environ 28:1567–1578

Arora R, Rowland LJ, Tanino K (2003) Induction and release of bud dormancy in woody perennials: a science comes of age. HortScience 38:911–921

Badelescu R, Ernst M (2006) Changes of temperature exotherms and soluble sugars in grapevines (Vitis vinifera L) buds during winter. J Appl Bot Food Qual 80:165–170

Bagnaresi P, Moschella A, Beretta O, Vittuli F, Ranalli P, Perata P (2008) Heterologous microarray experiments allow identification of the early events associated with potato tuber cold sweetening. BMC Genomics 9:176–119

Ben Mohamed H, Vadel AM, Geuns JMC, Khemira H (2010) Biochemical changes in dormant grapevines shoot tissues in response to chilling: possible role in dormancy release. Sci Hortic 124:440–447

Bethke PC, Schuurink R, Jones RL (1997) Hormonal signalling in cereal aleurone. J Exp Bot 48:1337–1356

Burke MJ, Gusta LV, Quamme HA, Weiser CJ, Li PH (1976) Freezing and injury in plants. Annu Rev Plant Physiol 27:507–528

Chang S, Puryear J, Cairney, JA (1993) Simple and efficient method for isolating RNA from pine trees. Plant Mol Biol Rep 11:113–116

Cutler SR, Rodriguez PL, Finkelstein RR, Abrams SR (2010) Abscisic acid: emergence of a core signalling network. Annu Rev Plant Biol 61:651–679

Dokoozlian NK, Williams LE, Neja RA (1995) Chilling exposure and hydrogen cyanamide interact in breaking dormancy of grape buds. HortScience 30:1244–1247

Erez A (1995) Means to compensate for insufficient chilling to improve bloom and leafing. Acta Hortic 395:81–95

Fan S, Bielenger DG, Zhebentyayeva T, Reighard GL, Okie WR, Holland D, Abbott AG (2010) Mapping quantitative trait loci associated with chilling requirements, heat requirement and bloom date in peach (Prunus persica). New Phytologist 185:917–930

Ferguson JC, Tarara JM, Mills LJ, Grove GG, Keller M (2011) Dynamic thermal time model of cold hardiness for dormant grapevine buds. Ann Bot 107:389–396

Finkelstein R, Reeves W, Ariizumi T, Steber C (2008) Molecular aspects of seed dormancy. Annu Rev Plant Biol 59:387–415

Grant TNL, Gargrave J, Dami IE (2013) Morphological physiological, and biochemical changes in Vitis genotype in response to photoperiod regimes. Am J Enol Vitic 64:466–475

Gusta LV, Wisniewski M (2013) Understanding plant cold hardiness: an opinion. Physiol Plantarum 147:4–14

Hansen J, Moller IB (1975) Percolation of starch and soluble carbohydrates from plant tissue for quantitative determination with anthrone. Anal Biochem 68:87–94

Kim TH, Böhmer M, Hu H, Nishimura N, Schoroeder JI (2010) Guard cell signal transduction network: advances in understanding abscisic acid, CO2 and Ca2+ signalling. Annu Rev Plant Biol 61:56–91

Kühn N, Ormeño J, Jaque-Zamora G, Pérez FJ (2009) Photoperiod modifies the diurnal expression profile of *VvPHYA* and *VvPHYB* transcript in field-grown grapevines. J Plant Physiol 166:1172–1180

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real time quantitative PCR and the ΔΔCT method. Methods 25:402–408

Maruyama K, Takeda M, Kidokoro S, Yamada K (2009) Metabolic pathways involved in cold acclimation identified by integrated analysis and transcript regulated by DREB1A and DREB2A. Plant Physiol 150:1972–1980

Mathiason, K, Dong HE, Grimplet J, Venkateswari J, Galbraith DW, Or E, Fennell A (2009) Transcript profiling in Vitis riparia during chilling requirements fulfillmenmt reveals coordination of gene expression patterns with optimized bud-break. Funct Integr Genomics 9:81–96

Mills LJ, Ferguson JC, Keller M (2006) Cold-hardiness evaluation of grapevine buds and cane tissues. Am J Enol Vitic 57:194–200

Noriega X, Burgos B, Pérez FJ (2007) Short-day photoperiod triggers and low temperature increase expression of peroxidase RNA transcripts and basic peroxidase isoenzyme activity in grape buds. Phytochemistry 68:1376–1383

Olsen JE, Junttila O, Nilsen J, Eriksson M, Martinussen I, Olsson O, Sandberg G, Moritz T (1997) Ectopic expression of phytochrome A in hybrid aspen changes critical day-length for growth and prevents cold acclimation. Plant J. 12:1339–1350

Pérez FJ, Rubio S, Ormeño-Nuñez J (2007) Is erratic bud-break in grapevines grown in warm winter areas related to disturbances in mitochondrial respiratory capacity and oxidative metabolism? Funct Plant Biol 34:624–632

Pérez FJ, Ormeño J, Reynaert B, Rubio S (2008) Use of the dynamic model for the assessment of winter chilling in a temperate and a subtropical climatic zone of Chile. Chil J Agric Res 68:198–206

Pérez FJ, Kühn N, Ormeño-Nuñez J, Rubio S (2009) The expression of VvPHYA and VvPHYB transcripts is differently regulated by photoperiod in leaves and buds of grapevines. Plant Signal Behav 4:7–13

Pierquet P, Stushnoff C (1980) Relationship of low temperature exotherms to cold injury in *Vitis riparia* Michx. Am J Enol Vitic 31:1–6

Possingham JV (1992) Viticulture in Australia. Proc Intl Symp on Recent Advances in Vitic and Enol. Hyderabad, India

Possingham JV (1994) Production of table grapes in India. Proc Intl Symp on table grapes, Anaheim Calif USA, Pub Am Soc Enol Vit Davies Calif, 1994

Rinne PLH, Kaikuranta PM, van der Schoot (2001) The shoot apical meristem restores its symplastic organization during chilling induced release from dormancy. The Plant J 26:249–264

Rohde A, Bhalerao RP (2007) Plant dormancy in the perennial context. Trends Plant Sci 12:217–223

Rozen S, Skaletsky H (2000) Primer3 on the www for general users and for biologist programmers. Methods Mol Biol 132:365–386

Rubio S, Donoso A, Pérez FJ (2014) The dormancy-breaking stimuli "chilling, hypoxia and cyanamide exposure" up-regulate the expression of α-amylase genes in grapevine buds. J Plant Physiol 171:373–381

Sakai A, Larcher W (1987) Frost survival of plants; response and adaptations to freezing stress. Springer, Berlin

Salzman R, Bressan RA, Hasegawa PM, Ashworth EN, Bordelon BP (1996) Programmed accumulation of LEA-like proteins during dessication and cold acclimation of overwintering grape buds. Plant Cell Environ 19:713–720

Seki M, Umezawa T, Urano K, Shinozaki K (2007) Regulatory metabolic networks in drought stress responses. Curr Opin Plant Biol 10:296–302

Sherman WB, Beckman TG (2003) Climatic adaptations in fruit crops. Acta Hortic 622:411–428

Srinivasan C, Dardick C, Callahan A, Scorza R (2012) Plum (Prunus domestica) trees transformed with poplar FT1 result in altered architecture, dormancy requirement and continuous flowering. PLoS ONE 7:e40715, 1–11

Topp BL, Sherman WB, Raseira MCB (2008) Low-chill cultivar development. In: Layne DR, Bassi D (eds) The peach botany, production and uses. CABI, Wallingford, pp 106–138

Tränker C, Lehmann S, Hoenicka H, Hanke M, Fladung M, Lenhardt D, Dunemann F, Gau A, Schlangen K, Malnoy M, Flachowsky H (2010) Over-expression of and FT homologous gene of apple induces early flowering in annual and perennial plants. Planta 232:1309–1324

Welling A, Moritz T, Palva ET, Junttila O (2002) Independent activation of cold acclimation by low temperature and short photoperiod in hybrid aspen. Plant Physiol 129:1633–1641

Xin H, Zhu W, Wang L, Xiang Y, Fang L, Li J, Sun X, Wang N, Londo JP, Li S (2013) Genome wide transcriptional profile analysis of *Vitis amurensis* and *Vitis vinifera* in response to cold stress. PLoS ONE 8(3):e57740

Zhang Y, Dami IE (2012) Foliar application of abscisic acid increases freezing tolerance of field-grown Vitis vinifera Cabernet franc grapevines. Am J Enol Vitic 63:377–384

Zhang J, Liu N, Niu R,Liu Y, Zhai H, Xu W, Wang Y (2013) Construction of a cDNA library of the Chinesewild Vitis amurensis under cold stress and analysis of potential hardiness related expressed sequence tags. Gen.Mol. Res. 12 (2) 182–193

Part III
Dormancy Mechanisms in Buds of Underground Structures

Chapter 11
Bridging Dormancy Release and Apical Dominance in Potato Tuber

Dani Eshel

List of Abbreviations Used

AD	Apical dominance
BE	Bromoethane
CIS	Cold-induced sweetening
PCD	Programmed cell death
TAM	Tuber apical meristem

Introduction

The potato (*Solanum tuberosum* L.) tuber is a swollen underground stem formed by swelling of subapical underground stolons (Harris 1992). As the tuber elongates, a growing number of lateral bud meristems (termed eyes) are formed in a spiral arrangement on its surface (Goodwin 1967). After harvest, tuber buds are generally dormant and will not sprout or grow, even if the tubers are placed under optimal conditions for sprouting (i.e., warm temperature, darkness, high humidity). The dormancy observed in postharvest potato tubers is defined as endodormancy (Lang et al. 1987) and is due to an unknown endogenous signal(s) that mediates suppression of meristem growth (Suttle 2004b). Dormancy is thought to be a physiological adaptation to intermittent periods of environmental limitations and is therefore a survival mechanism that prevents sprouting when tubers would be exposed to, for example, extreme temperatures (Suttle 2007). The duration of endodormancy is primarily dependent on the genotype, but other factors, such as environmental conditions during the crop growth and storage conditions after tuber harvest, are also important (Turnbull and Hanke 1985; Wiltshire and Cobb 1996). Following a

D. Eshel (✉)
Department of Postharvest Science of Fresh Produce, The Volcani Center, ARO,
Bet-Dagan, Israel
e-mail: dani@agri.gov.il

© Springer International Publishing Switzerland 2015

J. V. Anderson (ed.), *Advances in Plant Dormancy,* DOI 10.1007/978-3-319-14451-1_11

transition period of between 1 and 15 weeks depending on storage conditions and genotype, dormancy is broken and apical buds start to grow (Wiltshire and Cobb 1996). Typically, one eye/sprout becomes dominant and inhibits the growth of the other eyes that are ecodormant (meristem arrested by external environmental factors) (Suttle 2007). Tubers stored at optimal temperature (~ 14 °C) will sprout weeks before those stored in the cold, typically (but not always) with a single long bud. Tuber sprouting is usually initiated from its apical bud, located distal to the tuber–stolon junction. Although the potato tuber is used as a model system for the study of metabolic processes associated with dormancy progression, sprouting, and aging, very few studies have been done on the regulation of apical dominance (AD) during these processes (Michener 1942; Kumar and Knowles 1993; Holmes et al. 1970).

Hormonal Regulation

Hormonal control of tuber dormancy release and sprouting has been investigated in several studies but is still incompletely understood (reviewed by Sonnewald and Sonnewald 2014). The degree of each bud's autonomy in terms of timing of dormancy release and sprouting, and its interactions with other buds on the same tuber, is still unclear. Endogenous plant hormones and their relative balance within the tuber are suggested to regulate endodormancy progression, bud activation, and sprouting (Turnbull and Hanke 1985; Suttle 2004a; Sorce et al. 2009; Hartmann et al. 2011; Suttle 2009; Ji and Wang 1988).

In general, abscisic acid (ABA) and ethylene have been mainly linked to the onset and maintenance of tuber dormancy (Suttle and Hultstrand 1994; Suttle 2004b). Levels of ABA are highest in deeply dormant tubers and decline during storage (Destefano-Beltran et al. 2006b; Biemelt et al. 2000). Molecular analysis has indicated that the expression of genes associated with the catabolism of ABA correlates with dormancy release of bud meristems in potato tubers (Simko et al. 1997; Destefano-Beltran et al. 2006b; Ewing et al. 2004; Campbell et al. 2010). Nevertheless, continuous exposure to diniconazole (an inhibitor of ABA catabolism) had no effect on microtuber dormancy duration and exposure to 8'-acetylene ABA during microtuber development significantly increased the sprouting rate. This suggests that although a decrease in ABA content is a hallmark of tuber dormancy progression, the decline in ABA levels is not a prerequisite for dormancy release (Suttle et al. 2012).

Gibberellins (GAs) and cytokinins (CKs) have been associated with dormancy release and sprouting in postharvest potato tubers or their detached buds (Hartmann et al. 2011; Rentzsch et al. 2011; Suttle 2004a). By applying 6-benzylaminopurine (a synthetic CK), Hartmann et al. (2011) concluded that CK stimulates bud break and an additional dose of GA_3 is needed to induce further growth. GAs are inducers of bud activation and elongation after dormancy release, but their endogenous levels are probably not associated with maintenance of dormancy (Suttle 2004a; Hartmann et al. 2011). The endogenous contents of GA_{19}, GA_{20}, and GA_1 were relatively high immediately after harvest, declined during storage, and rose to their

highest levels during the period of robust sprout growth (Suttle 2004a). Interestingly, at the time of initial sprouting, internal levels of these bioactive GAs were lower than those found in deeply dormant tubers (Suttle 2007). Hartman et al. (2011) showed that transgenic potato plants with modified GA biosynthesis—expressing *Arabidopsis GA 20-oxidase* under the control of the chimeric STLS1/CaMV35 promoter—exhibit early tuber sprouting. These results showed that endogenous GA is able to terminate tuber dormancy and promote sprout outgrowth.

Biologically active cis- and trans-CKs increase over time in dormant potato tissues, suggesting a role for this class of hormones in bud activation (Suttle 2009; Hartmann et al. 2011). Expression of isopentenyltransferase from *Agrobacterium tumefaciens* in potato tubers to enhance endogenous CK levels, or cytokinin oxidase/dehydrogenase 1 (CKX) to reduce them, produced an earlier sprouting phenotype compared to the wild type or a prolonged dormancy period, respectively (Hartmann et al. 2011). This result supports an essential role for CKs in bud activation and shows that GA alone is not sufficient to break dormancy in the absence of CK.

Exogenous application of auxin, as indole-3 acetic acid (IAA) or 1-naphthaleneacetic acid, has a dual effect, with high doses inhibiting and low doses stimulating tuber bud growth (Suttle 2007; Hemberg 1985). There is evidence that the most abundant naturally occurring auxin, IAA, is at its highest level at the early stages of tuber dormancy and later decreases in the buds during storage (Sorce et al. 2000; Sorce et al. 2009; Sukhova et al. 1993). Feeding experiments have indicated that changes in IAA biosynthesis are a major cause of auxin variation in buds (Sorce et al. 2009). In dormant buds from freshly harvested tubers, the free IAA was found to accumulate mostly in the apical meristem, leaf, and lateral bud primordia, and in the differentiating vascular tissues underlying the apical meristem, whereas at the end of the storage period, only lateral bud primordia from growing buds displayed appreciable auxin levels (Sorce et al. 2009). Since AD is gradually lost during storage, auxin might be the link between bud activation and subsequent AD. Strigolactones have an inhibitory effect as well, with the synthetic strigolactone GR24 inhibiting the sprout-inducing activity of CK and GA in combined application (Pasare et al. 2013).

Tuber Sweetening and Sprouting

During tuber development, the storage parenchyma converts soluble assimilates (i.e., sucrose and amino acids) into polymeric reserves (starch and storage proteins, respectively Prat et al. 1990; Visser et al. 1994). At maturity, over 70 % of tuber carbohydrate is sequestered as starch, which must be converted into transport-compatible solutes for sprouting initiation and growth (Sonnewald 2001; Viola et al. 2007). Within the tuber, a rapid shift from storage metabolism (starch synthesis) to reserve mobilization accompanies sprouting and suggests a transition from sink to source. Sucrose synthesis appears as a dominant anabolic pathway in the storage parenchyma of dormant and sprouting tubers (Viola et al. 2007). Cold-induced

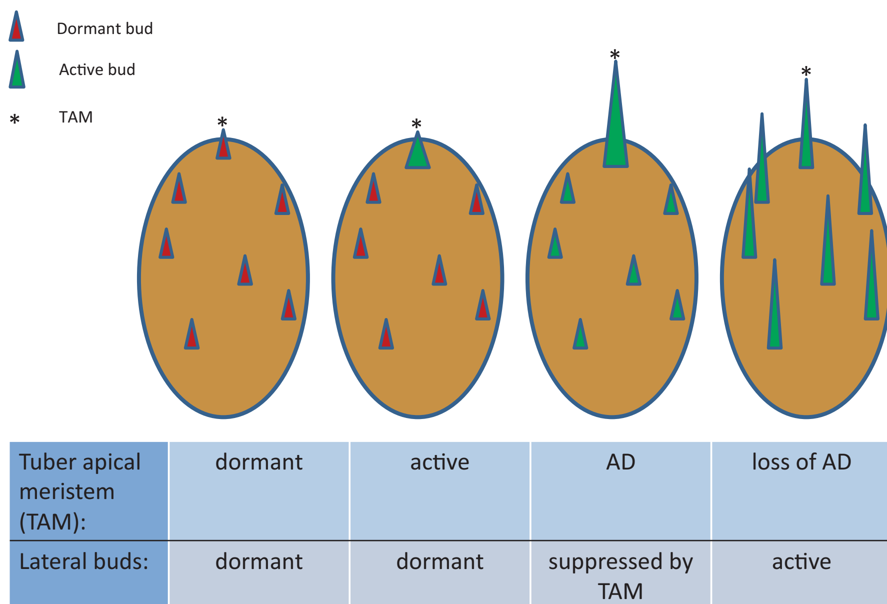
sweetening (CIS), a result of the accumulation of reducing sugars in cold-stored potato tubers, has been mainly studied for its relation to potato processing (Dale and Bradshaw 2003; Sowokinos 2001). Another form of reducing-sugar accumulation is sugar-end defect formation, which is typically associated with exposure of the plant to high temperatures during tuber initiation and bulking (Sowokinos et al. 2000; Kincaid et al. 1993). This form of reducing-sugar accumulation does not require low-temperature storage of tubers and cannot be removed by reconditioning at relatively warm temperatures, as can CIS (reviewed by Thompson et al. 2008). During CIS, sucrose synthesis increases, and some of it is transported to the vacuole where it is hydrolyzed to glucose and fructose (Isla et al. 1998; Sowokinos 2001; Isherwood 1973). In most common types of sugar-end defect, there is greater accumulation of reducing sugars at the tuber stem end than in the tuber apical bud complex, where the TAM is located (Iritani et al. 1973).

Viola et al. (2007) suggested that sprout growth is initially prevented by substrate limitation mediated via the symplastic connection in each bud. A recent additional discovery is that sugars, rather than auxin, are necessary and sufficient to regulate the earliest stages of bud outgrowth in pea plants following decapitation (Mason et al. 2014). The demand for sugars by the intact shoot tip was shown to override the effects of auxin depletion by preventing the initial outgrowth of axillary buds.

AD in Potato Tubers

AD in potato tubers results in control of the apical bud over lateral bud outgrowth. It is similar to the AD condition exerted by the shoot tip in many different plants (for review see Dun et al. 2006; Leyser 2009; Phillips 1975; Cline 1991). Cline (1997) suggested that AD and its release may be divided into four developmental stages: lateral bud formation (stage I), imposition of inhibition (AD; stage II), initiation of lateral bud outgrowth following decapitation (stage III), and subsequent elongation and development of lateral buds into branches (stage IV). He suggested that there is some overlap between the four stages and that the degree of inhibition imposed in stage II may vary among species (Cline 1997). In this review, a modified developmental process is suggested that starts with TAM activation following dormancy release, and subsequently, the TAM becomes dominant over lateral buds only after they are activated (Fig. 11.1).

Michener (1942) showed that when the intact potato tuber begins to grow after dormancy release, one or more apical buds grow, but the lateral buds usually do not. If, however, lateral buds and apical buds are excised, both start to grow at the same time. Moreover, in non-dormant tubers, any first-growing, large bud usually inhibits the growth of the later-growing, smaller ones (Michener 1942). Teper-Bamnolker et al. (2012) observed three main types of AD loss in stored potato: loss of dominance of the apical buds over those situated more basipetally on the tuber (“type I”), loss of dominance of the main bud in any given eye over the subtending axillary buds within the same eye (“type II”), and loss of dominance of the develop-



Tuber apical meristem (TAM):	dormant	active	AD	loss of AD
Lateral buds:	dormant	dormant	suppressed by TAM	active

Fig. 11.1 Schematic representation of dormancy release and loss of apical dominance (AD) as a result of potato tuber storage. Tuber apical meristem (TAM)

ing sprout apical meristem over more basal buds, meaning that side stems do not emerge from the base of the sprout as in type II ("type III").

Type I loss of dominance has been shown to exhibit classical stem-like behavior, but the developing apical bud suppresses only mature or dormancy-released buds. Removing the apical bud induces early sprouting of all other mature buds in the same tuber; after 30, 60, and 90 days in cold storage, an average of 1, 2, and 9 buds sprouted, respectively (Teper-Bamnolker et al. 2012), suggesting the need for each bud to reach maturity and autonomous dormancy release before it is controlled by the TAM (Fig. 11.1). Cline (1997) distinguished between initiation of axillary bud growth and subsequent axillary shoot elongation, which may be under the control of different hormone factors, as shown by Hartmann et al. (2011). Removal of a lateral meristem complex or wounding between buds did not impact AD or sprouting rate (Teper-Bamnolker et al. 2012). These experiments emphasize the importance of TAM presence and viability in the control of lateral bud meristem growth, before sprouting is observed.

AD and Tuber Physiological Age

The physiological age of the seed tuber is the physiological stage influencing its sprouting behavior (Struik 2007). The physiological status of a seed tuber at any given time is determined by genotype, chronological age, and environmental condi-

tions from tuber initiation until new plant emergence (reviewed by Caldiz 2009). Struik (2007) suggested that the summed temperature during storage is the predominant factor affecting physiological aging, although its effect is moderated by light conditions and genetic factors. The physiological age of seed tubers affects future crop performance, i.e., stem emergence rate, percentage of emergence, number of emerged stems per mother tuber, time to tuber initiation, crop vigor and growth, dry matter distribution, and tuber yield (O'Brien et al. 1983; Vakis 1986; Van Loon 1987; Moll 1994).

Sprouting behavior is one of the earliest morphophysiological indicators of a seed physiological age. Krijthe (1962) described four stages of sprouting shape in storage after dormancy is released: (i) AD where only one sprout develops, (ii) additional multiple buds sprouting as a result of reduced AD, (iii) branching of the sprouting stems, and (iv) in the aging mother tubers, sprout replacement by daughter tubers.

Sprouting Control and AD

Previous studies have shown that immediately after harvest, during their dormant period, potato tubers cannot be induced to sprout without some form of stress or exogenous hormone treatment (Struik and Wiersema 1999; Suttle 2009; Hartmann et al. 2011). On a large commercial scale, Rindite (a mixture of ethylene chlorohydrin, ethylene dichloride, and carbon tetrachloride) (Rehman et al. 2001), bromoethane (BE) (Coleman 1984), CS_2 (Meijers 1972; Salimi et al. 2010), and GA_3 (Rappaport et al. 1957) have been used to break seed tuber dormancy. Michener (1942) found that in dormant tubers treated with ethylene chlorohydrin, much of the auxin disappears. Auxin then reappears within 2 to 3 days after treatment termination. Michener (1942) also observed loss of AD after the chemical treatment and its restoration by application of IAA to the apex of the tubers. He concluded that auxin inhibits bud growth in the dormant tuber and that removal of the auxin by the action of ethylene chlorohydrin allows growth to proceed.

The phytotoxic chemical BE shortens the natural dormancy period from 2–4 months to approximately 10 days (Campbell et al. 2008; Alexopoulos et al. 2009; Destefano-Beltran et al. 2006a). Campbell et al. (2008) observed that transcript profiles during BE-induced cessation of dormancy are similar to those observed in natural dormancy release, suggesting that both follow a similar biological pattern during this transition. Thus, BE treatment can be used to compress and synchronize release from the dormant period, which is an advantage from an experimental standpoint (Campbell et al. 2008). Teper-Bamnolker et al. (2012) showed that BE application induces early sprouting in freshly harvested "Nicola" and "Désirée" tubers, as well as loss of AD. Buds surrounding the apical buds tended to grow faster than those located in more distant segments of the tuber. Loss of type I AD as a result of BE treatment was followed by loss of type III dominance, expressed as excessive branching of the growing shoots (Teper-Bamnolker et al. 2012). Teper-Bamnolker

et al. (2010) showed that very low doses of the sprout inhibitor R-carvone can also induce early sprouting and loss of AD. Whereas high doses of this inhibitor were shown to damage cell membranes in the apical meristem, no such damage was detected when the sprout-inducing low dose was used, suggesting a signaling effect (Teper-Bamnolker et al. 2010). At both R-carvone doses, the final result was loss of all types of AD when the tuber sprouted, leading to a bushlike pattern of growing buds.

The mode of action of phytotoxic chemicals in inducing dormancy release and altering apical bud dominance is poorly understood. Teper-Bamnolker et al. (2012) proposed programmed cell death (PCD) in the TAM as one of the mechanisms regulating AD. Hallmarks of PCD were identified in the TAM during normal growth, and these were more extensive when AD was lost following either extended cold storage or BE treatment. Hallmarks included DNA fragmentation, induced gene expression of vacuolar processing enzyme 1 (VPE1), and elevated VPE activity (Teper-Bamnolker et al. 2012). Treatment of tubers with BE and then VPE inhibitor induced faster growth and AD recovery in detached and non-detached apical buds, respectively, suggesting that PCD is associated with weakening of tuber AD, allowing early sprouting of mature lateral buds (Teper-Bamnolker et al. 2012).

Cold storage is the main tool used worldwide to delay sprouting of stored tubers. When the tuber is exposed to cool temperatures during its dormancy period, the number of sprouting buds after dormancy is released increases with time of exposure. In other words, an increase in the number of weeks of exposure to cool temperatures reduces AD (Struik 2007). Fauconnier et al. (2002) found that AD can last for up to approximately 60 days in storage in cvs. Bintje and Désirée. Between 60 and 240 days of storage, sprout number per tuber increased linearly with time due to loss of AD. Low temperature (4 °C as compared to 12 °C) reduces sprouting capacity and AD and increases the number of stems when the tubers eventually do sprout (Hartmans and Van Loon 1987).

To date, none of the sprouting control agents studied in potato have been shown to delay loss of AD. Dyson and Digby (1975) suggested that calcium is necessary to maintain AD of the sprout and prevent some of the changes attributed to physiological aging. Calcium application probably delays loss of AD by preventing the subapical necrosis typical to sprouting of potato tubers in dark storage.

Conclusions and Perspectives

The number of stems emerging from the soil is affected by mother tuber genetics, growing, and storage conditions. Dormancy release, AD, and stem branching are sequential events that are probably affected by hormonal regulation and the available energy in the tuber's storage tissue. Potato tubers exhibit AD behavior that is very similar to that of other stems. Apical bud dominance may serve as a marker for tuber physiological age. However, it can be altered by a number of abiotic stresses, including storage temperature and chemical sprouting control agents. Some of these

factors have been shown to induce PCD in the potato TAM. Decapitation experiments performed with sprouting tubers have shown the importance of keeping the TAM cells viable for maintenance of AD. Controlling seed tuber stem number is desirable for optimization of daughter tubers' size distribution (for seed tubers, table use, or processing).

References

Alexopoulos AA, Aivalakis G, Akoumianakis KA, Passam HC (2009) Bromoethane induces dormancy breakage and metabolic changes in tubers derived from true potato seed. Postharvest Biol Technol 54 (3):165–171

Biemelt S, Hajirezaei M, Hentschel E, Sonnewald U (2000) Comparative analysis of abscisic acid content and starch degradation during storage of tubers harvested from different potato varieties. Potato Res 43 (4):371–382

Caldiz DO (2009) Physiological age research during the second half of the twentieth century. Potato Res 52 (4):295–304

Campbell M, Segear E, Beers L, Knauber D, Suttle J (2008) Dormancy in potato tuber meristems: chemically induced cessation in dormancy matches the natural process based on transcript profiles. Funct Integr Genom 8(4):317–328

Campbell MA, Gleichsner A, Alsbury R, Horvath D, Suttle J (2010) The sprout inhibitors chlorpropham and 1, 4-dimethylnaphthalene elicit different transcriptional profiles and do not suppress growth through a prolongation of the dormant state. Plant Mol Biol 73(1):181–189

Cline MG (1991) Apical dominance. The Botanical Review 57(4):318–358

Cline MG (1997) Concepts and terminology of apical dominance. American J Bot 84(8):1064–1064

Coleman WK (1984) Large scale application of bromoethane for breaking potato tuber dormancy. Am J Potato Res 61(9):587–589

Dale MFB, Bradshaw JE (2003) Progress in improving processing attributes in potato. Trends Plant Sci 8(7):310–312

Destefano-Beltran L, Knauber D, Huckle L, Suttle J (2006a) Chemically forced dormancy termination mimics natural dormancy progression in potato tuber meristems by reducing ABA content and modifying expression of genes involved in regulating ABA synthesis and metabolism. J Exp Bot 57(11):2879–2886

Destefano-Beltran L, Knauber D, Huckle L, Suttle JC (2006b) Effects of postharvest storage and dormancy status on ABA content, metabolism, and expression of genes involved in ABA biosynthesis and metabolism in potato tuber tissues. Plant Mol Biol 61(4):687–697

Dun EA, Ferguson BJ, Beveridge CA (2006) Apical dominance and shoot branching. Divergent opinions or divergent mechanisms? Plant Physiol 142(3):812–819

Dyson P, Digby J (1975) Effects of calcium on sprout growth and sub-apical necrosis in Majestic potatoes. Potato Res 18(2):290–305

Ewing EE, Simko I, Omer EA, Davies PJ (2004) Polygene mapping as a tool to study the physiology of potato tuberization and dormancy. Am J Potato Res 81(4):281–289

Fauconnier ML, Rojas Beltrán J, Delcarte J, Dejaeghere F, Marlier M, Jardin P (2002) Lipoxygenase pathway and membrane permeability and composition during storage of potato tubers (*Solanum tuberosum* L. cv Bintje and Desiree) in different conditions. Plant Biol 4(1):77–85

Goodwin P (1967) The control and branch growth on potato tubers: I. Anatomy of buds in relation to dormancy and correlative inhibition. J Exp Bot 18(1):78–86

Harris PM (1992) The potato crop: the scientific basis for improvement, vol edn 2. Chapman and Hall, London

Hartmans KJ, Van Loon C (1987) Effect of physiological age on growth vigour of seed potatoes of two cultivars. I. Influence of storage period and temperature on sprouting characteristics. Potato Res 30(3):397–410

Hartmann A, Senning M, Hedden P, Sonnewald U, Sonnewald S (2011) Reactivation of meristem activity and sprout growth in potato tubers require both cytokinin and gibberellin. Plant Physiol 155(2):776–796

Hemberg T (1985) Potato rest. In: Li PHW CJ (ed) Potato physiology, vol 01-244-76600. Academic, Orlando, pp 353–388

Holmes JC, Lang RW, Singh AK (1970) The effect of five growth regulators on apical dominance in potato seed tubers and on subsequent tuber production. Potato Res 13(4):342–352

Iritani W, Weller L, Russell T (1973) Relative differences in sugar content of basal and apical portions of Russet Burbank potatoes. Am Potato J 50(1):24–31

Isherwood FA (1973) Starch-sugar interconversion in *Solanum tuberosum*. Phytochemistry 12(11):2579–2591

Isla MI, Vattuone MA, Sampietro AR (1998) Hydrolysis of sucrose within isolated vacuoles from *Solanum tuberosum* L. tubers. Planta 205(4):601–605

Ji ZL, Wang SY (1988) Reduction of abscisic acid content and induction of sprouting in potato, *Solanum tuberosum* L., by thidiazuron. J Plant Growth Regul 7(1):37–44

Kincaid D, Westermann D, Trout T (1993) Irrigation and soil temperature effects on Russet Burbank quality. Am Potato J 70(10):711–723

Krijthe N (1962) Observations on the sprouting of seed potatoes. Potato Res 5(4):316–333

Kumar G, Knowles NR (1993) Involvement of auxin in the loss of apical dominance and plant growth potential accompanying aging of potato seed tubers. Can J Bot 71(4):541–550

Lang GA, Early JD, Martin GC, Darnell RL (1987) Endo, para-and ecodormancy: physiological terminology and classification for dormancy research. Hort Sci 22:371–377

Leyser O (2009) The control of shoot branching: an example of plant information processing. Plant Cell Environ 32(6):694–703

Mason MG, Ross JJ, Babst BA, Wienclaw BN, Beveridge CA (2014) Sugar demand, not auxin, is the initial regulator of apical dominance. Proceedings of the National Academy of Sciences 111(16):6092–6097

Meijers CP (1972) Effect of carbon-disulphide on the dormancy and sprouting of seed-potatoes. Potato Res 15(2):160–165

Michener HD (1942) Dormancy and apical dominance in potato tubers. Am J Bot 20:558–568

Moll A (1994) The effects of physiological ageing of seed tubers on growth characteristics of eight potato cultivars tested under controlled conditions. Potato Res 37:11–20

O'Brien P, Allen E, Bean J, Griffith R, Jones SA, Jones J (1983) Accumulated day-degrees as a measure of physiological age and the relationships with growth and yield in early potato varieties. J Agric Sci 101(3):613–631

Pasare SA, Ducreux LJ, Morris WL, Campbell R, Sharma SK, Roumeliotis E, Kohlen W, Krol S, Bramley PM, Roberts AG (2013) The role of the potato (*Solanum tuberosum*) CCD8 gene in stolon and tuber development. New Phytologist 198(4):1108–1120

Phillips IDJ (1975) Apical dominance. Ann Rev Plant Phys 26(1):341–367

Prat S, Frommer WB, Hofgen R, Keil M, Kossmann J, Koster-Topfer M, Liu XJ, Muller B, Pena-Cortes H, Rocha-Sosa M (1990) Gene expression during tuber development in potato plants. FEBS Lett 268(2):334–338

Rappaport L, Lippert LF, Timm H (1957) Sprouting, plant growth, and tuber production as affected by chemical treatment of white potato seed pieces. Am J Potato Res 34(9):254–260

Rehman F, Lee SK, Kim HS, Jeon JH, Park J, Joung H (2001) Dormancy breaking and effects on tuber yield of potato subjected to various chemicals and growth regulators under greenhouse conditions. J Biol Sci 1(9):818–820

Rentzsch S, Podzimska D, Voegele A, Imbeck M, Müller K, Linkies A, Leubner-Metzger G (2011) Dose-and tissue-specific interaction of monoterpenes with the gibberellin-mediated release of potato tuber bud dormancy, sprout growth and induction of α-amylases and β-amylases. Planta 235:137–151

Salimi K, Tavakkol Afshari R, Hosseini MB, Struik PC (2010) Effects of gibberellic acid and carbon disulphide on sprouting of potato minitubers. Scientia Hort 124:14–18

Simko I, McMurry S, Yang HM, Manschot A, Davies PJ, Ewing EE (1997) Evidence from polygene mapping for a causal relationship between potato tuber dormancy and abscisic acid content. Plant Physiol 115(4):1453–1459

Sonnewald U (2001) Control of potato tuber sprouting. Trends Plant Sci 6(8):333–335
Sonnewald S, Sonnewald U (2014) Regulation of potato tuber sprouting. Planta 239(1):27–38
Sorce C, Lorenzi R, Ceccarelli N, Ranalli P (2000) Changes in free and conjugated IAA during dormancy and sprouting of potato tubers. Funct Plant Biol 27(4):371–377. doi:10.1071/PP99150
Sorce C, Lombardi L, Giorgetti L, Parisi B, Ranalli P, Lorenzi R (2009) Indoleacetic acid concentration and metabolism changes during bud development in tubers of two potato (*Solanum tuberosum*) cultivars. J Plant Physiol 166(10):1023–1033
Sowokinos JR (2001) Biochemical and molecular control of cold-induced sweetening in potatoes. Am J Potato Res 78(3):221–236
Sowokinos J, Shock C, Stieber T, Eldredge E (2000) Compositional and enzymatic changes associated with the sugar-end defect in Russet Burbank potatoes. Am J Potato Res 77(1):47–56
Struik P (2007) The canon of potato science: 40. Physiological age of seed tubers. Potato Res 50(3):375–377
Struik PC, Wiersema SG (1999) Seed potato technology. CSIRO, Wageningen
Sukhova LS, MachÁČkovÁ I, Eder J, Bibik ND, Korableva NP (1993) Changes in the levels of free IAA and cytokinins in potato tubers during dormancy and sprouting. Biol Planta 35(3):387–391
Suttle JC (2004a) Involvement of endogenous gibberellins in potato tuber dormancy and early sprout growth: a critical assessment. J Plant Physiol 161(2):157–164
Suttle JC (2004b) Physiological regulation of potato tuber dormancy. Am J Potato Res 81(4):253–262
Suttle JC (2007) Dormancy and sprouting. In Vreugdenhil D, ed, Potato Biology and Biotechnology: Advances and Perspectives. Elsevier, Amsterdam, pp 288–309.
Suttle JC (2009) Ethylene is not involved in hormone-and bromoethane-induced dormancy break in Russet Burbank minitubers. Am J Potato Res 86(4):278–285
Suttle JC, Hultstrand JF (1994) Role of endogenous abscisic acid in potato microtuber dormancy. Plant Physiol 105(3):891–896
Suttle JC, Abrams SR, Stefano-Beltrán D, Huckle LL (2012) Chemical inhibition of potato ABA-8′-hydroxylase activity alters in vitro and in vivo ABA metabolism and endogenous ABA levels but does not affect potato microtuber dormancy duration. J Exp Bot 63(15):5717–5725
Teper-Bamnolker P, Dudai N, Fischer R, Belausov E, Zemach H, Shoseyov O, Eshel D (2010) Mint essential oil can induce or inhibit potato sprouting by differential alteration of apical meristem. Planta 232(1):179–186
Teper-Bamnolker P, Buskila Y, Lopesco Y, Ben-Dor S, Saad I, Holdengreber V, Belausov E, Zemach H, Ori N, Lers A, Eshel D (2012) Release of apical dominance in potato tuber is accompanied by programmed cell death in the apical bud meristem. Plant Physiol 158:2053–2067
Thompson AL, Love SL, Sowokinos JR, Thornton MK, Shock CC (2008) Review of the sugar end disorder in potato (Solanum tuberosum, L.). Am J Potato Res 85(5):375–386
Turnbull CGN, Hanke DE (1985) The control of bud dormancy in potato tubers. Planta 165(3):359–365
Vakis N (1986) Influence of physiological ageing of seed potatoes on yield and earliness. Potato Res 29(3):417–425
Van Loon C (1987) Effect of physiological age on growth vigour of seed potatoes of two cultivars. 4. Influence of storage period and storage temperature on growth and yield in the field. Potato Res 30(3):441–450
Viola R, Pelloux J, van der Ploeg A, Gillespie T, Marquis N, Roberts AG, Hancock RD (2007) Symplastic connection is required for bud outgrowth following dormancy in potato (*Solanum tuberosum* L.) tubers. Plant, Cell & Environment 30(8):973–983
Visser RGF, Vreugdenhil D, Hendriks T, Jacobsen E (1994) Gene expression and carbohydrate content during stolon to tuber transition in potatoes (*Solanum tuberosum*). Physiol Planta 90(2):285–292
Wiltshire JJJ, Cobb AH (1996) A review of the physiology of potato tuber dormancy. Ann Appl Biol 129(3):553–569

Chapter 12
Meta-Analysis Identifies Potential Molecular Markers for Endodormancy in Crown Buds of Leafy Spurge; a Herbaceous Perennial

Münevver Doğramacı, David P. Horvath and James V. Anderson

Introduction

Dormancy in Underground Adventitious Buds of Leafy Spurge

Leafy spurge (*Euphorbia esula* L.) is a herbaceous perennial that is not considered invasive in its native range of Europe and Asia. However, after introduction through shipping, commerce, and migration of immigrants in the eighteenth and nineteenth centuries, it has become an invasive weed in North American ecosystems (Chao and Anderson 2004). Reproduction and spread occurs by both seeds and underground adventitious buds (UABs, commonly referred to as crown and root buds; see Fig. 12.1). However, the perennial nature of leafy spurge is attributed to vegetative production from an abundance of UABs that undergo well-defined phases of seasonally induced para-, endo- and ecodormancy (Anderson et al. 2005), which help optimize distribution of new shoots from the soil bud bank over time (Anderson et al. 2010). Because dormancy in UABs involves arrested development of the shoot apical meristems (Horvath et al. 2003; Horvath and Anderson 2009), similar to that reported in buds of perennial tree species (Cooke et al. 2012; Rinne et al. 2010; Rohde and Bhalerao 2007), it is a key factor allowing herbaceous perennial weeds to escape many control measures and periods of severe abiotic stress (Anderson et al. 2001, 2010; Doğramacı et al. 2014).

As defined by Lang et al. (1987), paradormancy and ecodormancy involve growth cessation controlled by physiological and environmental factors, respectively,

---

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer.

M. Doğramacı (✉) · D. P. Horvath · J. V. Anderson
Sunflower and Plant Biology Research Unit, USDA-Agricultural Research Service,
1605 Albrecht Blvd., N., Fargo, ND 58102-2765, USA
e-mail: munevver.dogramaci@ars.usda.gov

© Springer International Publishing Switzerland 2015

J. V. Anderson (ed.), *Advances in Plant Dormancy,* DOI 10.1007/978-3-319-14451-1_12

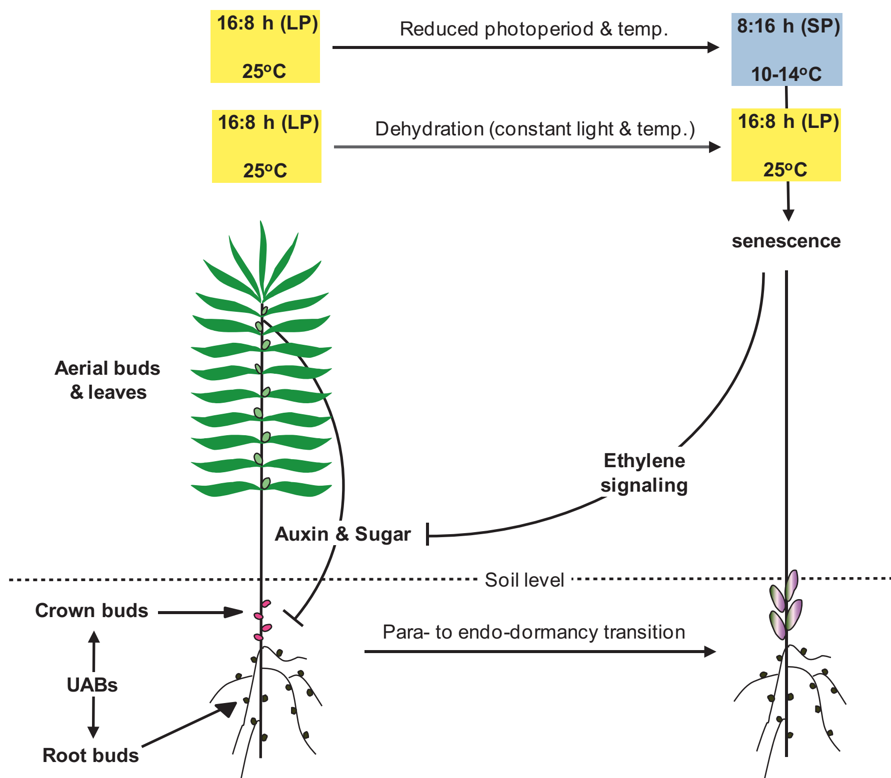


Fig. 12.1 Diagram of leafy spurge anatomy and environmental factors associated with transition from para- to endodormancy. Underground adventitious buds (UABs) are located on the underground stem (crown buds) and on the lateral roots (root buds). Basipetal movement of leaf-derived sugar and auxin under long photoperiod (LP) and growth-conducive temperature maintains paradormancy in UABs. A shift from long to short photoperiod (SP) and summer to autumn temperatures (°C), or extended periods of severe dehydration stress, induces senescence of aerial tissues, reduces sugars and auxin signaling from the aerial tissues, and coincides with a transition of UABs from a state of para- to endodormancy

external to the affected structure, whereas endodormancy involves growth cessation controlled by physiological factors internal to the affected structure. The environmental parameters required for inducing well-defined phases of dormancy in UABs of leafy spurge have previously been determined under field (Anderson et al. 2005) or controlled environmental conditions (Foley et al. 2009). As illustrated in Fig. 12.1, the transition from para- to endodormancy coincides with senescence of the aerial tissues, which is induced by decreasing photoperiod and temperature during the seasonal transition to autumn, or by extended periods of dehydration alone. The transition from endo- to ecodormancy requires an extended period of cold temperatures and usually occurs in late November to early December in the Northern Hemisphere. The discovery that transition from endo- to ecodormancy is also the point at which crown buds become flower competent (Anderson et al. 2005; Foley et al. 2009) provided evidence to support the hypothesis that cross-talk occurs

between mechanisms regulating both dormancy and flowering (Horvath 2009; Horvath et al. 2003). Based on average seasonal bare soil temperatures in Fargo, North Dakota, the transition of leafy spurge UABs from para- to endodormancy generally occurs at ~10–15°C and the transition from endo- to ecodormancy generally occurs at ~0°C under natural field conditions (Anderson et al. 2005).

A report by Mason et al. (2014) provides evidence that the preference for partitioning of leaf-derived sugar to growing shoot tips plays a pivotal role in regulating axillary bud outgrowth in pea (*Pisum sativum* L.). Specifically, their results indicate that sugar, not polar auxin transport from the apical meristem, is the early signaling mechanism regulating axillary bud outgrowth through repression of *BRANCHED1* (*BRC1*) by sucrose, at least in an annual species such as pea. These results are somewhat consistent with a leaf-derived signal also being involved in regulating paradormancy in UABs of leafy spurge (Horvath 1999; Horvath and Anderson 2000) as illustrated in Fig. 12.1. However, starch appears to be the main total non-structural carbohydrate (TNC) observed in leaves, stems, and UABs of leafy spurge during the paradormant period of June–September (Gesch et al. 2007). During autumn-induced senescence, photosynthetic capacities of aerial tissues dissipate and UABs transition from para- to endodormancy (Fig. 12.1) and the amount of TNC decrease in aerial shoots (leaves and stems) and increase in UABs (Gesch et al. 2007). However, in UABs, the increase in TNC is also paralleled by a shift from starch to sucrose (Anderson et al. 2005). Collectively, these results provide support for a leaf-derived sugar signal regulating dormancy in both annual and herbaceous perennial species, but sucrose appears to induce, not repress, bud dormancy in UABs of leafy spurge. This observation is further supported by a study demonstrating that exogenous application of sucrose to leafy spurge roots inhibited initiation of new shoot growth from paradormant UABs, whereas application of gibberellic acid (GA) was able to override this inhibitory effect (Chao et al. 2006).

However, auxin still appears to have a role in the regulation of paradormancy through apical dominance, because UABs of leafy spurge will not initiate new vegetative shoots unless all aerial tissues, including leaves, stems, and all meristems, are removed, whereas removing just leaves from aerial tissue of leafy spurge induced expression of GA-responsive (*GA-STIMULATED ARABIDOPSIS*), glucose-responsive (*BINDING PROTEIN*), and cell cycle (*HISTONE H3* and *CYCLIN D3-2*) genes in UABs (Horvath and Anderson 2002; Horvath et al. 2002, 2005). These studies led to a proposed model (Horvath et al. 2002, 2003) for two organ-specific signals to regulate paradormancy in UABs that include (1) a photosynthetic-dependent, leaf-derived signal (sugar) impacting GA perception to block the G1/S phase of the cell cycle and (2) meristem-derived signaling (auxin) inhibiting the G2/M phase of the cell cycle.

Leafy Spurge as a Model for Studying Well-Defined Phases of Dormancy

Global transcriptome profiling provided a comprehensive approach to investigate components of molecular mechanisms during well-defined phases of environmentally induced dormancy in UABs. Indeed, development of Euphorbiaceae-specific

microarrays (>23,000 elements) from EST databases (Anderson et al. 2007; Lokko et al. 2007) led to the first reports describing molecular processes for well-defined phases of dormancy in invasive weeds under field (Horvath et al. 2006, 2008) or controlled environments (Doğramacı et al. 2010). To eliminate environmental variability under field conditions, standardized growth chamber conditions for follow-up transcriptome studies included exposing 3-month-old greenhouse-propagated plants to a ramp down (RD) in photoperiod (16-h → 8-h light) and temperature (27 → 10˚C) over 12 weeks. This treatment induced a para- to endodormant transition in UABs, whereas an additional 8–12 weeks of vernalizing cold treatment (5–7˚C) induced a transition from endo- to ecodormancy (Foley et al. 2009). However, leafy spurge plants exposed to a RD in temperature alone (RDt; 27 → 10˚C) under a constant photoperiod of 16 h (Doğramacı et al. 2013) or a RD in photoperiod alone (RDp; 16-h → 8-h light) at a constant temperature of 26ºC (Foley et al. 2009) did not induce endodormancy in UABs. Additionally, exposing leafy spurge plants to 14 days of continuous dehydration induced a transition from para- to endodormancy in UABs (Doğramacı et al. 2014), whereas short-term (3 days) dehydration induced growth competence in UABs, which were previously forced into endodormancy by the RDtp treatment (Doğramacı et al. 2011).

In other perennial systems, growth cessation, bud set, and bud dormancy in *Populus spp*. (Welling et al. 1997), birch (*Betula papyrifera*; Downs and Bevington 1981), and grape (*Vitis riparia*; Fennel and Hoover 1991) are influenced by photoperiod, whereas in apple (*Malus spp.*), pear (*Pyrus spp.*; Heide and Prestrude 2005), and grape (*V. vinifera*; Fennel and Hoover 1991), they are influenced by temperature alone, but in peach (*Prunus persica*), apricot (*P. mume*; Yamane 2014), sour cherry (*P. cerasus*), and sweet cherry (*P. avium*; Heide 2008), they are influenced by both photoperiod and temperature. More comprehensive reviews describing the influence of environmental factors on growth cessation, bud set, and induction and release of bud dormancy in perennial systems including forest trees, fruit trees, shrubs, vines, and forbs are available (Anderson et al. 2010; Cooke et al. 2012; Horvath 2009; Horvath et al. 2003; Rios et al. 2014; Rohde and Bhalerao 2007; Tanino et al. 2010). However, because the transition to endodormancy is critical for inhibiting new vegetative shoot growth from UABs during autumn, when conditions can still be conducive for growth, our focus is to identify molecular processes involved in induction and maintenance of endodormancy. Results from such studies could provide new targets and insights for enhancing integrated weed management programs.

Working Models of Endodormancy Induction in UABs of Leafy Spurge

A role for *DEHYDRATION-RESPONSIVE ELEMENT BINDING*/*C-REPEAT BINDING FACTOR* (*DREB*/*CBF*) family members has been proposed as central regulators of molecular networks involved in endodormancy induction (Doğramacı

et al. 2010). *DREBs* belong to the ETHYLENE RESPONSE FACTOR (ERF) family of transcription factors involved in abiotic and biotic stress signaling, which has been extensively reviewed (Khan 2011; Nakashima et al. 2009; Xu et al. 2011). The observation that overexpression of a peach *CBF1* in apple resulted in short photoperiod-induced dormancy and cold acclimation (Wisniewski et al. 2011) provides evidence for *DREB*/*CBFs* playing a role in photoperiod-induced processes leading to bud endodormancy. These results are also consistent with long photoperiod repression of *DREB*/*CBFs* and thus repression of cold acclimation, through interactions with PHYTOCHROME B (PHYB), PHYTOCHROME INTERACTING FACTOR (PIF)-4 (PIF4), and -7 (PIF7) in Arabidopsis under warm environments (Lee and Thomashow 2012). It is still unclear whether short photoperiods play a role in cold acclimation of herbaceous perennials under warm environments. However, a decrease in temperature was determined to be the main environmental factor driving expression of numerous *DREB*/*CBFs* in UABs of leafy spurge, although photoperiod was proposed to have, as yet, unknown synergistic effects to induce endodormancy (Doğramacı et al. 2013).

Interestingly, soil applied 1-aminocyclopropane-1-carboxylate (ACC), the precursor to ethylene, induced a dwarfed phenotype from paradormant crown buds of treated plants after decapitation of aerial tissue (Doğramacı et al. 2013). Numerous leafy spurge transcripts with putative homology to Arabidopsis *DREBs* were differentially expressed in response to the ACC treatment, consistent with the expression observed in endodormant buds (Doğramacı et al. 2013). These results support the hypothesis that a transient spike in ethylene is a prerequisite to induction of endodormancy (Horvath et al. 2003; Ruttink et al. 2007; Suttle 1998), likely through cross-talk with abscisic acid (ABA) signaling in perennials (Anderson et al. 2010). As illustrated in Fig. 12.1, environmental factors (photoperiod and temperature, or dehydration) leading to senescence of aerial tissues are proposed to shift the balance of physiological signals (sugar and auxin) that impact molecular processes in UABs to induce endodormancy. The increase in abundance of a transcript coding for ACC OXIDASE and decreased abundance of a transcript coding for LIGHT HARVESTING CHLOROPHYLL a/b BINDING 1 (LHCB1) in aerial tissues in response to cold, dehydration, and xenobiotic stress (Fig. 12.2) would be consistent with this previous hypothesis.

A model illustrating potential interaction between leafy spurge DREB/CBFs and *DAM* has been proposed, based on the fact that the promoter of a leafy spurge *DAM1* homolog contains a CCGAC *cis*-regulatory element (CRE) in its upstream promoter (Horvath et al. 2013). Although not yet functionally confirmed in leafy spurge, a similar mechanism for regulation of Japanese pear (*Pyrus pyrifolia*) *PpDAM13-1* by PpCBF2 was proposed (Saito et al. 2013) and later confirmed using a transient reporter assay, indicating that *PpMADS13-1* transcription is enhanced via interaction of PpCBF2 with the *PpMADS13-1* promoter (Saito et al. 2014). This interaction is proposed to impact vegetative growth responses through DAM's regulation of the floral integrator *FLOWERING LOCUS T* (*FT*) as shown in Fig. 12.3. In poplar, *FT1* and *FT2* are involved in regulating reproductive versus vegetative growth, respectively (Hsu et al. 2011).

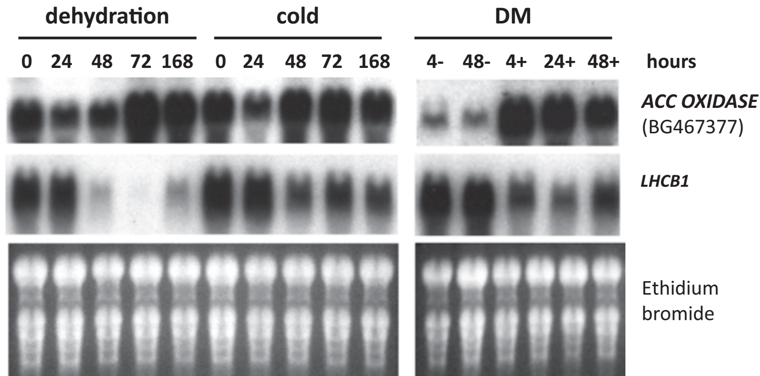


Fig. 12.2 Transcript abundance of *ACC OXIDASE* and *LIGHT HARVESTING CHLOROPHYLL B1* (*LHCB1*) in aerial tissue of leafy spurge in response to dehydration, cold, and xenobiotic stress. Methods and materials are the same as described in Anderson and Davis (2004). Briefly, soil applied water was withheld for dehydration, and plants were subjected to 4–7 °C in a cooling chamber under 16-h light, or sprayed with (+) or without (−) 5 mM technical grade diclofop-methyl (DM) in combination with an emulsified carrier

A role for a DAM/*FT* interaction to regulate vegetative growth and flowering, bud set, and dormancy in perennial fruit trees has been reported (Bielenberg et al. 2008; Yamane 2014). Thus, it is not surprising that chromatin immunoprecipitation (ChIP) assays indicate that leafy spurge DAM1 binds the promoter of a leafy spurge gene most similar to *FT2* of poplar, and increased expression of leafy spurge *DAM1* is inversely correlated with decreased expression of the putative *FT2* homolog (Hao and Horvath unpublished). Likewise, because *DAM* and *FT* are differentially regulated under short photoperiod conditions in perennials (Böhlenius et al. 2006; Cooke et al. 2012; Ruttink et al. 2007) and *DREBs* are known to be gated by the circadian clock (Dong et al. 2011; Fowler et al. 2005), it seems reasonable to assume that the impact of photoperiod and/or temperature on the circadian clock (see reviews by Cooke et al. 2012) could also affect DREB/DAM/FT interactions. Equally intriguing, a report by Chow et al. (2014) demonstrated that Arabidopsis DREB1B/CBF1 binds to a C-repeat (CRT)/dehydration-responsive element (DRE) in the promoter of *LUX ARRYHTHMO* (*LUX*) to mediate cold input into the circadian clock. Although not included in the proposed model (Fig. 12.3), this process may involve SUPPRESSOR OF CONSTANS 1 (SOC1) as part of a negative feedback loop to regulate *DREB1*/*CBFs* (Seo et al. 2009) and, thus, the cold response regulon. In this same context, cold-induced expression of *DREB1*/*CBF* impacts expression of *FLOWERING LOCUS C* (*FLC*), thereby providing a mechanism for repression of *FT* and *SOC1* in Arabidopsis (Thomashow 2010).

Objectives

The long-term goal of our research program is to provide insights into developing next-generation weed management strategies by identifying new targets for manipulation of plant growth and development. As part of this goal, our current objective

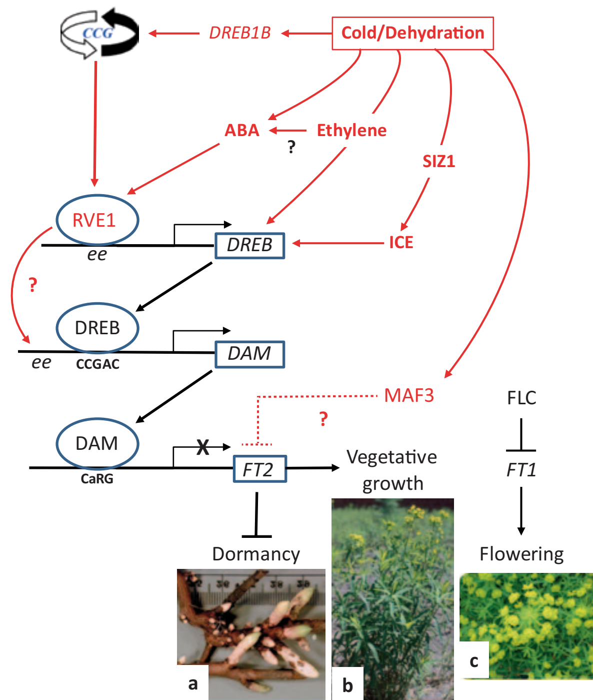


Fig. 12.3 A proposed model for regulation of endodormancy in UABs of leafy spurge. **a** Autumn-induced endodormancy in crown buds of leafy spurge, **b** vegetative regrowth of aerial tissues from UABs in spring and early summer, and **c** leafy spurge in full bloom spring and early summer. Abbreviations and rational for model are provided within the text. *Question marks* and *dashed lines* indicate hypothetical deductions as outlined in the text, and *orange lines* indicate updates on our previous existing models

is to identify CREs within promoters of putative endodormancy marker genes and determine the transcriptional machinery that interacts with these elements.

Materials and Methods

Plant Material and Experimental Designs

Leafy spurge plants were propagated as previously described by Anderson and Davis (2004), and standardized treatments for inducing well-defined phases of dormancy in UABs were employed (Foley et al. 2009). In brief, leafy spurge plants were propagated from a genetically uniform biotype (1984-ND001) and maintained in a greenhouse (~25–27 °C with 16h:8h light:dark photoperiod) for 3 months. Prior to the start of each experiment, plants were entrained in a growth chamber for 1 week at 27 °C, 16h:8h light:dark photoperiod. Each experiment was replicated three

or four times, and each replicate included 30–40 plants. Six to eight plants from each replicate were used to determine the dormancy status of buds by measuring the vegetative growth and flowering potential of crown buds after removal of existing aerial tissues by decapitation at soil level (see Fig. 12.1); the remaining plants were used to collect crown buds for studying transcriptome profiles (Doğramacı et al. 2010, 2011, 2013, 2014). All samples were collected between 1100 and 1300 Central Standard Times to avoid diurnal variation. Various environmental treatments (photoperiod, temperature, dehydration) were used to determine their impact on induction or release of endodormancy as summarized in Table 12.1.

RNA Extraction and Transcript Analyses

At the end of each treatment (Table 12.1), crown bud samples were collected and flash-frozen in liquid N_2. RNA was extracted according to the pine tree RNA extraction protocol (Chang et al. 1993), and RNA quality and quantity was confirmed by spectrophotometry and agarose gel electrophoresis. Microarray hybridizations were performed as described in detail by Doğramacı et al. (2010). Various bioinformatics tools were utilized for analyses of transcriptome data reported in Doğramacı et al. (2010, 2011, 2013, 2014). Specifically, GeneMaths XT 5.1 (Applied Maths Inc., Austin, TX, USA) was used for normalization and statistical analyses, and Pathway Studio (Ariadne Genomics Inc., Rockville, MD, USA) was used for Gene Set Enrichment Analysis and Sub Network Enrichment Analysis. Expression data are deposited at Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/) as GEO dataset queries GSE19217 (Doğramacı et al. 2010), GSE28047 (Doğramacı et al. 2011), GSE37477 (Doğramacı et al. 2013), and GSE55133 (Doğramacı et al. 2014).

Incorporation of Meta-Analysis to Detect Marker Genes for Endodormancy

Data from transcriptome studies (Table 12.1) were evaluated to detect genes showing consistent trends during endodormancy induction. Genes with increased transcript abundance in endodormant crown buds induced by the various environmental treatments but having opposite expression patterns during para- or ecodormancy were selected for further investigations.

Quantitative Real-Time PCR Analysis (qRT-PCR)

A leafy spurge EST database (Anderson et al. 2007) was used to design primer pairs (Table 12.2) employing the Primer Select program of DNASTAR Lasergene 11. cDNA synthesis and qRT-PCR were prepared as described in Doğramacı et al. (2010, 2011, 2013, 2014). Transcript abundance was measured from at least three biological replicates and three technical replicates using a LightCycler 480 II

Table 12.1 Summary of environmental treatments, treatment length (d: day, h: hour, wk: week), and associated publications demonstrating specific environmental signals impacting endodormancy induction or release in leafy spurge

Referred to as	Treatment	Temperature (°C)	Photoperiod (light)	Treatment period	Pretreatment bud status	Post-treatment bud status	Reference
Para	Plants entrained in growth chamber	27 °C	16 h	1 wk	Paradormant	Paradormant	Doğramacı et al. 2010
RDtp	Plants exposed to ramp down in temperature and photoperiod	27 °C → 10 °C	16 h → 8 h	12 wk	Paradormant	Endodormant	Foley et al. 2009; Doğramacı et al. 2010
RDp	Plants exposed to ramp down in photoperiod under constant temperature	27 °C	16 h → 8 h	12 wk	Paradormant	Paradormant	Foley et al. 2009
RDt	Plants exposed to ramp down in temperature under constant photoperiod	27 °C → 10 °C	16 h	12 wk	Paradormant	Paradormant	Doğramacı et al. 2013
FC Eco	Plants exposed to extended cold	7 °C	8 h	11 wk	Endodormant	Flower competent ecodormant	Doğramacı et al. 2010
NFC Eco	Plants exposed to extended cold	7 °C	8 h	11 wk	Paradormant	Non-flower competent ecodormant	Doğramacı et al. 2010
Endo->3-day dehydration stress	Plants exposed to dehydration stress	27 °C	16 h	3 d	Endodormant	Growth competent	Doğramacı et al. 2011
Three-day dehydration stress	Plants exposed to dehydration stress	27 °C	16 h	3 d	Paradormant	Paradormant	Doğramacı et al. 2014
Seven-day dehydration stress	Plants exposed to dehydration stress	27 °C	16 h	7 d	Paradormant	Para- to endodormant transition	Doğramacı et al. 2014
Fourteen-day dehydration stress	Plants exposed to dehydration stress	27 °C	16 h	14 d	Paradormant	Endodormant	Doğramacı et al. 2014

Table 12.2 Primer sequences for selected leafy spurge genes (Primer Sequence). Annotation of homologs of leafy spurge transcripts most similar to Arabidopsis were identified using the Arabidopsis Information Resource (TAIR) and to obtain gene identifications (TAIR ID) and abbreviations (Abv.)

TAIR ID	Abv.	5′F/R	Primer Sequence	Leafy spurge ID
CONSTITUTIVE PHOTOMORPHOGENIC 1 (AT2G32950)	*COP1*	5′F	TCTTGTTTTTCTTCCCCTC-TATCT	DV130469
		5′R	AGCACGTTTTTCAT-GTTCTTCA	
ELONGATED HYPOCOTYL 5 (AT5G11260)	*HY5*	5′F	CTCAA-CAAGCAAGGGAAAGGAAGA	DV157454
		5′R	CTAGCCAACGAAGAAACG-GAAAAT	
MADS AFFECTING FLOWERING 3 (AT5G65060)	*MAF3*	5′F	ATCGAAGAAAAGAGCATC-CGTCAG	CV03083A2E08
		5′R	TCTTCAAGTTGCATGTCAG-TAGTT	
RESPONSIVE TO DESSICATION 22 (AT5G25610)	*RD22*	5′F	AATCAAACCCC-GAAGCAAAAGTAT	DV131779
		5′R	CCTGAGGAAGAAAATG-GCAAACC	
REVEILLE 1 (AT5G17300)	*RVE1*	5′F	CGGATTGAAGAGCATGTAG-GTAGC	DV113864
		5′R	AGTTGGGGATTGATTTTCGT-GTTC	

(Roche). Transcript values were normalized using reference genes (*ARF2*, *MD-100*, *ORE9*, *PTB*, *SAND*) identified for leafy spurge (Chao et al. 2012).

Identification of Conserved Cis-Regulatory Elements

Assembly (*de novo*) of promoter sequence for candidate genes was accomplished as previously described (Doğramacı et al. 2014). Briefly, promoter sequence (~3000–7000 bases upstream of putative ATG start sites of CDS) was created using the program PriceTI (Ruby et al. 2013), which were used to identify the most conserved genes within the Malpighiales family (*Manihot esculenta*, cassava; *Ricinus communis,* castor bean; *Linum usitatissimum,* flax; *Populus trichocarpa,* poplar) using the program Phytozome (www.phytozome.net). The non-transcribed promoter regions for each family member were run in the MEME program (http://meme.nbcr.net/meme/cgi-bin/meme.cgi) to identify conserved promoter sequences. Conserved motifs identified by MEME were entered into Plant Promoter Analysis Navigator (http://plantpan.mbc.nctu.edu.tw/) to determine the function of putative CREs.

Results and Discussion

Identification of Potential Marker Genes

Meta-analysis of microarray data (Doğramacı et al. 2010, 2011, 2013, 2014) highlighted five leafy spurge transcripts with putative sequence homology to Arabidopsis *COP1*, *HY5*, *MAF3*, *RD22*, and *RVE1* that consistently had increased abundance in endodormant crown buds (Table 12.3), but had opposite expression in para- and often ecodormant buds of leafy spurge. Based on these results, we propose that PCR-amplified cDNA (see Table 12.2) for these transcripts may be used as endodormancy markers.

COP1

COP1 encodes for an E3 ubiquitin ligase that can target up to 20 % of the transcription factors in Arabidopsis (Moon et al. 2004), including HY5 and the floral promoter CONSTANS (CO) for degradation and stabilization of growth-promoting transcription factors such as PIF3 (Alabadí and Blázquez 2009; Henriques et al. 2009). Although COP1 targets HY5 for degradation in far-red and visible light-induced photomorphogenesis, it positively regulates HY5 in UV-B-induced photomorphogenesis (Favory et al. 2009). Further, in Arabidopsis, *COP1* expression is regulated by HY5 and FHY3 via a positive feedback loop (Huang et al. 2012). Thus, the simultaneous

Table 12.3 Transcript abundance of potential marker genes. Values (log2 scale) represent averages of four biological and three technical replicates relative to controls. Red indicates positive values, and blue indicates negative. Arabidopsis genes are used to annotate homologs of leafy spurge transcripts

Reference	Status of Buds	Treatment Summary	COP1	HY5	MAF3	RD22	RVE1
Doğramacı et al. 2010, 2013	Para	Para--Control	0	0	0	0	0
	Endo	RDtp	1.77	1.30	9.27	0.68	0.97
	FC Eco	RDtp + Cold	0.62	-0.24	17.35	-1.20	-0.35
	NFC Eco	Para + Cold	0.46	-0.43	17.51	-0.98	0.46
	RDt	RDt + Constant light	-0.23	-0.15	-1.38	-0.33	-0.55
Doğramacı et al. 2014	Para	Para--Control	0	0	0	0	0
	Day 3	Para--3 day dehydration	0.25	1.01	-0.67	0.65	0.11
	Day 7	Para--7 day dehydration	-0.13	0.96	1.08	2.35	0.62
	Day 14 (Endo)	Para--14 day dehydration	2.30	2.58	3.15	2.42	2.05
Doğramacı et al. 2011	Endo	Endo--Control	0	0	0	0	0
	Day-01	Endo--1 day dehydration	-2.11	-1.58	-4.53	-1.95	-5.17
	Day-03	Endo--3 day dehydration	-2.74	-1.55	-4.75	-2.30	-4.13
	FC Eco	Endo (RDtp) + Cold	-0.65	-1.38	7.44	-1.69	-0.74

increase in abundance of putative leafy spurge *HY5* and *COP1* transcripts by RDtp or dehydration alone indicates that normal light-mediated regulation of COP1–HY5 interactions is disrupted in endodormant UABs. This might be a consequence of RDtp or dehydration treatments on senescence of aerial tissue, suggesting that senescence-induced signaling could be a common factor leading to endodormancy in UABs.

The decreased abundance for a transcript with putative homology to an Arabidopsis *CO-like* (At2g33500) in endodormant crown buds of leafy spurge (Doğramacı et al. 2010) could imply that COP1 is targeting some members of this transcription factor family in endodormant crown buds. Although CO is a positive regulator of *FT*, and the *CO-FT* module has been associated with growth cessation and bud set in poplar (Böhlenius et al. 2006), data for leafy spurge *FT-like* transcripts were not available for all the samples included in our meta-analysis. However, transcript abundance for a putative leafy spurge *FT1-like* homolog in endodormant buds was reported to be induced by RDtp (Doğramacı et al. 2013) and 14-day dehydration stress treatments (Doğramacı et al. 2014). Because leafy spurge DAM1 has been shown to bind the promoter of a gene with putative homology to poplar *FT2* (Hao and Horvath unpublished), we propose that increased transcript abundance of leafy spurge *DAM1* and *DAM2* (Doğramacı et al. 2010) does not lead to repression of *FT1-like* transcripts in endodormant buds. Instead, it is proposed to repress transcripts with functional similarity to poplar *FT2* (see Fig. 12.3).

HY5

HY5 encodes for a bZIP transcription factor involved in the positive regulation of photomorphogenesis and the PHYA-mediated inhibition of hypocotyl elongation in Arabidopsis (Jiao et al. 2007; Saijo et al. 2003). Studies using *hy5* mutants indicate that HY5 promotes the expression of negative regulators of auxin signaling, thus linking hormone and light signaling pathways (Cluis et al. 2004). Because HY5 can bind to targets involved in regulating circadian rhythms, flowering, and hormone signaling in Arabidopsis, it has been proposed that HY5 likely has other roles in plant growth and development beyond light regulation (Lee et al. 2007). Indeed, Catalá et al. (2011) reported that HY5 levels in Arabidopsis are regulated by low temperature transcriptionally, via a CBF- and ABA-independent pathway, and post-translationally, via protein stabilization through nuclear depletion of COP1. Thus, increased expression of a leafy spurge transcript with putative homology to *HY5* could be acting as one of the central modulators of gene expression that helps coordinate light and cold signaling to promote endodormancy by the RDtp treatment.

Interestingly, induction of endodormancy in crown buds by 14-day dehydration stress, where plants were under ambient greenhouse conditions (~25–27 °C with 16-h photoperiod) prior to and during the dehydration stress treatment, also caused an increase in abundance of the putative leafy spurge *HY5* transcript (Table 12.3). As previously mentioned above, perhaps senescence-associated signaling (e.g., sugars, auxin) of aerial tissues by 14-day dehydration stress also causes an effect similar to that occurring under cold temperature–short photoperiod conditions

(RDtp). Therefore, the increase in putative leafy spurge *HY5* and *COP1* transcripts in response to 14-day dehydration demonstrates that this process also can occur independent from changes in cold and light signaling. If the product of this putative *HY5* transcript has similar functions in leafy spurge as in Arabidopsis, HY5 could be involved in negatively modulating auxin signaling in endodormant crown buds. These results also suggest that endodormancy induction by dehydration or RDtp likely involves overlapping mechanisms.

MAF3

In Arabidopsis, *MAF3* encodes for a MADS-box domain protein and flowering regulator that is closely related to the floral repressor *FLC* (Caicedo et al. 2009; Ratcliffe et al. 2003). Induction of endodormancy by RDtp treatment and 14-day dehydration stress increased abundance of this putative leafy spurge *MAF3* transcript relative to paradormant controls (Table 12.3). Transcript abundance of this *MAF3-like* was even greater in ecodormant buds (FC Eco), and it was also increased in NFC Eco buds even though these buds did not go through the endodormant phase. Thus, this putative *MAF3* transcript appears to be a marker for both endo- and ecodormant crown buds. As shown in Table 12.3, this putative *MAF3* transcript was not induced by a ramp down in temperature under a constant 16-h photoperiod (RDt), but was induced by ramp down in temperature and photoperiod (RDtp) or extended cold treatment under 8 h of short photoperiod (NFC Eco). These results suggest that expression of this putative *MAF3* transcript is induced by either short photoperiod alone or an interaction between short photoperiod and cold temperature signaling. However, no transcript data are currently available for short photoperiods under constant temperature to confirm this hypothesis in leafy spurge. Interestingly, abundance of this *MAF3-like* transcript was also induced by 14-day dehydration stress (Table 12.3), which further supports the hypothesis that senescence of aerial tissues by RDtp and dehydration induces an overlapping response that impacts the transition from para- to endodormancy in UABs.

RD22

RD22 is generally associated with abiotic stress responses (such as dehydration and salt stress) mediated by ABA in Arabidopsis (Yamaguchi-Shinozaki and Shinozaki 1993). Induction of endodormancy in crown buds of leafy spurge by RDtp treatment or 14-day dehydration stress caused an increase in transcript abundance of *RD22* (Table 12.3). Further, release of endodormancy by 3-day dehydration or extended cold treatment (FC Eco) caused a decrease in transcript abundance of *RD22*. These results are consistent with increased abundance of RD22 class proteins (along with other ABA-inducible transcripts) in dormant potato tuber meristems, which were decreased when meristem dormancy was terminated (Campbell et al. 2008).

RVE1

RVE1 encodes a clock-regulated MYB-like transcription factor that, in Arabidopsis, is homologous to *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*) and *LATE ELONGATED HYPOCOTYL 1* (*LHY1*), but inactivation of RVE1 does not affect the circadian rhythm (Rawat et al. 2009). More specifically, RVE1 is an output component of the circadian clock and has been shown to regulate hypocotyl growth by modulating free auxin levels in a time-of-day-specific manner in Arabidopsis (Rawat et al. 2009). Because RVE1 appears to modulate plant growth through regulation of auxin levels, while CCA1 and LHY1 likely control growth via different mechanisms, *RVE1* is considered an important node connecting circadian- and auxin-signaling pathways (Rawat et al. 2009). In leafy spurge crown buds, induction of endodormancy by the RDtp treatment or 14-day dehydration stress (Table 12.3) caused increased abundance of a transcript for this putative *RVE1* homolog. Further, release of endodormancy by 3-day dehydration or extended cold treatment (FC Eco) caused a decrease in transcript abundance of this *RVE1* transcript. In endodormant buds of leafy spurge, increased abundance of *RVE1* (Table 12.3) and a moderate increase in auxin levels (unpublished data), compared to paradormant controls, are consistent with RVE1-modulated free auxin levels in Arabidopsis. Because exogenous auxin treatment to Arabidopsis enhances hypocotyl elongation, while higher concentrations inhibit hypocotyl growth (Rawat et al. 2009), perhaps the putative leafy spurge RVE1 homolog plays a role in modulating auxin levels in endodormant UABs of leafy spurge, assuming that the product of the putative leafy spurge *RVE1* homolog performs the same function as in Arabidopsis.

Characterization of Proposed Marker Genes

Identification of promoter sequence for putative leafy spurge endodormancy marker genes (Table 12.4) was accomplished through *de novo* assembly as previously described in Doğramacı et al. (2014). Currently, sufficient promoter sequence is only available for *RVE1* and *HY5* among the five proposed endodormancy markers for leafy spurge. The validity of *de novo* assembly for *RVE1* was confirmed by comparing it to the sequence of leafy spurge genomic clones for *RVE1*; comparison of the 5′ upstream promoter sequences was >95 % conserved (Doğramacı et al. 2014). The promoter of the leafy spurge genomic clone for *RVE1* contains a conserved ABA-responsive element (ABRE)-like sequence that in other plant systems is involved in early response to dehydration and calcium (Whalley et al. 2011). The *RVE1* promoter also contained a putative MYC consensus sequence, common to dehydration-responsive genes, and a PIF3 binding element (Table 12.4). Because the circadian clock is disrupted in perennials by cold temperatures (Ramos et al. 2005; Ibáñez et al. 2008), uncoupling of the circadian clock by dehydration and/or temperature in UABs of leafy spurge may be compensated through ABA signaling involving ABREs to regulate circadian clock outputs.

Table 12.4 Conserved *cis*-acting elements (colored and underlined) within the promoters of putative leafy spurge homologs (genes) in the Malpighiales family of sequenced genomes. Element location (position) within promoters is upstream of proposed ATG start of coding sequence

Genes	Element	Strand	Position	Sequences		
RVE1	ABRE-Like	+	2178	GAAGGGCGGC	CTGACGTGGC	TAAAAGCGCA
RVE1	MYC Cons	+	3620	GACAAACTAA	CGCCACGTGTCT	GACTTGTCTT
RVE1	PIF3	+	3620	GACAAACTAA	CGCCACGTGTCT	GACTTGTCTT
HY5	ARR1	+	2662	ACCATCATAC	CACGATTCTCTC	CCACTATCCT
HY5	GT1 Cons	+	2820	AATTAATATC	CCGCTCTTTTTC	TCTCCCCAAA
ABR1	GCC Core	+	2658	TCTTGTAATA	AGGCGGC	GTTTACTTGT
ABR1	LTRE Core	+	2411	TCCAAAAAAA	CTCAGCACCGAC	TGAATGTGCA
ABR1	LTRE Core	+	2371	ATATCTAAAT	GGGCCGACC	TGGTTGTCGG
ABR1	LTRE Core	+	2187	CTCGATTAGA	CCCGACCC	AATCCACGAA
ERF1	ABRE-Like	-	126	CAGATGTTTA	GCTGACGTGGC	AGATTTTTAG
ERF1	ASF-1	-	126	CAGATGTTTA	GCTGACGTGGC	AGATTTTTAG
ERF1	GCC Core	+	2432	TATAAATGAA	GGCCGCC	TGATTGTATA
ERF1	MYC Cons	+	1782	TTATAAACAA	GTGGAGCAACC	AAACCAATAC
PIF3	ABRERATCAL	+	919	TATTAAATAT	GTCCACGTGGAC	CAATGAGATA
PIF3	CBF2	+	919	TATTAAATAT	GTCCACGTGGAC	CAATGAGATA
PIF3	MYC Cons	+	919	TATTAAATAT	GTCCACGTGGAC	CAATGAGATA

GCCGCC = GCC CORE - ethylene-responsive element.
CAAGTG = MYC recognition sequence in CBF3 promoter.
LTRE = Low temperature responsive element core.
ASF-1 binds to TGACG motifs - involved in transcriptional activation by auxin/salicylic acid.
GT-1 is a binding site in many light-regulated genes.
ARR1 is a typeB cytokinin response element.

The *de novo*-assembled promoter of leafy spurge *HY5* contains a conserved CRE that, in Arabidopsis, interacts with B-type ARABIDOPSIS RESPONSE REGULATOR 1 (ARR1) (Sharma et al. 2011). Because B-type ARR1 regulates transcription of target genes in response to cytokinin (Heyl et al. 2008; Sharma et al. 2011), our results suggest that cytokinin signaling could be playing some role in the increased abundance of *HY5* in endodormant crown buds of leafy spurge. However, because silencing of *ARR1* in Arabidopsis induced over a 4-fold increase in the expression of *COP1* (Heyl et al. 2008), it appears that repression of cytokinin signaling is likely required to induce *COP1* and *HY5* expression in endodormant buds, at least in UABs of leafy spurge.

The assembled promoter sequences for several other genes of interest included putative homologs of *APETALA2*/*ERF* family members (*ABR1* and *ERF1*) and *PIF3*. The promoters of putative leafy spurge *ERF1* and *PIF3* genes also contained ABRE-like and MYC CREs, whereas these same binding elements do not appear to be conserved in the promoters of putative leafy spurge *HY5* and *ABR1* genes (Table 12.4). However, *ABR1* and *ERF1* do contain ethylene-responsive GCC core elements, which have been reported to play important roles in regulating jasmonate-responsive gene expression (Ohme-Tagaki et al. 2000). In addition, the putative leafy spurge *ABR1* promoter sequence contained low temperature-responsive elements (LTRE), which also includes the core C-repeat/dehydration-responsive element (C/DRE; -CCGAC-), and light signaling mediated by phytochrome is necessary for cold- or drought-induced gene expression through the C/DRE in Arabidopsis (Kim

et al. 2002). Collectively, these results suggest that timing of transcriptional activation or repression of target genes involved in the regulation of seasonal dormancy is likely modulated by a complex set of binding interactions that are responsive to environmental cues and phytohormones.

Updated Hypothetical Model for Endodormancy Induction

A hypothetical model for endodormancy induction (Fig. 12.3) shows how cold or dehydration stress impacts circadian clock genes, as previously reported in leafy spurge (Doğramacı et al. 2010, 2013, 2014) and other species (Ibáñez et al. 2008; Ramos et al. 2005; Rios et al. 2014). Indeed, in the case of cold, *DREB1B*/*CBF1* has been shown to bind to a C/DRE in the promoter of Arabidopsis *LUX* to regulate oscillation and mediate cold input into the circadian clock (Chow et al. 2014). Cold uncoupling of the circadian clock in perennials (Ibáñez et al. 2008; Ramos et al. 2005) would be expected to impact circadian clock output genes such as *RVE1*. Thus, regulation of a homolog of *RVE1* in endodormant UABs of leafy spurge in response to cold and dehydration could involve regulation through ABA signaling (Doğramacı et al. 2014). Indeed, RVE1 can bind *cis*-acting evening elements (*ee*) in genes (Franco-Zorrilla et al. 2014; Mizoi et al. 2012), and these *ee* have been linked to circadian-regulated and cold-induced expression, and coupling with ABRE-like can enhance the cold-induced expression in Arabidopsis (Mikkelsen and Thomashow 2009).

Because some *AP2*/*ERF*s are gated by the circadian clock as previously described, it is possible that the impact of cold or dehydration on circadian clock components in leafy spurge also affects abundance of these transcription factors. We have considered DREBs/CBFs as a central component that impact GA catabolism, sugar signaling, and other processes associated with various transitional phases of dormancy in leafy spurge (Doğramacı et al. 2010, 2014; Horvath et al. 2013). Indeed, DREBs can affect GA catabolism and signaling in Arabidopsis (Magome et al. 2009), and GA catabolism would be expected to impact downstream GA signaling of DELLAs, which, in turn, could affect vegetative growth through its repression of growth-promoting transcription factors such as PIFs (see review by Hirsch and Oldroyd 2009). We hypothesize that DREBs could also be playing a role in dormancy processes as shown in Fig. 12.3, based on their known involvement in expression of genes similar to *FLC*, which in turn leads to repression of *FT* in Arabidopsis (Seo et al. 2009).

Ethylene's impact on some *AP2*/*ERF* family members in leafy spurge has been proposed to have a role in regulating the transition from para- to endodormancy (Doğramacı et al. 2013). Indeed, overexpression of several *AP2*/*ERFs*, similar to those induced by ethylene in leafy spurge (Doğramacı et al. 2013), causes dwarfed phenotypes or induces endodormancy in other plant systems (Khan 2011; Wisniewski et al. 2011; Xu et al. 2011). Because DREBs are known to bind CREs similar to those identified in leafy spurge *DAM* genes (Horvath et al. 2013), and leafy spurge DAM1 has been shown to bind the promoter of a putative leafy spurge *FT2-like* gene (Hao and Horvath unpublished), we propose that RVE1 may func-

tion through binding to *ee* of *DREB/CBFs* or directly to the promoter of *DAM1* (see Fig. 12.3). Although ACC synthase is the rate-limiting step of ethylene biosynthesis in Arabidopsis (Wang et al. 2004), the last step to ethylene biosynthesis involving ACC oxidase might also play a role in the senescence-induced spikes in ethylene that has been proposed to induce endodormancy in UABs (Anderson et al. 2010; Horvath et al. 2003). The observed increase in abundance of transcript coding for ACC oxidase in response to abiotic and xenobiotic stress in leafy spurge leaves (Fig. 12.2) would be consistent with this concept. In Arabidopsis, ethylene production also induces the nuclear transcription factor *ETHYLENE INSENSITIVE 2*, which impacts ABA signaling (Wang et al. 2007). Thus, we propose that senescence-induced ethylene signaling impacts mobile auxin and sugar signaling from the aerial tissues (see Fig. 12.1) and could impact cross-talk with ABA signaling pathways in leafy spurge (see Fig. 12.3).

A previous study (Doğramacı et al. 2014) also suggested a potential role for post-translational modification through interactions between SIZ1 (an E3 SUMO ligase) and INDUCER OF CBF EXPRESSION1 (ICE1) to impact *DREB* expression in endodormant UABs of leafy spurge. Because SIZ1 can stabilize ICE1 through sumoylation (Mizoi et al. 2013) and ICE1 binds the promoter of *DREBs* (Chinnusamy et al. 2006), it is also possible that cold- and dehydration-induced expression of *DREBs* in UABs of leafy spurge involves similar post-translational modification mechanisms as illustrated in Fig. 12.3.

Another major outcome from this meta-analysis was identification of a putative leafy spurge *MAF3-like* transcript, one of the several *FLC-like* genes in Arabidopsis (Ratcliffe et al. 2003), as a molecular marker for endo- and ecodormancy in crown buds of leafy spurge. In Arabidopsis, FLC is known to inhibit *FT* to block flowering (Ratcliffe et al. 2003; Reeves et al. 2007), and in perennial tree species, several members of DAMs (also members of the MADS-box domain family of proteins) are known to block *FT2* and induce growth cessation and bud set (Cooke et al. 2012; Rios et al. 2014). Based on the strong increase in abundance of a leafy spurge transcript with putative homology to Arabidopsis *MAF3* in endo- and ecodormant UABs (Table 12.3), we propose that the product of leafy spurge *MAF3-like* could inhibit *FT2-like* expression as part of a mechanism involved in maintaining endo- and ecodormancy (Fig. 12.3). However, leafy spurge *MAF3-like* transcript in endo- and ecodormant UABs appears to display alternative splicing (unpublished). Since the product of spliced variants of *MAFs* have been reported to interact with SHORT VEGETATIVE PHASE to regulate flowering in Arabidopsis in a temperature dependent manner (Posé et al. 2013; Severing et al. 2012), it is also plausible that spliced variants of the leafy spurge *MAF3-like* transcript could potentially interact with DAM-like MADS-box proteins to affect dormancy in UABs.

Future Direction

Conceptual models, such as proposed in Fig. 12.3, provide a starting point to test the functionality of these putative leafy spurge homologs and to determine their potential role in endodormancy maintenance in leafy spurge or other model perennial

systems. Here, we update a working hypothetical model to include new components for regulation of endodormancy in UABs of leafy spurge that involves (1) the potential interaction of MAF3 with *FT2* to inhibit vegetative growth and (2) an ABA-dependent signaling mechanism to regulate a putative homolog of the circadian clock output gene *RVE1* that may impact downstream genes containing evening elements, similar to that described in other systems, or to modulate auxin levels. The overlap between dehydration- and photoperiod/temperature-induced endodormancy in UABs of leafy spurge may involve senescence-induced ethylene signaling. Further research into well-defined phase of dormancy in UABs of leafy spurge would certainly benefit from studies that determine the impact of molecular and physiological signaling mechanisms associated with aerial tissues, for example, determining whether FT is produced in aerial tissues and whether it is mobile and transported to the underground adventitious buds. Likewise, further studies are needed to determine if spliced variants of *MAF3-like*, or other MAF family members, affect *FT2* directly or function through interaction with DAM-like MADS-box proteins.

However, relying on orthologous genomes to annotate genomes of weedy species has pitfalls associated with proposing biological interactions and processes. Spurious assumptions that transcripts with the best sequence homology to genes of other plant species have conserved functionality may lead to confounded models. Thus, meta-analysis of the leafy spurge transcripts based on annotation to other genomes only provides for the first step in building testable hypotheses. Future research will be needed to functionally characterize these leafy spurge marker genes and determine the upstream binding complexes that drive their expression.

Acknowledgments The authors wish to thank Brant B. Bigger and Cheryl A. Huckle for their technical assistance.

References

Alabadí D, Blázquez MA (2009) Molecular interactions between light and hormone signaling to control plant growth. Plant Mol Biol 69:409–417

Anderson JV, Davis DG (2004) Abiotic stress alters transcript profiles and activity of glutathione S-transferase, glutathione peroxidase, and glutathione reductase in *Euphorbia esula*. Physiol Plant 120:421–433

Anderson JV, Chao WS, Horvath DP (2001) A current review on the regulation of dormancy in vegetative buds. Weed Sci 49:581–589

Anderson JV, Gesch RW, Jia Y, Chao WS, Horvath DP (2005) Seasonal shifts in dormancy status carbohydrate metabolism and related gene expression in crown buds of leafy spurge. Plant Cell Environ 28:1567–1578

Anderson JV, Horvath DP, Chao WS, Foley ME, Hernandez AG, Thimmapuram J, Liu L, Gong GL, Band M, Kim R, Mikel MA (2007) Characterization of an EST database for the perennial weed leafy spurge: an important resource for weed biology research. Weed Sci 55:193–203

Anderson JV, Horvath DP, Chao WS, Foley ME (2010) Bud dormancy in perennial plants: a mechanism for survival. In: Lubzens E, Cerda J, Clark M (eds) Dormancy and resistance in harsh environments. Topics in current genetics 21, Chapter 5, Hohmann S (series ed). Springer, Berlin, pp 69–90

Bielenberg DG, Wang YE, Li Z, Zhebentyayeva T, Fan S, Reighard GL, Scorza R, Abbott AG (2008) Sequencing and annotation of the evergrowing locus in peach [*Prunus persica* (L.) Batsch] reveals a cluster of six MADS-box transcription factors as candidate genes for regulation of terminal bud formation. Tree Genetics Genomes 4:495–507

Böhlenius H, Huang T, Charbonnel-Campaa L, Brunner AM, Jansson S, Strauss SH, Nilsson O (2006) CO/FT regulatory module controls timing of flowering and seasonal growth cessation in trees. Science 312:1040–1043

Caicedo AL, Richards C, Ehrenreich IM, Purugganan MD (2009) Complex rearrangements lead to novel chimeric gene fusion polymorphisms at the *Arabidopsis thaliana MAF2-5* flowering time gene cluster. Mol Biol Evol 26:699–711

Campbell M, Segear E, Beers L, Knauber D, Suttle J (2008) Dormancy in potato tuber meristems: chemically induced cessation in dormancy matches the natural process based on transcript profiles. Funct Integr Genom 8:317–328

Catalá R, Medina J, Salinas J (2011) Integration of low temperature and light signaling during cold acclimation response in *Arabidopsis*. Proc Natl Acad Sci USA 108:16475–16480

Chang S, Puryear J, Cairney J (1993) A simple and efficient method for isolating RNA from pine trees. Plant Mol Biol Rep 11:113–116

CAB International. (2004) Euphorbia esula[original text by W. Chao & J. V. Anderson]. In: Crop Protection Compendium, 2004 Edition, pp. 1–33. CAB International, Wallingford, UK.

Chao WS, Serpe MD, Anderson JV, Gesch RW, Horvath DP (2006) Sugars, hormones, and environment affect the dormancy status in underground adventitious buds of leafy spurge (*Euphorbia esula* L.). Weed Sci 54:59–68

Chao WS, Doğramacı M, Foley ME, Horvath DP, Anderson JV (2012) Selection and validation of endogenous reference genes for qRT-PCR analysis in leafy spurge (*Euphorbia esula*). PLoS ONE 7:e42839

Chinnusamy V, Zhu J, Zhu J-K (2006) Gene regulation during cold acclimation in plants. Physiol Plant 126:52–61

Chow BY, Sanchez SE, Breton G, Pruneda-Paz JL, Krogan NT, Kay SA (2014) Transcriptional regulation of LUX by CBF1 mediates cold input to the circadian clock in *Arabidopsis*. Curr Biol 24(13):1518–1524

Cluis CP, Mouchel CF, Hardtke CS (2004) The *Arabidopsis* transcription factor HY5 integrates light and hormone signaling pathways. Plant J 38:332–347

Cooke JEK, Eriksson ME, Junttila O (2012) The dynamic nature of bud dormancy in trees: environmental control and molecular mechanisms. Plant Cell Environ 35:1707–1728

Doğramacı M, Horvath DP, Chao WS, Foley ME, Christoffers MJ, Anderson JV (2010) Extended low temperature impacts dormancy status, flowering competence, and transcript profiles in crown buds of leafy spurge. Plant Mol Biol 73:207–226

Doğramacı M, Horvath DP, Christoffers MJ, Anderson JV (2011) Dehydration and vernalization treatments identify overlapping molecular networks impacting endodormancy maintenance in leafy spurge crown buds. Funct Integr Genom 11:611–626

Doğramacı M, Foley ME, Chao WS, Christoffers MJ, Anderson JV (2013) Induction of endodormancy in crown buds of leafy spurge (*Euphorbia esula* L.) implicates a role for ethylene and cross-talk between photoperiod and temperature. Plant Mol Biol 81:577–593

Doğramacı M, Horvath DP, Anderson JV (2014) Dehydration-induced endodormancy in crown buds of leafy spurge highlights involvement of MAF3- and RVE1-like homologs, and hormone signaling cross-talk. Plant Mol Biol 86:409–24, doi:10.1007/s11103-014-0237-2

Dong MA, Farré EM, Thomashow MF (2011) Circadian clock-associated 1 and late elongated hypocotyl regulate expression of the C-repeat binding factor (CBF) pathway in *Arabidopsis*. Proc Natl Acad Sci USA 108:7241–7246

Downs RJ, Bevington JM (1981) Effect of temperature and photoperiod on dormancy of *Betula papyrtfera*. Am J Bot 68:795–800

Favory J, Stec A, Gruber H, Rizzini L, Oravecz A, Funk M, Albert A, Cloix C, Jenkins GI, Oakeley EJ, Seidlitz HK, Nagy F, Ulm R (2009) Interaction of COP1 and UVR8 regulates UV-B-induced photomorphogenesis and stress acclimation in *Arabidopsis*. EMBO J 28:591–601

Fennell A, Hoover E (1991) Photoperiod influences growth, bud dormancy, and cold-acclimation in *Vitis labruscana* and *V. riparia*. J Am Soc Hortic Sci 116(2):270–273

Foley ME, Anderson JV, Horvath DP (2009) The effects of temperature, photoperiod, and vernalization on regrowth and flowering competence in *Euphorbia esula* (Euphorbiaceae) crown buds. Botany 87:986–992

Fowler SG, Cook D, Thomashow MF (2005) Low temperature induction of *Arabidopsis* CBF1, 2, and 3 is gated by the circadian clock. Plant Physiol 137:961–968

Franco-Zorrilla JM, Lopez-Vidriero I, Carrasco JL, Godoy M, Vera P, Solano R (2014) DNA-binding specificities of plant transcription factors and their potential to define target genes. Proc Natl Acad Sci USA 111:2367–2372

Gesch RW, Palmquist D, Anderson JV (2007) Seasonal photosynthesis and partitioning of non-structural carbohydrates in *Euphorbia esula*. Weed Sci 55:346–351

Heide OM (2008) Interaction of photoperiod and temperature in the control of growth and dormancy of *Prunus* species. Sci Hortic 115:309–314

Heide OM, Prestrud AK (2005) Low temperature, but not photoperiod, controls growth cessation and dormancy induction and release in apple and pear. Tree Physiol 25(1):109–114

Henriques R, Jang IC, Chua NH (2009) Regulated proteolysis in light-related signaling pathways. Curr Opin Plant Biol 12:49–56

Heyl A, Ramireddy E, Brenner WG, Riefler M, Allemeersch J, Schmulling T (2008) The transcriptional repressor ARR1-SRDX suppresses pleiotropic cytokinin activities in *Arabidopsis*. Plant Physiol 147:1380–1395

Hirsch S, Oldroyd GED (2009) GRAS-domain transcription factors that regulate plant development. Plant Signal Behav 4(8):698–700

Horvath DP (1999) Role of mature leaves in inhibition of root bud growth in *Euphorbia esula* L. Weed Sci 47:544–550

Horvath DP (2009) Common mechanisms regulate flowering and dormancy. Plant Sci 177:523–531

Horvath DP, Anderson JV (2000) The effects of photosynthesis on underground adventitious shoot bud dormancy/quiescence in leafy spurge (*Euphorbia esula* L.). In: Viemont J-D, Crabbe JJ (eds) 2nd International symposium on plant dormancy: short communications, Presses de L 'Universite d' Angers and CAB International, pp 30–34

Horvath DP, Anderson JV (2002) A molecular approach to understanding root bud dormancy in leafy spurge. Weed Sci 50:227–231

Horvath DP, Anderson JV (2009) Leafy spurge: an emerging model to study traits of perennial weeds. In: Stewart CN Jr, (ed) Weedy and invasive plant genomics, Wiley-Blackwell, pp 113–126. (ISBN: 978-0-8138-2288-4)

Horvath DP, Chao WS, Anderson JV (2002) Molecular analysis of signals controlling dormancy and growth in underground adventitious buds of leafy spurge. Plant Physiol 128:1439–1446

Horvath DP, Anderson JV, Chao WS, Foley ME (2003) Knowing when the grow signals regulating bud dormancy. Trends Plant Sci 8:534–539

Horvath DP, Anderson JV, Jia Y, Chao WS (2005) Cloning, expression, and regulation of CYCLIN D3-2 from leafy spurge (*Euphorbia esula*). Weed Sci 53:431–437

Horvath DP, Anderson JV, Soto-Suarez M, Chao WS (2006) Transcriptome analysis of leafy spurge (*Euphorbia esula*) crown buds during shifts in well-defined phases of dormancy. Weed Sci 54:821–827

Horvath DP, Chao WS, Suttle JC, Thimmapuram J, Anderson JV (2008) Transcriptome analysis identifies novel responses and potential regulatory genes involved in seasonal dormancy transitions of leafy spurge (*Euphorbia esula* L.). BMC Genomics 9:536

Horvath DP, Kudrna D, Talag J, Anderson JV, Chao WS, Wing R, Foley MA, Doğramacı M (2013) BAC library development, and clone characterization for dormancy-responsive *DREB4A*, *DAM*, and *FT* from leafy spurge (*Euphorbia esula* L.) identifies differential splicing and conserved promoter motifs. Weed Sci 61(2):303–309

Hsu CY, Adams JP, Kim H, No K, Ma C, Strauss SH, Drnevich J, Vandervelde L, Ellis JD, Rice BM (2011) FLOWERING LOCUS T duplication coordinates reproductive and vegetative growth in perennial poplar. Proc Natl Acad Sci USA 108:10756–10761

Huang X, Ouyang X, Yang P, Lau OS, Li G, Li J, Chen H, Deng XW (2012) *Arabidopsis* FHY3 and HY5 positively mediate induction of COP1 transcription in response to photomorphogenic UV-B light. Plant Cell 24:4590–4606

Ibáñez C, Ramos A, Acebo P, Contreras A, Casado R, Allona I, Aragoncillo C (2008) Overall alteration of circadian clock gene expression in the chestnut cold response. PLoS ONE 3:e3567. doi:10.1371/journal.pone.0003567

Jiao Y, Lau OS, Deng XW (2007) Light-regulated transcriptional networks in higher plants. Nat Rev Genet 8:217–230

Khan MS (2011) The role of DREB transcription factors in abiotic stress tolerance of plants. Biotechnol Biotechnol Equip 25:2433–2442

Kim HJ, Kim YR, Park JY, Kim J (2002) Light signaling mediated by phytochrome plays an important role in cold induced gene expression through the C-repeat/dehydration responsive element (C/DRE) in *Arabidopsis thaliana*. Plant J 6:693–704

Lang GA, Early JD, Martin GC, Darnell RL (1987) Endo-, para-, and ecodormancy: physiological terminology and classification for dormancy research. Hort Sci 22:371–377

Lee CM, Thomashow MF (2012) Photoperiodic regulation of C-repeat binding factor (CBF) cold acclimation pathway and freezing tolerance in *Arabidopsis thaliana*. Proc Natl Acad Sci USA 109:15054–15059

Lee J, He K, Stolc V, Lee H, Figueroa P, Gao Y, Tongprasit W, Zhao H, Lee I, Deng XW (2007) Analysis of transcription factor HY5 genomic binding sites revealed its hierarchical role in light regulation of development. Plant Cell 19:731–749

Lokko Y, Anderson JV, Rudd S, Raji A, Horvath D, Mikel MA, Kim R, Liu L, Hernandez A, Dixon AGO, Ingelbrecht I (2007) Characterization of an 18,166 EST dataset for cassava (*Manihot esculenta* Crantz) enriched for drought-responsive genes. Plant Cell Rep 26:1605–1618

Mason MG, Ross JJ, Babst BA, Wienclaw BN, Beveridge CA (2014) Sugar demand, not auxin, is the initial regulator of apical dominance. Proc Natl Acad Sci USA 111:6092–6097

Mikkelsen MD, Thomashow MF (2009) A role for circadian evening elements in cold-regulated gene expression in *Arabidopsis*. Plant J 60:328–339

Mizoi J, Shinozaki K, Yamaguchi-Shinozaki K (2012) AP2/ERF family transcription factors in plant abiotic stress responses. Biochim Biophys Acta 1819:86–96

Mizoi J, Ohori T, Moriwaki T, Kidokoro S, Todaka D, Maruyama K, Kusakabe K, Osakabe Y, Shinozaki K, Yamaguchi-Shinozaki K (2013) GmDREB2A;2, a canonical DEHYDRATION-RESPONSIVE ELEMENT-BINDING PROTEIN2-type transcription factor in soybean, is posttranslationally regulated and mediates dehydration-responsive element-dependent gene expression. Plant Physiol 161:346–361

Moon J, Parry G, Estelle M (2004) The ubiquitin-proteasome pathway and plant development. Plant Cell 16:3181–3195

Nakashima K, Ito Y, Yamaguchi-Shinozaki K (2009) Transcriptional regulatory networks in response to abiotic stresses in *Arabidopsis* and grasses. Plant Physiol 149:88–95

Ohme-Tagaki M, Suzuki K, Shinshi H (2000) Regulation of ethylene-induced transcription of defense genes. Plant Cell Physiol 41:1187–1192

Posé D, Verhage L, Ott F, Yant L, Mathieu J, Angenent GC, Immink RG, Schmid M (2013) Temperature-dependent regulation of flowering by antagonistic FLM variants. Nature 503: 414–417

Ramos A, Pérez-Solís E, Ibáñez C, Casado R, Collada C, Gomez L, Aragoncillo C, Allona I (2005) Winter disruption of the circadian clock in chestnut. Proc Natl Acad Sci USA 102:7037–7042. doi:10.1073/pnas.0408549102

Ratcliffe OJ, Kuminoto RW, Wong BJ, Riechmann JL (2003) Analysis of the *Arabidopsis* MADS affecting flowering gene family: MAF2 prevents vernalization by short periods of cold. Plant Cell 15:1159–1169

Rawat R, Schwartz J, Jones MA, Sairanen I, Cheng Y, Andersson CR, Zhao Y, Ljung K, Harmer SL (2009) REVEILLE1, a MYB-like transcription factor, integrates the circadian clock and auxin pathways. Proc Natl Acad Sci USA 106:16883–16888

Reeves PA, He Y, Schmitz RJ, Amasino RM, Panella LW, Richards CM (2007) Evolutionary conservation of the flowering locus C-mediated vernalization response: evidence from sugar beet (*Beta vulgaris*). Genetics 176:295–307

Rinne PLH, Welling A, van der Schoot C (2010) Perennial life style of *Populus*: dormancy cycling and overwintering. In: Jansson S, Bhalerao RP, Groover AT (eds) Genetics and genomics of *Populus*. Springer, New York, pp 171–200

Rios G, Leida C, Conejero A, Badenes ML (2014) Epigenetic regulation of bud dormancy events in perennial plants. Front Plant Sci 5:247

Rohde A, Bhalerao RP (2007) Plant dormancy in the perennial context. Trends Plant Sci 12:217–223

Ruby JG, Bellare P, DeRisi JL (2013) PRICE: software for the targeted assembly of components of (meta) genomic sequence data. G3 (Bethesda) 3:865–880

Ruttink T, Arend M, Morreel K, Storme V, Rombauts S, Fromm J, Bhalerao RP, Boerjan W, Rohde A (2007) A molecular timetable for apical bud formation and dormancy induction in poplar. Plant Cell 19:2370–2390

Saijo Y, Sullivan JA, Wang H, Yang J, Shen Y, Rubio V, Ma L, Hoecker U, Deng XW (2003) The COP1-SPA1 interaction defines a critical step in phytochrome A-mediated regulation of HY5 activity. Genes Dev 17:2642–2647

Saito T, Bai S, Ito A, Imai T, Nakajima I, Moriguchi T (2013) Regulatory mechanism of dormancy associated MADS-box gene in Japanese pear. In, Abstract and Programme Book, 5th International Plant Dormancy Symposium 2013, Auckland, New Zealand, p 44

Saito T, Bai S, Imia T, Ito A, Nakajima I, Moriguchi T (2014) Histone modification and signalling cascade of the dormancy-associated MADS-box gene, *PpMADS13-1*, in Japanese pear (*Pyrus pyrifolia*) during endodormancy. Plant, Cell & Environment. doi: 10.1111/pce.12469

Seo E, Lee H, Jeon J, Park H, Kim J, Noh YS, Lee I (2009) Crosstalk between cold response and flowering in *Arabidopsis* is mediated through the flowering-time gene *SOC1* and its upstream negative regulator FLC. Plant Cell 21:3185–3197

Severing EI, van Dijk ADJ, Morabito G, Busscher-Lange J, Immink RGH, van Ham RCHJ (2012) Predicting the impact of alternative splicing on plant MADS domain protein function PLoS ONE 7: e30524

Sharma N, Russell SD, Bhalla PL, Singh MB (2011) Putative cis-regulatory elements in genes highly expressed in rice sperm cells. BMC Res Notes 4:319

Suttle JC (1998) Involvement of ethylene in potato microtuber dormancy. Plant Physiol 118:843–848

Tanino KK, Kalcsits L, Silim S, Kendall E, Gray GR (2010) Temperature-driven plasticity in growth cessation and dormancy development in deciduous woody plants: a working hypothesis suggesting how molecular and cellular function is affected by temperature during dormancy induction. Plant Mol Biol 73:49–65

Thomashow MF (2010) Molecular basis of plant cold acclimation: insights gained from studying the CBF cold-response pathway. Plant Physiol 154:571–577

Wang KLC, Yoshida H, Lurin C, Ecker JR (2004) Regulation of ethylene gas biosynthesis by the *Arabidopsis* ETO1 protein. Nature 428:945–950

Wang Y, Liu C, Li K, Sun F, Hu H, Li X, Zhao Y, Han C, Zhang W, Buan Y, Liu M, Li X (2007) *Arabidopsis* EIN2 modulates stress response through abscisic acid responses pathway. Plant Mol Biol 64:633–644

Welling A, Kaikuranta P, Rinne P (1997) Photoperiodic induction of dormancy and freezing tolerance in *Betula pubescens*. Involvement of ABA and dehydrins. Physiol Plant 100:119–125

Whalley HJ, Sargeant AW, Steele JF, Lacoere T, Lamb R, Saunders NJ, Knight H, Knight MR (2011) Transcriptomic analysis reveals calcium regulation of specific promoter motifs in *Arabidopsis*. Plant Cell 23:4079–4095

Wisniewski M, Norelli J, Bassett C, Artlip T, Macarisin D (2011) Ectopic expression of a novel peach (*Prunus persica*) CBF transcription factor in apple (*Malus* × *domestica*) results in short-day induced dormancy and increased cold hardiness. Planta 233:971–83

Xu ZS, Chen M, Li LC, Ma YZ (2011) Functions and application of the AP2/ERF transcription factor family in crop improvement. J Integr Plant Biol 53:570–585

Yamaguchi-Shinozaki K, Shinozaki K (1993) The plant hormone abscisic acid mediates the drought-induced expression but not the seed-specific expression of rd22, a gene responsive to dehydration stress in *Arabidopsis thaliana*. Mol Gen Genet 238:17–25

Yamane H (2014) Regulation of bud dormancy and bud break in Japanese apricot (*Prunus mume* Siebold & Zucc.) and peach [*Prunus persica* (L.) Batsch]: a summary of recent studies. J Japan Soc Hort Sci 83(3):187–202

Part IV
Comparison of Seed and Bud Dormancy Mechanisms

Chapter 13
A Comparison of Transcriptomes Between Germinating Seeds and Growing Axillary Buds of Arabidopsis

Dawei Yan, Kiyoshi Tatematsu, Kazumi Nakabayashi, Akira Endo, Masanori Okamoto and Eiji Nambara

Abbreviations

ABA	Abscisic acid
ABRE	Abscisic acid responsive element
ABRC	ABA-response complex
BRC1	BRANCHED1
CE	Coupling element
FR	Far-red light
GA	Gibberellin
GO	Gene ontology
MAX	MORE AXILLARY GROWTH
R	Red light
TCP	TEOSINTE BRANCHED1/CYCLOIDEA/PCF

E. Nambara (✉) · D. Yan · A. Endo
Department of Cell & Systems Biology, University of Toronto, Toronto, Canada
email: eiji.nambara@utoronto.ca

K. Tatematsu
Laboratory of Plant Organ Development, National Institute for Basic Biology, Okazaki, Japan

K. Nakabayashi
Department of Plant Breeding and Genetics, Max Planck Institute for Plant Breeding Research, Köln, Germany

A. Endo
Present address; Crop Breeding Research Division, National Agriculture and Food Research Organization (NARO), Naro, Japan

M. Okamoto
Arid Land Research Center, Tottori University, Tottori, Japan

© Springer International Publishing Switzerland 2015

J. V. Anderson (ed.), *Advances in Plant Dormancy,* DOI 10.1007/978-3-319-14451-1_13

Part I: Growth Regulation in Seeds and Buds

Growth regulation is essential for both plant architecture and acquisition of stress tolerance. Plant growth in an organ is influenced by other organs in the same body as well as by changing environment. Dormancy is a type of growth arrest and is recognized as an adaptive trait to control timing of the growth for better survival and production of offspring. Dormancy was defined as "the temporary suspension of visible growth of any plant structure containing a meristem" (Lang et al. 1987). In plants, dormancy is observed in several organs such as seeds and axillary buds. The mechanisms that control growth and dormancy in seeds and buds have been investigated with great interest, and more recent functional genomics research has revealed novel regulators of growth and their interactions. This article summarizes the current knowledge on growth regulation in seeds and buds with focus on Arabidopsis.

Germination of mature dry seeds involves resumption of embryo growth after a period of dry quiescent state (Bewley 1997). Seed dormancy is the inability of seeds to germinate even under favorable conditions. Germination of mature dry seeds starts with water uptake and ends with the emergence of radicle. Germination proceeds when the embryonic growth overcomes the mechanical constraint imposed by the endosperm/seed coat. Seed germination is regulated by finely orchestrated mechanisms involving two antagonistic plant hormones, abscisic acid (ABA), and gibberellins (GAs), and environmental cues including light, temperature, nutrients, and water (Yamaguchi 2008; Nambara et al. 2010). One of the target sites of these germination regulators is the endosperm that plays an important role in regulating the embryonic growth by supplying nutrients during seed development and restricting embryo growth (Sørensen et al. 2002; Bethke et al. 2007), and mediating the communication between the embryo and surrounding environment (Lee et al. 2010; Lee et al. 2012; Yan et al. 2014). In contrast to the role of the endosperm, molecular mechanisms that regulate embryonic growth potential and seed vigor remain unclear.

In Arabidopsis, the radicle and embryonic axis (hypocotyl) are the primary sites for the regulation of embryonic growth during germination. These tissues express cell wall-modifying enzymes required for cell elongation during germination (Ogawa et al. 2003; Barroco et al. 2005; Iglesias-Fernandez et al. 2011). In addition, the radicle is thought to play a role in weakening the micropylar endosperm and genetic evidence has shown that endosperm plays an active role in the regulation of seed germination (Penfield et al. 2006; Muller et al. 2006). The lower part of hypocotyl and the adjacent transition zone were observed to be active in cell elongation during germination (Sliwinska et al. 2009), while cotyledons are the storage tissue for seed reserves and act as a nutrient source. Cell elongation is a primary outcome of embryonic growth potential and seed vigor during Arabidopsis germination. Most embryonic cells in dry Arabidopsis seeds are arrested in the G1 phase, followed by

DNA replication initiated at the start of radicle protrusion, then, mitotic cell division occurs after the completion of seed germination (Barroco et al. 2005). Random organization of microtubules (MT) is formed shortly after the start of imbibition, and MTs are progressively aligned in a transverse orientation in the radicles of imbibed seeds, followed by the increase in the number of MTs during radicle protrusion (Barroco et al. 2005). It is worth mentioning that timing of activating each cell cycle process during germination varies among seed tissues and plant species, as well as physiological and imbibition conditions (Liu et al. 1997; Gornik et al. 1997; Sliwinska et al. 1999; de Castro et al. 2000).

Similar to seed germination, axillary bud outgrowth is under the control of both endogenous signals and environmental factors (Horvath et al. 2003). The axillary bud lies at the axil of a leaf and is capable of developing into a branch shoot or flower cluster. Axillary buds outgrowth is inhibited by auxin derived from the shoot apical meristem, which is known as apical dominance (Cline 1991, 1997; Dun et al. 2006). Plant hormones, in particular, auxin, cytokinins, and strigolactones, are involved in this process (Shimizu-Sato and Mori 2001). Axillary bud dormancy has been extensively analyzed in garden pea. Mason et al. (2014) reported that sugar availability to axillary buds is the determinant for the maintenance of apical dominance of pea. In pea, the primary checkpoint of the cell cycle regulation in the axillary buds seems to be at the G1 phase, and decapitation induces DNA synthesis followed by mitotic division (Devitt et al. 1995; Shimizu and Mori 1998). Importantly, the checkpoints of cell cycle arrest in dormant buds depend on developmental maturity (Shimizu and Mori 1998, references therein). Molecular markers whose expression is associated with bud dormancy have been reported in pea. The dormant axillary bud of pea accumulates abundant transcripts for PsDRM1, PsDRM2, PsAD1, and PsAD2 (Stafstrom et al. 1998; Madoka and Mori 2000). The homologues of these genes are often used as dormancy markers in other plant species including Arabidopsis (Tatematsu et al. 2005; Rae et al. 2013).

The molecular mechanisms of growth regulation are complex and have multiple checkpoints, although are recognized by the simple outputs such as the increase in cell numbers, cell elongation, or the increase in biomass. In order to dissect these complex phenomena, it is essential to define robust markers that reflect the output response (i.e., growth). Transcriptome analysis is suitable for this purpose, because this enables simultaneous quantification in the levels of a large number of transcripts (markers). Another merit of transcriptome data is to allow comparison of transcript profiles among different organs or species. The comparison of different growing organs provides common and organ-specific expression patterns of the markers. Moreover, co-expressed genes in different growing organs provide an insight into how these genes are regulated through over-representation of particular *cis*-acting elements in their promoters.

Part II: Comparison of Growth Regulation Between Seed Germination and Axillary Bud Outgrowth

Genetic analysis has revealed that some growth regulators modulate both seed germination and axillary bud outgrowth. ABA is required for the induction and maintenance of seed dormancy and the inhibition of germination (Finkelstein et al. 2008; Nambara et al. 2010). ABA also inhibits lateral and axillary bud growth and shoot branching (Chatfield et al. 2000; Reddy et al. 2013). Plants sense red light (R) and far-red light (FR) by photoreceptor phytochromes. A change in the ratio of R to FR regulates seed germination through promoting the degradation of PIF3-like 5 (PIL5) bHLH transcription factor, which promotes ABA accumulation and represses GA accumulation in Arabidopsis seeds (Oh et al. 2006). Light also serves as an indicator of impending shading by neighboring vegetation and repress bud outgrowth. Low R/FR represses axillary shoot outgrowth, and the increase in the R/FR promotes the growth. The fact that ABA levels correlate negatively with bud growth and increasing R/FR causes a reduction in ABA levels indicates that ABA regulates this process in response to R/FR (Reddy et al. 2013).

Strigolactones were originally identified as germination stimulators of parasitic weeds produced by host plants. They were later shown to be the growth regulator of the host plant itself and involved in inhibiting axillary bud outgrowth (Gomez-Roldan et al. 2008; Umehara et al. 2008; Domagalska and Leyser 2011). Characterization of *MORE AXILLARY GROWTH* (*MAX*) in Arabidopsis and their orthologous genes in other species demonstrates that strigolactones regulate axillary bud outgrowth and shoot branching in an auxin-dependent manner (Brewer et al. 2009). A TEOSINTE BRANCHED1/CYCLOIDEA/PCF (TCP) transcription factor, BRANCHED1 (BRC1), is an Arabidopsis orthologue of maize *teosinte branched1* (*tb1*) that represses axillary bud growth (Studer et al. 2011; Aguilar-Martinez et al. 2007; Gonzalez-Grandio et al. 2013). The expression of *BRC1* is suppressed in *max* mutants indicating that it acts downstream of strigolactones to repress shoot branching in Arabidopsis (Aguilar-Martinez et al. 2007). Genetic analysis indicates that strigolactone-deficient and insensitive mutants of Arabidopsis show germination phenotypes; thus, it promotes seed germination under some conditions (Tsuchiya and McCourt 2009; Tsuchiya et al. 2010; Toh et al. 2012).

Available microarray data are useful for comparison of transcriptomes between seed germination and axillary bud outgrowth. Two studies have reported transcriptome analysis of Arabidopsis axillary bud outgrowth (Tatematsu et al. 2005; Reddy et al. 2013). Axillary buds outgrowth is induced by decapitation of the main shoot (Tatematsu et al. 2005) or by increasing the R/FR ratio (Reddy et al. 2013), in which the changes in transcription profile are observed within 6 h and 3 h after the treatment, respectively. Many research groups have reported transcriptome analysis of Arabidopsis seed germination. In germinating seeds, reprograming the expression of genes from seed maturation to germination such as those involved in DNA processing, transcription and protein synthesis has been seen as early as 6 h postimbibition (Nakabayashi et al. 2005). Further, transcriptome data from germinating

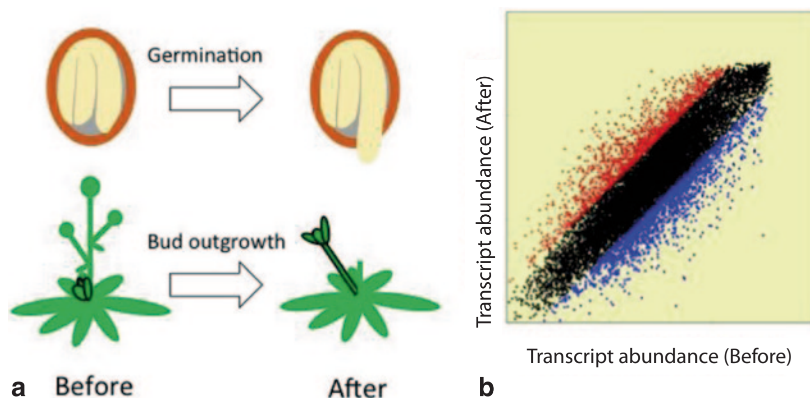


Fig. 13.1 Comparative transcriptome analysis. **a** Germination (*top*) and axillary bud outgrowth (*bottom*). Analysis of the microarray data was performed on two physiological processes. Seed germination (before: dry seed; after: 24-h imbibed seed), axillary bud outgrowth (before: axillary buds from intact plants; after: axillary buds from plants 24-h after decapitation). **b** A typical microarray result plotted by the expression level before treatment and 24-h after treatment. Each dot represents the expression level of a single gene. *Blue* and *red dots* represent genes with increased transcript abundance before and after treatment, respectively

seeds and axillary bud outgrowth were compared (Tatematsu et al. 2005, 2008a, b). Both dry seeds and axillary buds of intact plants are in the quiescent state, and each growth-inducing treatment (i.e., imbibition for seeds and decapitation for axillary buds) triggers resumption of growth (Fig. 13.1a). Down-regulated genes represent those that are highly expressed in the quiescent organ, and up-regulated genes are associated with resumption of growth (Fig. 13.1b). There are significant overlaps in changes of transcriptomes observed during seed germination and axillary buds outgrowth.

In silico promoter analysis identified two *cis*-acting elements, Up1 and Up2, overrepresented in the 500-bp promoters of genes whose transcripts showed increased abundance, by microarray analysis, after decapitation (Tatematsu et al. 2005). A synthetic promoter (proU1U2) made from repeats of Up1 and Up2 responded to decapitation and activated the reporter gene expression; however, the Up1 or Up2 repeat alone was unaffected by decapitation (Fig. 13.2; Tatematsu et al. 2005). It is worth noting that both Up1 and Up2 were also significantly over-represented in the upstream regions of up-regulated genes during germination (Tatematsu et al. 2008b). Mutational analysis of a native Up1/Up2-containing promoter of *RPL15B* has shown that both Up1 and Up2 are necessary for the induction of gene expression in axillary buds, while Up1 alone is sufficient to activate germination-associated transcription (Tatematsu et al. 2005, 2008b). Some conclusions can be drawn by using the synthetic promoters containing Up1 and Up2 (Fig. 13.2). Up1 is similar to the site II motif known to be a potential target for a TCP transcription factor, while Up2 resembles the *telo*-box, the target site of the AtPure, a transcriptional regulator for protein synthesis and cell cycle-related

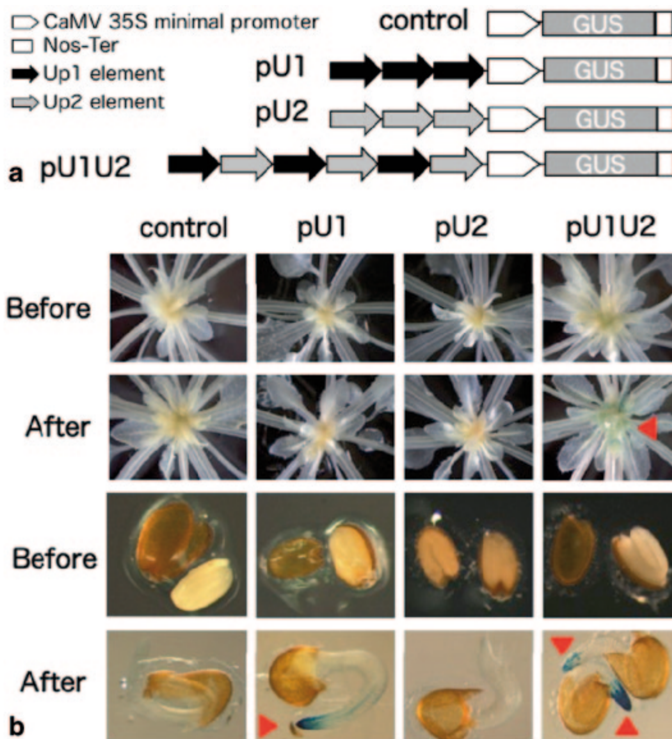


Fig. 13.2 Functional analysis of synthetic promoters driven by Up1 alone, Up2 alone, and both Up1 and Up2 elements. **a** Structure of synthetic promoter: β-glucuronidase (GUS). Synthetic promoters are made with three tandem repeats of Up elements with a Cauliflower Mosaic Virus 35S minimal promoter (90-bp). GUS reporter gene contains the Nos terminator at the 3′ end. **b** GUS expression in the transgenic lines harboring control, proU1, proU2, and proU1U2 genes. From top to bottom: axillary buds of intact plants (before), axillary buds of decapitated plants (after), dry seeds (before), 48-h imbibed germinated seedlings (after). *Arrowheads* indicate GUS expression

genes. These two motifs coexist in the promoters of approximate 70 % of 216 Arabidopsis ribosomal protein genes (Tremousaygue et al. 2003). Indeed, up-regulated genes in buds and seeds include a large number of ribosomal protein and cell cycle-related genes (Tatematsu et al. 2008a).

Genetic analysis also indicates the involvement of TCP transcription factors in growth regulation of axillary buds and seeds. The Arabidopsis genome contains 24 TCP genes, which are divided into two groups, class I and class II. Rice PCFs, positive regulators of cell proliferation, are the class I TCPs (Kosugi and Ohashi, 1997), while maize TB1 and snapdragon CYC, growth repressors of reproductive organs, belong to the class II TCPs (Doebley et al. 1997; Howarth and Donoghue 2006). Arabidopsis TCP12/BRC1 and TCP18/BRC2, members of the class II TCPs, were shown to be involved in the inhibition of axillary bud outgrowth (Aguilar-Martinez et al. 2007), similar to maize TB1. Arabidopsis TCP14, a

member of the class I TCPs, that is expressed most abundantly in germinating seeds promotes embryonic growth during germination (Tatematsu et al. 2008b).

Genes that were down-regulated during germination contain an over-represented number of ABA-responsive elements (ABREs) in their promoters (Nakabayashi et al. 2005). Over-representation of the ABRE is prominent for genes with abundant mRNA accumulation in the dry seed, while these genes are down-regulated in response to seed imbibition. At the same time, no over-representation of ABREs can be seen in the promoters of genes highly expressed in 6-h imbibed seeds. This is consistent with the remarkable reduction in ABA levels observed within 6 hours after the start of seed imbibition (Nakabayashi et al. 2005). The ABRE is known to form a functional ABA response complex (ABRC) with other *cis*-acting elements or with itself (Shen and Ho 1995). The seed-specific enhancer RY/Sph motif and the coupling element (CE) were experimentally shown to function synergistically with the ABRE (Shen et al. 1996). Both ABREs and RY/Sph motif are overrepresented in genes highly expressed in the dry seed while the CE alone does not follow this pattern (Nakabayashi et al. 2005). The association of ABRE with CE, RY motif, or ABRE itself facilitates strong gene expression in the dry seed.

Reddy et al. (2013) reported transcriptome analysis on axillary bud outgrowth of Arabidopsis triggered by the increase in R/FR ratio. Down-regulated genes were over-represented in the GO category of ABA function. Consistent with the transcriptome data, ABA-deficient mutants display enhanced axillary bud outgrowth under nonpermissive (low R/FR ratio) condition. Indeed, the decapitation-induced transcriptome of axillary buds was also over-represented with ABRE among down-regulated genes, but with abundant expression in the quiescent bud (Fig. 13.3).

Part III: Conclusions and Prospective

Understanding growth regulation is essential for plant biology and biotechnology. Recent genome sequencing efforts demonstrate that the complex plant life is programmed within only 30,000–50,000 genes. Therefore, it is reasonable to speculate that each gene functions in multiple processes in plant life. Genetic analysis is a powerful approach to find a growth regulator commonly acting in multiple physiological processes. For example, *max2* mutants display phenotypes in both germination and axillary bud outgrowth, indicating that MAX2 is a common regulator for these processes. Although genetic and reverse-genetic approach is powerful, it is sensitive to gene redundancy. In addition, some plant species are not suitable for genetic analysis or making transgenic plants. Other complementary approaches might help to better understand the growth regulation in plants. Comparison of transcriptomes is suitable for this approach, because it provides the common molecular markers and *cis*-acting elements from different sets of microarray data rather than simply providing differentially expressed gene lists. Transcriptomes from different organs can be compared to find common processes associated with growth

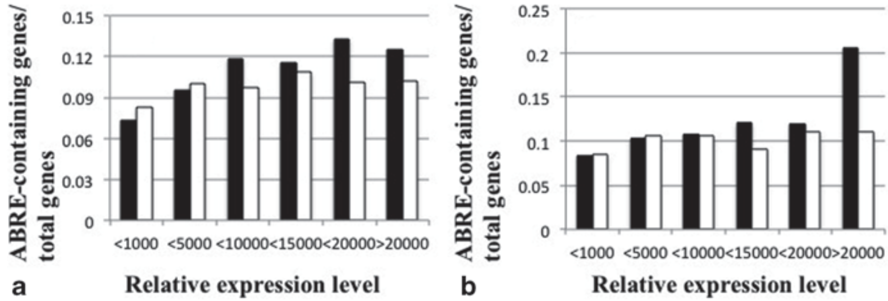


Fig. 13.3 Enrichment of ABRE in the promoters of genes highly expressed in quiescent organs. X-axis indicates the range of expression levels. Ratios of the number of genes with ABRE-containing promoters to the total number of genes in each expression range. The 1000-bp promoters having ABRE (MCACGTGK) were searched at the Patmatch Web site (http://www.arabidopsis.org/cgi-bin/patmatch/nph-patmatch.pl). *Black* and *white bars* indicate the ratios of ABRE-containing genes before and after treatment, respectively. **a** Axillary bud outgrowth. Genes down-regulated in axillary buds 24 h after decapitation were used. Expression levels were obtained from Tatematsu et al. (2005). **b** Seed germination. Gene down-regulated in 24 h-imbibed seeds were used. Expression levels were obtained from Nakabayashi et al. (2005)

regulation. Furthermore, it has a potential to identify transcription factors that play a role through *cis*-acting elements. Noteworthy, transcriptome-based comparison and genetic analysis have different merits and act complementary to each other for understanding molecular mechanisms of growth.

Comparison of transcriptomes is useful for filling a gap of knowledge on molecular mechanisms that control plant growth. It provides us molecular markers for dissecting common and organ-specific mechanisms. In addition, comparison of transcriptomes from fully genome-sequenced plants allows examining *cis*-acting elements that are involved in determining transcriptome patterns. It is likely that currently identified *cis*-acting elements are not sufficient for explaining transcriptional regulation associated with plant growth. Therefore, improvement of tools for in silico analysis will lead to a better prediction of the novel *cis*-acting elements that are involved in common and organ-specific growth regulation. It is important to link these regulatory elements to actual downstream growth regulation. Functional analysis of identified genes/regulators in combination with cell biology and physiological analysis will be necessary to validate the findings from comparative transcriptome analysis.

Acknowledgements The authors acknowledge Professor Belay Ayele (Department of Plant Sciences, University of Manitoba) for valuable comments on this manuscript. This work was financially supported by the Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant and NSERC Strategic Project Grant to E.N. [grant number: RGPIN-2014-03621, STPGP 397474-10]

References

Aguilar-Martinez JA, Poza-Carrion C, Cubas P (2007) Arabidopsis BRANCHED1 acts as an integrator of branching signals within axillary buds. Plant Cell 19:458–472

Barroco RM, Van Poucke K, Bergervoet JH, De Veylder L, Groot SP, Inze D et al (2005) The role of the cell cycle machinery in resumption of postembryonic development. Plant Physiol 137:127–140

Bethke PC, Libourel IGL, Aoyama N, Chung Y-Y, Still DW, Jones RL (2007) The Arabidopsis aleurone layer responds to nitric oxide, gibberellin, and abscisic acid and is sufficient and necessary for seed dormancy. Plant Physiol 143:1173–1188

Bewley JD (1997) Seed germination and dormancy. Plant Cell 9:1055–1066

Brewer PB, Dun EA, Ferguson BJ, Rameau C, Beveridge CA (2009) Strigolactone acts downstream of auxin to regulate bud outgrowth in pea and Arabidopsis. Plant Physiol 150:482–493

Chatfield SP, Stirnberg P, Forde BG, Leyser O (2000) The hormonal regulation of axillary bud growth in Arabidopsis. Plant J 24:159–169

Cline MG (1991) Apical dominance. Bot Rev 57:318–358

Cline MG (1997) Concepts and terminology of apical dominance. Am J Bot 84:1064–1069

de Castro RD, van Lammeren AAM, Groot SPC, Bino RJ, Hilhorst HWM (2000). Cell division and subsequent radicle protrusion in tomato seeds are inhibited by osmotic stress but DNA synthesis and formation of microtubular cytoskeleton are not. Plant Physiol 122:327–335

Devitt ML, Stafsrom JP (1995) Cell cycle regulation during growth-dormancy cycles in pea axillary buds. Plant Mol Biol 29:255–265

Doebley EA, Stec A, Hubbard L (1997) The evolution of apical dominance in maize. Nature 386:485–488

Domagalska MA, Leyser O (2011) Signal integration in the control of shoot branching. Nat Rev Mol Cell Biol 12:211–221

Dun EA, Ferguson BJ, Beveridge CA (2006) Apical dominance and shoot branching. Divergent opinions or divergent mechanisms? Plant Physiol 142:812–819

Finkelstein R, Reeves W, Ariizumi T, Steber C (2008) Molecular aspects of seed dormancy. Annu Rev Plant Biol 59:387–415

Gomez-Roldan V, Fermas S, Brewer PB, Puech-Pages V, Dun EA, Pillot JP, Letisse F, Matusova R, Danoun S, Portais JC, Bouwmeester H, Becard G, Beveridge CA, Rameau C, Rochange SF (2008) Strigolactone inhibition of shoot branching. Nature 456:189–194

Gonzalez-Grandio E, Poza-Carrion C, Sorzano CO, Cubas P (2013) BRANCHED1 promotes axillary bud dormancy in response to shade in Arabidopsis. Plant Cell 25:834–850

Gornik K, de Castro RD, Liu Y, Bino YL, Groot SPC (1997). Inhibition of cell division during cabbage (Brassica oleracea L.) seed germination. Seed Sci Res 7:333–340

Penfield S, Alison YL, Gilday AD, Graham S, Graham IA (2006) Arabidopsis ABA INSENSITIVE4 regulates lipid mobilization in the embryo and reveals repression of seed germination by the endosperm. Plant Cell 18:1887–1899

Horvath DP, Anderson JV, Chao WS, Foley ME (2003) Knowing when to grow: signals regulating bud dormancy. Trends Plant Sci 8:534–540

Howarth DG, Donoghue MJ (2006) Phylogenetic analysis of the "ECE" (CYC1/TB1) clade reveals duplications predating the core eudicots. Proc Natl Acad Sci U S A 103:9101–9106

Iglesias-Fernandez R, Rodrıguez-Gacio MC, Barrero-Sicilia C, Carbonero P, Matilla A (2011) Three endo- β-mannanase genes expressed in the micropylar endosperm and in the radicle influence germination of *Arabidopsis thaliana* seeds. Planta 233:25–36

Kosugi S, Ohashi Y (1997) PCF1 and PCF2 specifically bind to cis elements in the rice proliferating cell nuclear antigen gene. Plant Cell 9:1607–1619

Lang GA, Early JD, Martin GC, Darnell RL (1987) Endo-, Para-, and eco-dormancy: physiological terminology and classification for dormancy research. Hortscience 22:371–377

Lee KP, Piskurewicz U, Tureckova V, Strnad M, Lopez-Molina L (2010) A seed coat bedding assay shows that RGL2-dependent release of abscisic acid by the endosperm controls embryo growth in Arabidopsis dormant seeds. Proc Natl Acad Sci U S A 107:19108–19113

Lee KP, Piskurewicz U, Tureckova V, Carat S, Chappuis R, Strnad M, Fankhauser C, Lopez-Molina L (2012) Spatially and genetically distinct control of seed germination by phytochromes A and B. Genes Dev 26:1984–1996

Liu Y, Hilhorst HWM, Groot SPC, Bino R (1997) Amounts of nuclear DNA and internal morphology of gibberellin- and abscisic acid-deficient tomato (*Lycopersicon esculentum* Mill.) seeds during maturation, imbibition and germination. Annals Bot 79:161–168

Modoka Y, Mori H (2000) Two novel transcripts expressed in pea dormant axillary buds. Plant Cell Physiol 41:274–281

Mason MG, Ross JJ, Babst BA, Wienclaw BN, Beveridge CA (2014) Sugar demand, not auxin, is the initial regulator of apical dominance. Proc Natl Acad Sci U S A. doi:10.1073/pnas.1322045111

Muller K, Tintelnot S, Leubner-Metzger G (2006) Endosperm-limited Brassicaceae seed germination: abscisic acid inhibits embryo-induced endosperm weakening of *Lepidium sativum* (cress) and endosperm rupture of cress and *Arabidopsis thaliana*. Plant Cell Physiol 47:864–877

Nakabayashi K, Okamoto M, Koshiba T, Kamiya Y, Nambara E (2005) Genome-wide profiling of stored mRNA in *Arabidopsis thaliana* seed germination: epigenetic and genetic regulation of transcription in seed. Plant J 41:697–709

Nambara E, Okamoto M, Tatematsu K, Yano R, Seo M, Kamiya Y (2010) Abscisic acid and the control of seed dormancy and germination. Seed Sci Res 20:55–67

Oh E, Yamaguchi S, Kamiya Y, Bae G, Chung WI, Choi G (2006) Light activates the degradation of PIL5 protein to promote seed germination through gibberellin in Arabidopsis. Plant J 47:124–139

Ogawa M, Hanada A, Yamauchi Y, Kuwahara A, Kamiya Y, Yamaguchi S (2003) Gibberellin biosynthesis and response during Arabidopsis seed germination. Plant Cell 15:1591–1604

Rae GM, David K, Wood M (2013) The dormancy marker DRM1/ARP associated with dormancy but a broader role *in planta*. Dev Biol J. ID 632524

Reddy SK, Holalu SV, Casal JJ, Finlayson SA (2013) Abscisic acid regulates axillary bud outgrowth responses to the ratio of red to far-red light. Plant Physiol 163:1047–1058

Shen OX, Ho THD (1995) An abscisic-acid response complex consists of a g-box and a novel coupling element. Plant Physiol 108:46–46

Shen QX, Zhang PN, Ho THD (1996) Modular nature of abscisic acid (ABA) response complexes: composite promoter units that are necessary and sufficient for ABA induction of gene expression in barley. Plant Cell 8:1107–1119

Shimizu S, Mori H (1998) Analysis of cycles of dormancy and growth in pea axillary buds based on mRNA accumulation patterns of cell cycle-related genes. Plant Cell Physiol 39:255–262

Shimizu-Sato S, Mori H (2001) Control of outgrowth and dormancy in axillary buds. Plant Physiol 127:1405–1413

Sliwinska E, Jing H-C, Job C, Job D, Bergervoet JHM, Bino RJ, Groot SPC (1999) Effect of harvest time and soaking treatment on cell cycle activitiy in sugarbeet seeds. Seed Sci Res 9:91–99

Sliwinska E, Bassel GW, Bewley JD (2009) Germination of *Arabidopsis thaliana* seeds is not completed as a result of elongation of the radicle but of the adjacent transition zone and lower hypocotyl. J Exp Bot 60:3587–3594

Sørensen MB, Mayer U, Lukowitz W, Robert H, Chambrier P, Jürgens G, Somerville C, Lepiniec L, Berger F (2002) Cellularisation in the endosperm of *Arabidopsis thaliana* is coupled to mitosis and shares multiple components with cytokinesis. Development 129:5567–5576

Stafstrom JP, Ripley BD, Devitt ML, Drake DB (1998) Dormancy-associated gene expression in pea axillary buds. Planta 205:547–552

Studer A, Zhao Q, Ross-Ibarra J, Doebley J (2011) Identification of a functional transposon insertion in the maize domestication gene *tb1*. Nat Genet 43:1160–1163

Tatematsu K, Ward S, Leyser O, Kamiya Y, Nambara E (2005) Identification of cis-elements that regulate gene expression during initiation of axillary bud outgrowth in Arabidopsis. Plant Physiol 138:757–766

Tatematsu K, Kamiya Y, Nambara E (2008a) Co-regulation of ribosomal protein genes as an indicator of growth status. Plant Signal Behav 3:450–452

Tatematsu K, Nakabayashi K, Kamiya Y, Nambara E (2008b) Transcription factor AtTCP14 regulates embryonic growth potential during seed germination in *Arabidopsis thaliana*. Plant J 53:42–52

Toh S, Kamiya Y, Kawakami N, Nambara E, McCourt P, Tsuchiya Y (2012) Thermoinhibition uncovers a role of strigolactones in Arabidopsis seed germination. Plant Cell Physiol 53:107–117

Tsuchiya Y, McCourt P (2009) Strigolactones: a new hormone with a past. Curr Opin Plant Biol 12:556–561.

Tsuchiya Y, Vidaurre D, Toh S, Hanada A, Nambara E, Kamiya Y et al (2010) A small-molecule screen identifies new functions for the plant hormone strigolactone. Nat Chem Biol 6:741–749

Tremousaygue D, Garnier L, Bardet C, Dabos P, Herve C, Lescure B (2003) Internal telomeric repeats and 'TCP domain' protein-binding sites cooperate to regulate gene expression in *Arabidopsis thaliana* cycling cells. Plant J 33:957–966

Umehara M, Hanada A, Yoshida S, Akiyama K, Arite T, Takeda-Kamiya N, Magome H, Kamiya Y, Shirasu K, Yoneyama K, Kyozuka J, Yamaguchi S (2008) Inhibition of shoot branching by new terpenoid plant hormones. Nature 455:195–200

Yamaguchi S (2008) Gibberellin metabolism and its regulation. Annu. Rev. Plant Biol 59:225–251

Yan D, Duermeyer L, Leoveanu C, Nambara E (2014) The functions of the endosperm during seed germination. Plant Cell Physiol. in press

Chapter 14
Dormancy Induction and Release in Buds and Seeds

Wun S. Chao, Münevver Doğramacı, David P. Horvath, Michael E. Foley and James V. Anderson

Introduction—Overall Differences Between Buds and Seeds

Dormancy in both buds and seeds is an important survival mechanism in the life cycle of plants. Seed dormancy ensures distribution of germination in time and space and prevents ill-timed germination either in the fruit or on the ground, whereas bud dormancy inhibits buds from initiating new vegetative growth under favorable or unfavorable environmental conditions.

Although both buds and seeds contain viable shoot meristems, they are very different anatomically. The major difference is that a radicle can be found in seeds, but not in buds; in addition, buds remain on the plants during dormancy induction and release. In contrast, seed dormancy is induced during seed maturation while pods are still attached to the plants, and mature seeds undergo dormancy release processes (after-ripening) after disseminating from the plants. There are also differences in genotypic inheritance; buds are 100 % maternal, whereas only the seed coat (testa) is 100 % maternal in seeds. Other constituents of seeds in flowering plants (Angiosperms) include endosperm and embryo, where endosperm is 66 % maternal/33 % paternal and embryo is 50 % maternal/50 % paternal (Steward 1991). These disparities in structural and genetic constituents might affect signal perception and response differently during dormancy induction and release in buds and seeds.

In *Arabidopsis*, seed dormancy involves at least two sequential phases, arrested embryo growth and embryo dormancy (Raz et al. 2001). Embryo growth (regulated by cell division) is arrested around 10 days after pollination when the embryo is at the mature stage. Embryo dormancy increases in the seed sac until the seed is fully developed and dormancy reaches a maximum in ripe seeds. Dormancy in seeds is defined as a developmental state in which a viable seed fails to germinate

W. S. Chao (✉) · M. Doğramacı · D. P. Horvath · M. E. Foley · J. V. Anderson
USDA-Agricultural Research Service, Biosciences Research Lab, Sunflower and Plant Biology Research Unit, 1605 Albrecht Boulevard N., Fargo, ND 58102, USA
e-mail: wun.chao@ars.usda.gov

© Springer International Publishing Switzerland 2015

J. V. Anderson (ed.), *Advances in Plant Dormancy,* DOI 10.1007/978-3-319-14451-1_14

under favorable environmental conditions (Bewley 1997). Seed dormancy is also determined by both morphological and physiological properties, and includes five dormancy classes, namely physiological (PD), morphological (MD), morphophysiological (MPD), physical (PY), and combinational (PY + PD) dormancy (Baskin and Baskin 2004; Nikolaeva 2004; Finch-Savage and Leubner-Metzger 2006). PD, including deep and non-deep dormancy, is the most common form of dormancy and can be released by cold or warm stratification. MD is caused by underdevelopment of the embryos, whereas MPD shows characteristics of both PD and MD. PY is caused by seed coat impermeability to water and thus requires mechanical or chemical scarification to break dormancy. PY + PD has characteristics of both PY and PD. In *Arabidopsis*, both testa and endosperm prevent germination of the embryo by providing a physical barrier for radical elongation, and dormancy can be released through moist chilling (stratification) or after-ripening (Debeaujon et al. 2000; Müller et al. 2006). Seed dormancy for leafy spurge (*Euphorbia esula* L.) is classified as PD, and dormancy varies between populations from little or no dormancy to moderate periods of dormancy (Bowes and Thomas 1978; Foley and Chao 2008).

Bud dormancy is defined as the temporary suspension of visible growth of any plant structure containing a meristem, which was further subdivided into three well-defined phases of para-, endo-, and eco-dormancy based on seasonal/environmental, dormancy-imposing stimuli (Lang et al. 1987). Paradormancy is growth cessation controlled by physiological factors within the plant but external to the affected structure, endodormancy is growth cessation controlled by physiological factors internal to the affected structure, and ecodormancy is growth cessation controlled by environmental factors external to the plant (Lang et al. 1987). Dormant buds also have specialized leaves (bud scales) which are known to filter red and far-red light reaching the apical dome (Pukacki et al. 1980).

Buds of annual plants such as pea and *Arabidopsis* have only a paradormancy phase. In contrast, woody perennials such as trees and shrubs have para-, endo-, and ecodormancy in primary and axillary buds. In the herbaceous perennial leafy spurge, all three phases of dormancy occur in underground adventitious buds (referred to in the literature as crown and root buds). However, unlike woody perennials, the aerial portion of this herbaceous perennial senesces and dies in the fall, but buds on the underground crown and root systems become dormant and over-winter potentiating renewed seasonal shoot growth (Anderson et al. 2005).

Although differences in dormancy mechanisms are evident due to drastic anatomical differences between bud and seed, similar pathways and mechanisms involved in controlling dormancy development and release including photoperiod, temperature, hormones, circadian clock, epigenetic regulation, and genes associated with these signals and mechanisms have been identified. This review describes the main findings in those areas, and the focus will be placed on the similarity between bud and seed dormancy (see also Table 14.1). The contents are not meant to be comprehensive but will highlight the analogous key pathways/genes regulating seed and bud dormancy. We will also use leafy spurge, a model herbaceous perennial, as an example to describe commonalities in gene expression and molecular mechanisms during bud and seed dormancy and release.

Table 14.1 Signals and genes affecting bud and seed dormancy

Signals and genes	Tissue	Genes involved (and mentioned in the text)	Roles	References
Temperature and light	Bud	*PHYA*	Endodormancy induction	Kircher et al. 2011
	Seed	*AtGA3OX1*, *AtGA2OX2* *PHYA*, *PHYB*, *PHYE*	Germination induction Germination induction	Yamauchi et al. 2004 Heschel et al. 2008
Phytochrome related	Bud	*PHYA*, *FT*, *CO*	SDP-mediated growth cessation and endodormancy induction	Eriksson and Moritz 2000; Böhlenius et al. 2006; Ruonala et al. 2008
	Seed	*PHYA* *PIL5/PIF1*	Germination suppression Germination induction through phytochrome-mediated degradation of PIL5	Heschel et al. 2008; Footitt et al. 2013 Oh et al. 2006
Circadian clock	Bud	*LHY*, *TOC1/PRR1* *CBF1-3*	Growth cessation, freezing tolerance, bud burst Freezing tolerance	Ibáñez et al. 2010 Dong et al. 2011
	Seed	*LHY*, *CCA1*, *GI*, *TOC/PRR1*, *ZTL*	Dormancy induction and release	Penfield and King 2009; Penfield and Hall 2009
ABA	Bud	*ABI3*	Bud set and dormancy induction	Rohde et al. 2002
	Seed	*ABA1*, *NCEDs*, *ABA2/GIN1/SDR1* *ABI3*, *ABI5* *FUS3*, *LEC1*, *LEC2*	ABA biosynthesis Seed maturation and dormancy induction Embryo growth arrest, seed maturation, dormancy induction, storage protein expression	Nambara and Marion-Poll 2003 Parcy et al. 1994; Lopez-Molina et al. 2002; Holdsworth et al. 2008 Raz et al. 2001; Kagaya et al. 2005; To et al. 2006
GA	Bud	*GA20OX*	SDP-mediated growth cessation and bud formation under low GA levels	Eriksson and Moritz 2002; Cooke et al. 2012
	Seed	*SPT*, *PIL5*, *CTS* *GA3OX*, *CYP707A2*, *MFT*	After-ripening process, inhibition of GA biosynthesis genes (*GA3OX1* and *GA3OX2*) Seed germination	Russell et al. 2000; Penfield et al. 2005; Carrera et al. 2007 Kushiro et al. 2004; Yamauchi et al. 2004; Xi et al. 2010

Table 14.1 (continued)

Signals and genes	Tissue	Genes involved (and mentioned in the text)	Roles	References
MADS domain genes	Bud	*FLC/DAM*	Endodormancy induction	Michaels and Amasino 1999; Bielenberg et al. 2004, 2008; Li et al. 2009
	Seed	*FLC*, *FT*, *SOC1*, *AP1*	Seed maturation and germination	Chiang et al. 2009
Epigenetic regulation	Bud	*HUB2*, *GCN5L* *DAM*, *DAM6*	Dormancy induction and release Dormancy release	Santamaría et al. 2009 Horvath et al. 2010; Leida et al. 2012
	Seed	*HUB1*, *HUB2* *DOG1*	Dormancy induction Dormancy induction	Liu et al. 2007, 2011 Liu et al. 2011

Signals and Genes Affecting Bud and Seed Dormancy

Temperature and Light

Bud Environmental signals, primarily temperature (cold) and light (photoperiod), are the most important signals for dormancy induction in buds, although the extent of these signals in regulating dormancy induction is species dependent. For example, many northern deciduous perennials including poplar (Howe et al. 1995; Jeknić and Chen 1999), birch (Li et al. 2004), red osier dogwood (*Cornus stolonifera* Michx) (Smithberg and Weiser 1968), wild grape (*Vitus riparia*) (Wake and Fennell 2000), and orpine (*Sedum telephium*) (Heide 2001) require short day photoperiods (SDP) at normal growing temperatures to induce endodormancy. Some domesticated grape (*Vitus ssp.*) (Wake and Fennell 2000), Scotch heather (*Calluna vulgaris* L.) (Kwolek and Woolhouse 1982), and leafy spurge (Foley et al. 2009; Doğramacı et al. 2013) require both cold and SDP to induce endodormancy. In some species such as apple, pear (*Pyrus spp.*), and willow (*Salix paraplesia*), temperature alone can induce endodormancy (Heide and Prestrud 2005; Li et al. 2005). Buds respond to photoperiod via PHYTOCHROME A (PHYA) to regulate dormancy development in *Populus* and *Arabidopsis* (Kozarewa et al. 2010; Kircher et al. 2011) and the expression of some cold-responsive genes in silver birch (*Betula pendula*) (Puhakainen et al. 2004).

These environmental signals alter the physiology of these buds, which alters phytohormone [ethylene and abscisic acid (ABA)] levels and other changes that thwart buds from initiating new vegetative growth under growth-conducive conditions (Allona et al. 2008; Chao et al. 2007; Franklin 2009; Rohde and Bhalerao 2007). Other phytohormones [auxin, gibberellic acid (GA), cytokinin], sugar, and developmental signals (flowering and senescence) are also associated with changes in dormancy status that occur when plants perceive environmental signals. The involvement and cross-talk among those signaling pathways during various phases of dormancy have been reviewed in several publications (Horvath et al. 2003; Chao et al. 2007; Rohde and Bhalerao 2007; Anderson et al. 2010; Tanino et al. 2010; Cooke et al. 2012).

Endodormancy release in buds is triggered by extended periods of low temperature (Horvath et al. 2003; Anderson et al. 2005; Rohde and Bhalerao 2007; Holdsworth et al. 2008). Endodormancy release and vernalization processes are needed for bud burst leading to vegetative growth and flowering. In general, bud growth does not occur immediately after endodormancy release in temperate field conditions because growth is inhibited by low temperatures through the winter months (ecodormancy).

Seed Temperature and light also are major environmental signals affecting seed dormancy (Donohue et al. 2008; Chiang et al. 2009). Temperature controls dormancy release during dry storage (after-ripening) and regulates the rate of hard-seed breakdown (Probert 2000). In general, the rate of dormancy removal increases with

increasing temperature, and the rate of germination can be predicted by thermal-time requirements between individual seeds within populations in hydrated non-dormant seeds (Allen et al. 1995; Favier 1995; Steadman et al. 2003). Temperature also affects hormone levels through transcriptional regulation of GA biosynthesis and degradation enzymes. For example, the GA biosynthesis gene *AtGA3OX1* can be induced by stratification at 4 °C in dark-imbibed seeds of *Arabidopsis*; in contrast, the GA degradation gene *AtGA2OX2* is down-regulated by low temperatures, coinciding with an increase in the level of GA (Yamauchi et al. 2004).

The effect of light on seed germination varies. Light appears to act as a depth-sensing signal by preventing seed from germination too deep in the soil (Benech-Arnold et al. 2000). In *Arabidopsis* and some other species, light affects seed germination through a photo-reversible regulation of GA biosynthesis genes as some GA biosynthesis genes are up-regulated by red light and down-regulated by far red light (Toyomasu et al. 1998; Yamaguchi et al. 1998; Yamauchi et al. 2004). Red light also decreases endogenous ABA levels in *Arabidopsis* seeds (Seo et al. 2006). Furthermore, temperature and light signals jointly regulate phytochrome-mediated seed germination pathways. Heschel et al. (2008) showed that PHYA had an important role in promoting germination at higher temperatures (above 25 °C), PHYE was important at low temperatures (below 10 °C) and PHYB was important across a range of temperatures. Temperature and light also regulate seed dormancy induction and release through controlled expression of temperature- and/or light-regulated genes such as *DELAY OF GERMINATION 1* (*DOG1*), *C-REPEAT BINDING FACTOR* (*CBF*) group, *PHYA*, *FLOWERING LOCUS C* (*FLC*), and *MOTHER OF FT AND TFL1* (*MFT*) (Chiang et al. 2009; Footitt et al. 2011, 2013; Kendall et al. 2011).

Phytochrome A (PHYA) and its Companion Genes

Bud Phytochromes have the capacity to rapidly sense changes of incident light composition and regulate all aspects of photomorphogenic development in plants (Kircher et al. 2011). In woody hybrid aspen species (*Populus tremula* × *P. tremuloides*), PHYA mediates length of daylight (photoperiod) detection (Olsen et al. 1997; Welling et al. 2002; Kozarewa et al. 2010). PHYA also responds to cold and regulates the expression of cold-responsive genes; for example, cold-responsive gene expression was greatly enhanced in silver birch by low temperatures under SDP conditions (Puhakainen et al. 2004). PHYA (and thus SDP) appears to also act through ABA and ethylene signaling pathways for endodormancy induction (Rohde et al. 2002; Ruonala et al. 2006; Ruttink et al. 2007).

Moreover, PHYA is involved in regulating the flowering-related protein FLOWERING LOCUS T (FT) through CONSTANS (CO) to control growth cessation and endodormancy (Böhlenius et al. 2006; Ruonala et al. 2008). CO and FT mediate SDP-induced signals leading to growth cessation (Böhlenius et al. 2006). In *Arabidopsis*, *FT* is positively regulated by photoperiod through PHYA and CO and is negatively regulated by FLOWERING LOCUS C (FLC) and SHORT VEGETATIVE

PHASE (SVP) (He and Amasino 2005; Helliwell et al. 2006); CO activates the expression of *FT*, promoting flowering under long-day photoperiods and inhibiting flowering under SDP (Yanovsky and Kay 2002). Down-regulation of *FT* in poplar appears to trigger subsequent events leading to dormancy, whereas over-expression of *FT* in poplar prevents SDP-induced growth cessation, which is required for endodormancy induction (Böhlenius et al. 2006). Transgenic poplar over-expressing *PHYA* also exhibit elevated levels of *FT* and do not enter endodormancy after extended periods of SDP treatments (Ruonala et al. 2008). Studies from several tree species have shown that transduction pathways regulating endodormancy and flowering appear to converge (Rohde and Bhalerao 2007). However, it remains to be resolved on how these pathways interact. Interestingly, Hsu et al. (2011) observed that two *FT* genes *(FT1* and *FT2*) regulate different processes in poplar; *FT2* is a negative regulator of seasonal growth cessation, bud set, and dormancy induction and is down-regulated in late summer, whereas *FT1* primarily regulates flowering and is up-regulated during ecodormancy.

Seed PHYA contributes to the suppression of seed germination (Heschel et al. 2008). *PHYA* expression is greatest in deeply dormant seeds (Footitt et al. 2013), and oat *PHYA* over-expression results in reduced bioactive GAs in tobacco (*Nicotiana tabacum*) (Jordan et al. 1995). Seeds respond to light via PHYA to determine the actual time of germination, and high *PHYA* expression appears to be linked to lengthened dark exposure and low temperature (Footitt et al. 2013). Interestingly, PHYA also promotes germination in imbibed seeds through phytochrome-mediated degradation of PHYTOCHROME-INTERACTING FACTOR3-LIKE5 (PIL5 or PIF1), which is a major component in seed light responses and a negative regulator of seed germination. PIL5 represses the expression of GA biosynthetic genes (*GA3OX1* and *GA3OX2*) and thus impedes seed germination. When imbibed seeds perceive light, activated phytochromes transmit the light signal in the nucleus, resulting in PIL5 degradation and GA synthesis (Oh et al. 2006). Barrero et al. (2014) revealed that CRYPTOCHROME1 (CRY1) photoreceptor mediates the effects of blue light on seed dormancy in barley, and the inhibition of seed germination coincides with induction of the ABA biosynthetic gene *9-cis-epoxycarotenoid dioxygenase* (*NCED1*) and increase in ABA levels.

Circadian Clock Genes

Bud The circadian clock plays important roles in coordinating control of bud development and dormancy. *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*), *LATE ELONGATED HYPOCOTYL* (*LHY*), and *TIMING OF CAB EXPRESSION 1* (*TOC1/PRR1*) are major components of the central oscillator. Reciprocal regulation between LHY/CCA1 and TOC1 forms the central loop for clock function in *Arabidopsis*. CCA1 and LHY are morning-induced MYB-domain transcription factors that bind directly to the *TOC1* promoter to inhibit *TOC1* expression in *Arabidopsis*, and TOC1 is an evening-expressed pseudo-response regulator (PRR) that binds to

the promoters of *CCA1* and *LHY* to repress expression of these two genes (Alabadí et al. 2001; Gendron et al. 2012; Pokhilko et al. 2012). In woody hybrid aspen species (*Populus tremula* × *P. tremuloides*), LHY sets the critical day length for growth cessation, since down-regulation of *LHY* delayed bud set, reduced freezing tolerance, and delayed bud burst. However, down-regulation of *TOC1* enhanced *LHY* expression in cold and increased freezing tolerance in poplar (Ibáñez et al. 2010), showing similar functional relationships to that of *Arabidopsis LHY* and *TOC1*, which mutually repress each other in gene expression to control clock output. CCA1 and LHY also regulate the CBF cold-response pathway by binding directly to promoters of *CBF1-3* genes to control cold response/acclimation and freezing tolerance in *Arabidopsis* (Dong et al. 2011).

Seed Circadian clock affects seed dormancy through a series of interlocking transcriptional loops involving *LHY*, *CCA1*, *GIGANTEA* (*GI*), *TOC1*/*PRR1*, and *ZEITLUPE* (*ZTL*) (Penfield and King 2009). *LHY*, *CCA1*, and *GI* are required for appropriate seed dormancy and the response to dormancy release signals since mutations in these three genes cause germination defects in response to low temperature, alternating temperatures, and dry after-ripening (Penfield and Hall 2009). In addition, clock gene expression is arrested in an evening-like state in dry seeds and entrains to light/dark cycles in ambient temperatures upon imbibitions (Penfield and Hall 2009). Furthermore, the 'evening loop' components GI and TOC1 were associated with dormancy release in seeds; they are important for regulating the expression of ABA- and GA-responsive genes (Penfield and Hall 2009).

ABA and GA—Two Major Phytohormones Regulating Bud and Seed Dormancy and Release

Bud ABA is essential in controlling bud development and maturation (Rohde et al. 2002; Rohde and Bhalerao 2007; Ruttink et al. 2007) and may contribute to the suppression of growth during bud formation (Ruttink et al. 2007). In fact, ABA levels increased in apical buds of poplar after 24 to 27 d, under SDP, coincident with the cessation of growth (Rohde et al. 2002). Likewise, increased endogenous ABA levels in apical and lateral buds of silver birch also suggested a role for ABA in endodormancy induction (Li et al. 2003). ABA-INSENSITIVE 3 (ABI3) is a transcription factor that positively regulates ABA signaling. Black cottonwood (*Populus trichocarpa*) *ABI3* is expressed in buds and its expression correlates well with natural bud set and dormancy, which occurs after plants perceive a critical photoperiod (Rohde et al. 2002). During bud development, ABI3 may be involved in increasing ABA sensitivity at critical times (3 to 4 weeks of SDP) when the ABA concentration reaches its peak (Ruttink et al. 2007). ABI3 may also influence light signal transduction; the expression of *FT* and its close family member *MFT* are strongly up-regulated in transgenic poplars over-expressing ABI3 after the onset of SDP (Ruttink et al. 2007). *FT* belongs to the small phosphatidylethanolamine-binding protein (PEBP) gene family and regulates flowering in *Arabidopsis* and

other perennial models (Cooke et al. 2012; Pin and Nilsson 2012). In general, *FT* is down-regulated under SDP to bring seasonal arrest (Böhlenius et al. 2006) and up-regulated during cold-induced dormancy release (Rinne et al. 2011).

During bud development, reduction in bioactive GA levels in response to SDP is required to control growth cessation and bud formation (Cooke et al. 2012). Under SDP in hybrid aspen, the activity of a GA biosynthetic enzyme, GA20 oxidase, is reduced (Eriksson and Moritz 2002). In addition, transgenic hybrid aspen plants over-expressing an oat *PHYA* do not perceive SDP and are unable to down-regulate GA levels (Olsen et al. 1997). However, low GA levels alone cannot induce endodormancy development (Olsen 2010). In contrast, ample bioactive GA is needed for shoot elongation and bud burst in poplar (Zawaski et al. 2011).

Seed ABA is produced by the seed itself where it is a positive regulator of seed dormancy. ABA is involved in the suppression of GA biosynthesis in developing seeds and during seed germination (Seo at al. 2006), whereas GA counteracts the effects of ABA by promoting dormancy release and germination. Several genes including *ABA DEFICIENT 1* (*ABA1*), 9-*cis-epoxycarotenoid dioxygenase* (*NCED*s), and *ABA2*/*GIN1*/*SDR1* are involved in ABA biosynthesis in seeds (Nambara and Marion-Poll 2003). ABA positively regulates the expression of *ABI3* and *ABI5* transcripts and the level/activity of their respective proteins (Lopez-Molina et al. 2002; Holdsworth et al. 2008). ABI3 and ABI5 are transcription factors required for seed maturation and dormancy induction (Holdsworth et al. 2008) as well as growth arrest in germinating seeds under adverse conditions (Giraudat et al. 1992; Finkelstein and Lynch 2000). ABI3 is involved in regulating embryo dormancy through control of several cellular processes such as ABA sensitivity, desiccation tolerance, and storage protein accumulation during late embryogenesis and seed maturation (Parcy et al. 1994). ABI5 acts downstream of *ABI3* implementing the ABA-dependent growth arrest (Lopez-Molina et al. 2002). ABI3 also interacts with *FUSCA3* (*FUS3*), *LEAFY COTYLEDON 1* (*LEC1*), and *LEC2* in the regulatory network to control embryo growth arrest, seed maturation, dormancy induction, and the expression of storage proteins in *Arabidopsis* (Raz et al. 2001; Kagaya et al. 2005; To et al. 2006).

GA biosynthesis may involve cross-talk with ABA, ethylene, or auxin pathways (Curaba et al. 2004). Recently, Toh et al. (2012) showed that strigolactones modulate the ABA/GA ratio in secondary dormancy control. A period of after-ripening (seed storage) is required for dormancy release following seed dissemination from the plant. The speed of after-ripening varies depending on environmental conditions during seed maturation, storage, and germination (Donohue et al. 2005). Several genes such as *SPATULA* (*SPT*), *PIL5*, and *COMATOSE* (*CTS*) (Russell et al. 2000; Penfield et al. 2005) are involved in the after-ripening process. SPT and PIL5 are basic helix-loop-helix transcription factors and involved in cold stratification. Both inhibit the GA biosynthesis genes *GIBBERELLIC ACID 3-OXIDASE 1* (*GA3OX1*) and *GA3OX2* expression, thus repressing seed germination. *CTS* encodes an ATP-binding cassette transporter that is required for the import of biologically important molecules into the peroxisome. Transcriptome analysis has provided some evidence of interactions between CTS and GA in the expression of germination-related tran-

scripts (Carrera et al. 2007). Embryo arrest is reversed upon germination after dry *Arabidopsis* seeds are imbibed in water (Liu et al. 2009). The signaling molecule nitric oxide (NO) releases seed dormancy in many species by decreasing ABA sensitivity and/or concentration of imbibed seeds (Bethke et al. 2004, 2006; Liu et al. 2009; Šírová et al. 2011). Also, *GA3OX* and *CYTOCHROME P450 707A2* (*CYP707A2*) genes are induced after imbibition of after-ripened seeds. CYP707A2 is required for ABA breakdown (Kushiro et al. 2004) and GA3OX catalyzes GA synthesis (Yamauchi et al. 2004). Moreover, MFT regulates seed germination via the ABA and GA signaling pathways. MFT is a negative regulator of ABA signaling in *Arabidopsis*, and it promotes embryo growth by directly repressing *ABI5* expression (Xi et al. 2010).

MADS Domain Genes

Bud The *FLOWERING LOCUS C* (*FLC*) gene encodes a MADS domain protein, which acts as a repressor of flowering by down-regulating *FT* in *Arabidopsis*. Late-flowering ecotypes of *Arabidopsis* containing dominant alleles of *FLC* suppress flowering (Michaels and Amasino 1999). Other flowering-related MAD domain proteins known as *DORMANCY ASSOCIATED MADS-BOX* (*DAM*) were identified based on their functional relationship with endodormancy in the *EVERGROWING* locus of peach (*Prunus persica*) (Bielenberg et al. 2004, 2008). Mutations in this locus prevent terminal buds from going into endodormancy, and map-based cloning revealed that it was caused by a deletion of 6 tandem repeated *DAM* genes. *DAM* gene expression is markedly affected by photoperiod and chilling signals (Li et al. 2009). Over-expressing *PmDAM6* from Japanese apricot in poplar enhanced dormancy induction and reduced *FT* transcript levels (Sasaki et al. 2011).

DAM genes are related to transcription factors *AGAMOUS-LIKE 24 (AGL24*) and *SHORT VEGETATIVE PHASE* (*SVP*) genes of *Arabidopsis*. *AGL24* and *SVP* have opposite effects on flowering in *Arabidopsis*; SVP inhibits flowering through negative regulation of the floral regulatory genes *FT*, and AGL24 promotes flowering through positive regulation of *LEAFY* (*LFY*) (Hartmann et al. 2000; Michaels et al. 2003). *SVP* is negatively regulated by the circadian regulatory proteins CCA1 and LHY (Fujiwara et al. 2008). *DAM* genes have a closer phylogenetic relationship with *AGL24*; however, their physiological roles appear to be more analogous to *SVP* and *FLC*. Leafy spurge *DAM* genes are preferentially expressed in shoot tips and buds in response to cold temperature and SDP-associated endodormancy induction (Horvath et al. 2010). Li et al. (2009) showed that peach *DAM* genes have distinct seasonal and photoperiodic expression patterns in that *DAM1*, *DAM2*, and *DAM4* are correlated with growth cessation and bud set, while *DAM5* and *DAM6* are correlated with dormancy.

Seed FLC is also involved in seed germination. Chiang et al. (2009) demonstrated that FLC acts through FT, SUPPRESSOR OF OVER-EXPRESSION OF CO1 (SOC1), and APETALA1 (AP1) to stimulate ABA degradation and GA synthesis

during seed development and germination. Both SOC1 and AP1 are members of the MADS-box family of transcription factor. FLC-mediated seed germination is considered maternally controlled because *FLC* expression peaks while *FT*, *SOC1*, and *AP1* levels decrease during seed maturation, which is associated with elevated levels of *CYP707A2* (ABA degradation) and *GA20OX1* (GA synthesis) expression. Interestingly, this physiological signal still affects the ABA and GA germination pathway after seeds are separated from maternal plants and promotes seed germination under cool temperatures (Chiang et al. 2009).

Epigenetic Regulation

Bud Epigenetic regulation appears to be required in bud dormancy. Santamaría et al. (2009) observed opposite patterns for acetylated H4 histone and genomic DNA methylation in buds of dormant and non-dormant chestnut tree, suggesting different forms of epigenetic arrangement monitor gene expression during transition between different phases of dormancy. They further observed that the expression of genes involved in epigenetic regulation is associated with dormancy induction and release as *HUB2* (histone monoubiquitinase) and *GCN5L* (histone acetyltransferase) transcripts peaked in late November and *AUR3* (histone H3 kinase) transcripts peaked during bud burst (Santamaría et al. 2011). *DAM* gene expression during vernalization and endodormancy could also be mediated by common epigenetic mechanisms. Prolonged cold exposure causes a decrease in trimethylation of H3 lysine 4 (H3K4me3) and an increase of H3K27me3 in the promoter of leafy spurge *DAM1* concomitantly with gene repression and dormancy release (Horvath et al. 2010). In peach, *DAM6* also shows epigenetic changes associated with gene repression after dormancy release; there is an overall modification of H3K27me3 in the *DAM6* promoter, coding region, and intron in addition to a decrease of H3 acetylation and H3K4me3 modification around the translation start region (Leida et al. 2012). These results implied that epigenetic regulation is involved in bud dormancy; however, the details of their mechanistic roles remain to be elucidated. Chromatin remodeling may also have a role during ecodormancy to release dormancy and could promote bud growth and flowering when environmental conditions are optimal; however, alteration in these molecular mechanisms is not understood (Horvath et al. 2003, Romeu et al. 2014).

Seed Epigenetic regulation of gene expression is also needed in *Arabidopsis* seed dormancy. Histone H2B monoubiquitination genes, *HUB1* and *HUB2*, appear to be involved in dormancy induction since mutation of these two genes leads to reduction in seed dormancy (Liu et al. 2007). HUB1 encodes a C3HC4 RING finger protein and may function as an E3 ligase for monoubiquitination of histone H2B and chromatin remodeling, affecting the expression of several dormancy-related genes (Liu et al. 2007, 2011). *DELAY OF GERMINATION 1* (*DOG1*) is a seed dormancy gene responsible for variations occurring in natural *Arabidopsis* populations (Bentsink et al. 2006). This protein has the greatest similarity to a wheat Histone DNA

Binding Protein-1b (HBP-1b) (Bentsink et al. 2006). HBP-1b is a leucine zipper-type transcription factor that binds to the hexameric motif ACGTCA in the regulatory region of the wheat *Histone H3* gene (Mikami et al. 1989). DOG1 is localized in the nucleus and accumulates during seed maturation, showing a positive correlation with dormancy levels (Nakabayashi et al. 2012). Nevertheless, this relationship is lost in after-ripened seeds because protein levels still remain relatively high. Nakabayashi et al. (2012) proposed that protein modification (a shift in the isoelectric point) may make DOG1 non-functional prior to and following after-ripening. The *DOG1* gene is also down-regulated in *Arabidopsis hub1-2* mutants, indicating that absence of functional HUB1, i.e. monoubiquitination of histone H2B, leads to altered expression of *DOG1*. This result further indicates that chromatin remodeling may be important for seed dormancy (Liu et al. 2011).

Commonalities on Gene Expression During Bud and Seed Dormancy Induction and Release in Leafy Spurge

Leafy spurge is considered an invasive perennial weed in the Upper Great Plains of North America and has been reported to cause significant economic losses (Leitch et al. 1996). Dormancy in both underground adventitious buds (UABs) and seeds is an important survival mechanism in the life cycle of leafy spurge. Leafy spurge UABs exhibit three well-defined phases of para-, endo-, and ecodormancy; however, seed dormancy for leafy spurge is classified as physiological dormancy that requires after-ripening and alternating temperature for maximal germination. Overlaps in transcriptome profiles between different phases of UAB and seed dormancy have not been determined. We thus compared various phases of dormancy between seeds and buds, namely (1) paradormant buds vs. growth-competent seeds and (2) ecodormant buds vs. growth-competent seeds to identify common genes and molecular processes. The expression profiles of 201 leafy spurge genes involved in growth, hormone, light, and temperature response/regulation were examined using quantitative real-time—PCR (qRT-PCR) on RNA samples prepared from seeds and buds (Chao et al. 2014). All leafy spurge transcripts discussed here are based on annotation against the *Arabidopsis* database (www.arabidopsis.org), and the results are summarized below.

In this comparison (Table 14.2), leafy spurge crown buds (designated as "buds" throughout this section) were prepared according to Doğramacı et al. (2010, 2013). Prior to the start of each experiment, intact plants were acclimated in a growth chamber for 1 week at 27 °C, 16:8 h light:dark photoperiod and subjected to four different treatments: I) Para: paradormant plants were kept under constant temperature and photoperiod (27 °C, 16 h light); II) 2d-growth: paradormant plants were decapitated to induce and allow bud growth for 2 days at 27 °C, 16:8 h light:dark photoperiod; III) Endo: paradormant plants were subjected to a ramp-down in temperature (27 → 10 °C) and photoperiod (16 h → 8 h light) for 12 weeks to induce endodormancy; and IV) Eco: endodormant plants were given extended cold treat-

Table 14.2 Abbreviations for bud (Para, 2d-growth, Endo, and Eco) and seed (1d C, 21d C, and 21d C + 2d A) dormancy statuses before and after treatments

	Referred to as	Treatment	Temperature (°C)	Photoperiod (light)	Treatment period	Pre-treatment bud/ seed status	Post-treatment bud/seed status	Reference
Buds	Para	3-month-old plants acclimated in a growth chamber	27 °C	16 h light 8 h dark	1 week	Paradormant	Paradormant	Doğramacı et al. 2010
	2d-growth	Growth induced by decapitation of aerial tissues	27 °C	16 h light 8 h dark	2 days	Paradormant	Growth initiated	Chao et al. 2014
	Endo	Plants with para-dormant buds were exposed to ramp down in temperature and photoperiod	27 °C → 10 °C	16 h → 8 h light	12 weeks	Paradormant	Endodormant	Doğramacı et al. 2010
	Eco	Plants with endodor-mant buds were exposed to extended cold	5–7 °C constant	8 h light 16 h dark	11 weeks	Endodormant	Ecodormant	Doğramacı et al. 2010
Seeds	1d C	Seeds Imbibed under constant temperature for 1 day	20 °C	in dark	1 day	Dormant	Dormant—not germinated	Foley et al. 2010; Chao et al. 2011
	21d C	Seeds imbibed under constant temperature for 21 days	20 °C	in dark	21 days	Dormant	Growth com-petent—not germinated	Foley et al. 2010; Chao et al. 2011
	21d C + 2d A	21d C seeds exposed to alternating tem-perature and light for 2 days	30 °C for 8 h 20 °C for 16 h	16 h light 8 h dark	2 days	Growth competent	Dormancy released—germi-nation initiated	Foley et al. 2010; Chao et al. 2011

ment for 11 weeks at 5–7 °C, under constant 8:16 h light:dark photoperiod to induce buds from endo- to ecodormancy.

Leafy spurge seed treatments were previously described (Foley et al. 2010; Chao et al. 2011). In this study, three germination treatments (Table 14.2) were prepared: I) 1d C: seeds incubated for 1 d at the constant temperature of 20 °C; II) 21d C: seeds incubated for 21 d at the constant temperature of 20 °C; and III) 21d C + 2d A: seeds imbibed for 21 d at 20 °C followed by 2 d at alternating temperature (20:30 °C/16:8 h).

As summarized in Table 14.2, seeds incubated for 1 day at the constant temperature of 20 °C (1d C) will not germinate at optimal growth conditions; however, seeds incubated at a constant temperature of 20 °C for 21 days (21d C) will germinate when subjected to alternating temperatures of 20:30 °C (Foley et al. 2010). Therefore, the physiological state of 21d C seeds could be comparable to paradormant buds if seed germination was inhibited by endosperm-generated signals. In contrast, the physiological state of 21d C seeds could also be comparable to ecodormant buds if seed germination was inhibited by mechanisms such as a requirement for diurnal temperature variation. The physiological state of endodormant buds could be comparable to 1d C, since neither will germinate at optimal growth conditions.

Cluster analysis on the expression profiles of 201 genes indicated that buds and seeds fall into two main groups. One group contained all bud samples: Eco, Endo, Para, and 2d-growth. The other group contained all seed samples: 1d C, 21d C + 2d A, and 21d C. These results indicate substantial transcriptomic divergence exists between buds and seeds, which was likely due to differences in tissue types or other physiological, developmental, or environmental states. Consequently, direct comparison between buds and seeds was implausible. We thus selected the endodormant phase as the baseline for buds and dormant seeds (1d C) as the baseline for seeds, due to their inability to grow at optimal growth conditions.

Differentially expressed genes within buds (i.e. 'Para to Endo' or 'Eco to Endo' or '2d-growth to Endo') and seeds (i.e. '21d C to 1d C' or '21d C + 2d A to 1d C') were determined first for the 201 genes by qRT-PCR. Transcript abundance for 48, 29, and 64 genes was significantly different ($p < 0.1$) in 'Para to Endo', 'Eco to Endo', and '21d C to 1d C' comparisons, respectively. After growth initiation, transcript abundance for 23 and 35 genes was significantly different ($p < 0.1$) in '2d-growth to Endo' and '21d C + 2d A to 1d C' comparisons, respectively. Common differentially expressed genes that have the same trend in expression pattern were then identified based on the following comparisons: (1) paradormant buds vs. growth-competent seeds ('Para to Endo' vs. '21d C to 1d C'), (2) ecodormant buds vs. growth-competent seeds ('Eco to Endo' vs. '21d C to 1d C'), and (3) 2d growth-induced buds vs. 2d germination-induced seeds ('2d-growth to Endo' vs. '21d C + 2d A to 1d C').

Comparison of transcript abundance for the 'Para to Endo buds' and '21d C to 1d C seeds' identified 9 common differentially expressed genes with the same trend in expression pattern. These 9 transcripts are associated with cell cycle (*HisH4*), stress response/transcription factors (*ICE2*, *ERFB4/ABR1*), ABA and auxin response (*ABA1*, *ARF1*, *IAA7*, *TFL1*), carbohydrate/protein degradation (*GAPDH_1*), and

transport (*ABCB2*). *TFL1* (*TERMINAL FLOWER1*) transcript level was significantly higher in dormant buds and seeds relative to growth competent buds and seeds. TFL1 protein is a key floral repressor that maintains indeterminate (vegetative) growth in the center of the shoot apical meristem in *Arabidopsis* (Ratcliffe et al. 1999). Although it is not known if TFL1 is involved in dormancy development, the up-regulation of a putative *TFL1* transcript in dormant buds and seeds is consistent with the findings that down-regulation of *FT*, which encodes a major component of the florigen, in poplar triggers subsequent events leading to dormancy (Böhlenius et al. 2006).

In addition, the ABA biosynthetic gene *ABA1* was down-regulated in both paradormant buds and 21d C seeds relative to endodormant buds and 1d C seeds, respectively, indicating that ABA synthesis was lower in paradormant buds and 21d C seeds relative to their baseline. Likewise, genes involved in auxin transport (*ABCB2*) and response (*IAA7*/*AXR2*, *ARF1*) were down-regulated in paradormant buds and 21d C seeds, suggesting that auxin signaling was lower as well in paradormant buds and 21d C seeds relative to their baseline. In contrast, only 3 common differentially expressed genes had the same trend in expression pattern for the 'Eco to Endo buds' and '21d C to 1d C seeds' comparison. These 3 genes are associated with ABA response (*ATEM6*), auxin response (*ARF1*), and cell cycle (*HisH4*). On the basis of these results, we conclude that the physiological state of 21d C seeds is more analogous to paradormant buds than that of ecodormant buds.

Comparison of transcript abundance for the '2d-growth to Endo' vs. '21d C+2d A to 1d C' identified transcripts associated with auxin transport (*PID*, *PIN3*) and growth (*EXP6*), indicating that growth initiation induces auxin response/transport and cell expansion processes in both buds and seeds. The combined results indicate that common molecular mechanisms involved in dormancy transitions of buds and seeds involve processes associated with ABA and auxin signaling and transport, cell cycle, and AP2/ERF transcription factors or their up-stream regulators.

Conclusion

The physiology and molecular mechanisms of dormancy are complex. Although a substantial number genes reviewed in this chapter were not among the common differentially expressed genes identified in bud and seed dormancy in leafy spurge, the observation that dormancy transitions in buds and seeds involved processes associated with abscisic acid- and auxin-signaling and transport, cell cycle, and AP2/ERF transcription factors is consistent with the literature review. There is no consistent hypothesis to account for induction, maintenance, and breaking of dormancy for buds and seeds across all species. However, dormancy researches have generated vast information on how plants respond to and interact with environmental signals and have identified many factors including light (photoperiod), temperature (cold), hormones, circadian clock, and epigenetic regulation that control dormancy-associated genes regulating induction and release of dormancy in buds and seeds.

On the basis of these findings, we conclude that dormancy mechanisms are difficult to compare due to intrinsic differences between terminal and axillary buds of annuals and perennials, underground adventitious buds of herbaceous perennials, and seeds from all three of the aforementioned species, even though buds and seeds perceive and respond to similar environmental and developmental signals. Additionally, our research was conducted using complete seeds/buds and did not take the individual contributions of various tissues into account. Different parts of the bud and seed play different roles during their dormancy cycle and, thus, it would be more informative if expression analyses were compared separately with analogous meristematic regions of bud and seed. However, these types of fine-scale studies are labor-intensive and technically challenging.

Nevertheless, bud and seed dormancy share many common physiological processes. The most obvious examples are that both require ABA to induce dormancy and GA to break dormancy. Low temperature also seems to be a common factor for releasing buds and seeds from dormancy in many species. Not surprisingly, some similar signals control different phases of dormancy between bud and seed; for example, light and temperature signals show a profound effect for dormancy induction in buds, but in contrast, these two signals predominately determine dormancy release in seeds—a possible phenomenon of position and anatomical differences. Using two common baselines, endodormant buds (Endo) and dormant seeds (1d C), we were also able to identify genes associated with similar dormancy physiology in leafy spurge buds and seeds based on commonalities in differentially expressed genes. According to the number of common genes showing the same trend in expression pattern, we conclude that physiological relatedness in some phases of dormancy does exist between buds and seeds, and these genes may be used as molecular markers for specific dormancy phases in both buds and seeds.

References

Anderson JV, Gesch RW, Jia Y, ChaoWS, Horvath DP (2005) Seasonal shifts in dormancy status, carbohydrate metabolism, and related gene expression in crown buds of leafy spurge. Plant Cell Environ 28:1567–1578

Anderson JV, Horvath DP, Chao WS, Foley ME (2010) Bud dormancy in perennial plants: a mechanism for survival. In: Lubzens E, Cerda J, Clark M (eds) Dormancy and resistance in harsh environments, topics in current genetics, vol 21. Springer-Verlag, Berlin, p 69–89

Alabadí D, Oyama T, Yanovsky MJ, Harmon FG, Más P, Kay SA (2001) Reciprocal regulation between TOC1 and LHY/CCA1 within the *Arabidopsis* circadian clock. Science 293:880–883

Allen PS, Meyer SE, Beckstead J (1995) Patterns of seed after-ripening in *Bromus tectorum* L. J Exp Bot 46:1737–1744

Allona I, Ramos A, Ibáñez C, Contreras A, Casado R, Aragoncillo C (2008) Review. Molecular control of winter dormancy establishment in trees. Span J Agric Res 6(Special issue):201–210

Barrero JM, Downie AB, Xu Q, Gubler F (2014) A role for barley cryptochrome1 in light regulation of grain dormancy and germination. Plant Cell 26:1094–1104

Baskin JM, Baskin CC (2004) A classification system for seed dormancy. Seed Sci Res 14:1–16

Benech-Arnold R.L, Sánchez RA, Forcella F, Kruk B, Ghersa CM (2000) Environmental control of dormancy in weed seed banks in soil. Field Crops Res 67:105–122

Bentsink L, Jowett J, Hanhart CJ, Koornneef M (2006) Cloning of DOG1, a quantitative trait locus controlling seed dormancy in *Arabidopsis*. Proc Natl Acad Sci U S A 103:17042–17047

Bethke PC, Gubler F, Jacobsen JV, Jones RL (2004) Dormancy of *Arabidopsis* seeds and barley grains can be broken by nitric oxide. Planta 219:847–855

Bethke PC, Libourel IGL, Jones RL (2006) Nitric oxide reduces seed dormancy in *Arabidopsis*. J Exp Bot 57:517–526

Bewley JD (1997) Seed germination and dormancy. Plant Cell 9:1055–1066

Bielenberg DG, Wang Y, Fan S, Reighard GL, Scorza R, Abbott AG (2004) A deletion affecting several gene candidates is present in the evergrowing peach mutant. J Hered 95:436–444

Bielenberg DG, Wang Y, Li ZG, Zhebentyayeva T, Fan SH, Reighard GL, Scorza R, Abbott AG (2008) Sequencing and annotation of the evergrowing locus in peach [*Prunus persica* (L.) Batsch] reveals a cluster of six MADS-box transcription factors as candidate genes for regulation of terminal bud formation. Tree Genet Genomes 4:495–507

Böhlenius H, Huang T, Charbonnel-Campaa L, Brunner AM, Jansson S, Strauss SH, Nilsson O (2006) CO/FT regulatory module controls timing of flowering and seasonal growth cessation in trees. Science 312:1040–1043

Bowes CG, Thomas AG (1978) Longevity of leafy spurge seeds in the soil following various control programs. J Range Manage 31:137–140

Carrera E, Holman T, Medhurst A, Peer W, Schmuths H, Footitt S, Theodoulou FL, Holdsworth MJ (2007) Gene expression profiling reveals defined functions of the ABC transporter COMATOSE late in phase II of germination. Plant Physiol 143:1669–1679

Chao WS, Foley ME, Horvath DP, Anderson JV (2007) Signals regulating dormancy in vegetative buds. Int J Plant Dev Biol 1:49–56

Chao WS, Foley ME, Doğramacı M, Anderson JV, Horvath DP (2011) Alternating temperature breaks dormancy in leafy spurge seeds and impacts signaling networks associated with HY5. Funct Integr Genomics 11:637–649

Chao WS, Doğramacı M, Horvath DP, Anderson JV, Foley ME (2014) The resemblance and disparity of gene expression in dormant and non-dormant seeds and crown buds of leafy spurge (*Euphorbia esula*). BMC Plant Biol, in press

Chiang GC, Barua D, Kramer EM, Amasino RM, Donohue K (2009) Major flowering time gene, Flowering locus C, regulates seed germination in *Arabidopsis thaliana*. Proc Natl Acad Sci U S A 106:11661–11666

Cooke JEK, Eriksson ME, Junttila O (2012) The dynamic nature of bud dormancy in trees: environmental control and molecular mechanisms. Plant Cell Environ 35:1707–1728

Curaba J, Moritz T, Blervaque R, Parcy F, Raz V, Herzog M, Vachon G (2004) AtGA3ox2, a key gene responsible for bioactive gibberellins biosynthesis, is regulated during embryogenesis by LEAFY COTYLEDON2 and FUSCA3 in *Arabidopsis*. Plant Physiol 136:3660–3669

Debeaujon I, Léon-Kloosterziel KM, Koornneef M (2000) Influence of the testa on seed dormancy, germination, and longevity in *Arabidopsis*. Plant Physiol 122:403–413

Doğramacı M, Horvath DP, Chao WS, Foley ME, Christoffers MJ, Anderson JV (2010) Low temperatures impact dormancy status, flowering competence, and transcript profiles in crown buds of leafy spurge. Plant Mol Biol 73:207–226

Doğramacı M, Foley ME, Chao WS, Christoffers MJ, Anderson, JV (2013) Induction of endodormancy in crown buds of leafy spurge (*Euphorbia esula* L.) implicates a role for ethylene and cross-talk between photoperiod and temperature. Plant Mol Biol 81:577–593

Dong MA, Farré EM, Thomashow MF (2011) CIRCADIAN CLOCK-ASSOCIATED 1 and LATE ELONGATED HYPOCOTYL regulate expression of the C-REPEAT BINDING FACTOR (CBF) pathway in *Arabidopsis*. Proc Natl Acad Sci U S A 108:7241–7246

Donohue K, Dorn L, Griffith C, Kim E, Aguilera A, Polisetty CR, Schmitt J (2005) The evolutionary ecology of seed germination of *Arabidopsis* thaliana: variable natural selection on germination timing. Evolution 59:758–770

Donohue K, Heschel MS, Butler CM, Baura D, Sharrock RA, Whitelam GC, Chiang GC (2008) Diversification of phytochrome contributions to germination as a function of seed-maturation environment. New Phytologist 177:367–379

Eriksson ME, Moritz T (2002) Daylength and spatial expression of a gibberellin 20-oxidase isolated from hybrid aspen (*Populus tremula* L. x *P. tremuloides* Michx.). Planta 214:920–930
Favier JF (1995) A model for germination rate during dormancy loss in *Hordeum vulgare*. Ann Bot 76:631–638
Finch-Savage WE, Leubner-Metzger G (2006) Seed dormancy and the control of germination. New Phytol 171:501–523
Finkelstein RR, Lynch TJ (2000). The *Arabidopsis* abscisic acid response gene *ABI5* encodes a basic leucine zipper transcription factor. Plant Cell 12:599–609
Foley ME, Chao WS (2008) Temperature and moisture status affect afterripening of leafy spurge (*Euphorbia esula*) seeds. Weed Sci:56:516–522
Foley ME, Anderson JV, Horvath DP (2009) The effects of temperature photoperiod and vernalization on regrowth and flowering competence in *Euphorbia esula* (Euphorbiaceae) crown buds. Botany 87:986–992
Foley ME, Anderson JV, Chao WS, Horvath DP (2010) Initial changes in the transcriptome of *Euphorbia esula* seeds induced to germinate with a combination of constant and diurnal alternating temperatures. Plant Mol Biol 73:131–142
Footitt S, Douterelo-Soler I, Clay H, Finch-Savage WE (2011) Dormancy cycling in *Arabidopsis* seeds is controlled by seasonally distinct hormone-signaling pathways. Proc Natl Acad Sci U S A 108:20236–20241
Footitt S, Huang ZY, Clay HA, Mead A, Finch-Savage WE (2013) Temperature, light and nitrate sensing coordinate *Arabidopsis* seed dormancy cycling, resulting in winter and summer annual phenotypes. Plant J 74:1003–1015
Franklin KA (2009) Light and temperature signal crosstalk in plant development. Curr Opin Plant Biol 12:63–68
Fujiwara S, Oda A, Yoshida R, Niinuma K, Miyata K, Tomozoe Y, Tajima T, Nakagawa M, Hayashi K, Coupland G, Mizoguchi T (2008) Circadian clock proteins LHY and CCA1 regulate SVP protein accumulation to control flowering in *Arabidopsis*. Plant Cell 20:2960–2971
Gendron JM, Pruneda-Paz JL, Doherty CJ, Gross AM, Kang SE, Kay SA (2012) *Arabidopsis* circadian clock protein, TOC1, is a DNA-binding transcription factor. Proc Natl Acad Sci U S A 109:3167–3172
Giraudat J, Hauge BM, Valon C, Smalle J, Parcy F, Goodman HM (1992) Isolation of the *Arabidopsis ABI3* gene by positional cloning. Plant Cell 4:1251–1261
Hartmann U, Hormann S, Nettesheim K, Wisman E, Saedler H, Huijser P (2000) Molecular cloning of SVP: a negative regulator of the floral transition in *Arabidopsis*. Plant J 21:351–360
He Y, Amasino RM (2005) Role of chromatin modification in flowering-time control. Trends Plant Sci 10:30–35
Heide OM (2001) Photoperiodic control of dormancy in Sedum telephium and some other herbaceous perennial plants. Physiol Plant 113:332–337
Heide OM, Prestrud AK (2005) Low temperature, but not photoperiod, controls growth cessation and dormancy induction and release in apple and pear. Tree Physiol 25:109–114
Helliwell CA, Wood CC, Robertson M, James Peacock W, Dennis ES (2006) The *Arabidopsis* FLC protein interacts directly in vivo with SOC1 and FT chromatin and is part of a high-molecular-weight protein complex. Plant J 46:183–192
Heschel MS, Butler CM, Barua D, Chiang GCK, Wheeler A, Sharrock RA, Whitelam GC, Donohue K (2008) New roles of phytochromes during seed germination. Int J Plant Sci 169:531–540
Holdsworth MJ, Bentsink L, Soppe WJJ (2008) Molecular networks regulating *Arabidopsis* seed maturation, after-ripening, dormancy and germination. New Phytol 179:33–54
Horvath DP, Anderson JV, Chao WS, Foley ME (2003) Knowing when to grow: signals regulating bud dormancy. Trends Plant Sci 8:534–540
Horvath DP, Sung S, Kim D, Chao W, Anderson J (2010) Characterization, expression and function of *DORMANCY ASSOCIATED MADS-BOX* genes from leafy spurge. Plant Mol Biol 73:169–179
Howe GT, Hackett WP, Furnier GR, Klevorn RE (1995) Photoperiodic responses of a northern and southern ecotype of black cottonwood. Physiol Plant 93:695–708

Hsu CY, Adams JP, Kim H, No K, Ma C, Strauss SH et al (2011). FLOWERING LOCUS T duplication coordinates reproductive and vegetative growth in perennial poplar. Proc Natl Acad Sci U S A 108:10756–10761

Ibáñez C, Kozarewa I, Johansson M, Ogren E, Rohde A, Eriksson ME (2010) Circadian clock components regulate entry and affect exit of seasonal dormancy as well as winter hardiness in Populus trees. Plant Physiol 153:1823–1833

Jeknić Z, Chen THH (1999) Changes in protein profiles of poplar tissues during the induction of bud dormancy by short-day photoperiods. Plant Cell Physiol 40:25–35

Jordan ET, Hatfield PM, Hondred D, Talon M, Zeevaart JAD, Vierstra RD (1995) Phytochrome-A overexpression in transgenic tobacco-correlation of dwarf phenotype with high-concentrations of phytochrome in vascular tissue and attenuated gibberellin levels. Plant Physiol 107:797–805

Kagaya Y, Toyoshima R, Okuda R, Usui H, Yamamoto A, Hattori T (2005) LEAFY COTYLEDON1 controls seed storage protein genes through its regulation of FUSCA3 and ABSCISIC ACID INSENSITIVE3. Plant Cell Physiol 46:399–406

Kendall SL, Hellwege A, Marriot P, Whalley C, Graham IA, Penfield S (2011) Induction of dormancy in *Arabidopsis* summer annuals requires parallel regulation of DOG1 and hormone metabolism by low temperature and CBF transcription factors. Plant Cell 23:2568–2580

Kircher S, Terecskei K, Wolf I, Sipos M, Adam E (2011) Phytochrome A-specific signaling in *Arabidopsis thaliana*. Plant Signal Behav 6:1714–1719

Kozarewa I, Ibanez C, Johansson M, Ogren E, Mozley D, Nylander E, Chono M, Moritz T, Eriksson ME (2010) Alteration of PHYA expression change circadian rhythms and timing of bud set in Populus. Plant Mol Biol 73:143–156

Kushiro T, Okamoto M, Nakabayashi K, Yamagishi K, Kitamura S, Asami T, Hirai N, Koshiba T, Kamiya Y, Nambara E (2004) The *Arabidopsis* cytochrome P450 CYP707A encodes ABA 8′-hydroxylases: key enzymes in ABA catabolism. EMBO J 23:1647–1656

Kwolek VA, Woolhouse HW (1982) Studies on the dormancy of *Calluna vulgaris* (L.) hull, during winter: The effect of photoperiod and temperature on the induction of dormancy and the annual cycle of development. Ann Bot 49:367–376

Lang GA, Early JD, Martin GC, Darnell RL (1987) Endo-, para-, and ecodormancy: physiological terminology and classification for dormancy research. HortScience 22:371–377

Leida C, Conesa A, Llácer G, Badenes ML, Ríos G (2012) Histone modifications and expression of *DAM6* gene in peach are modulated during bud dormancy release in a cultivar-dependent manner. New Phytol 193:67–80

Leitch JA, Leistritz FL, Bangsund DA (1996) Economic effect of leafy spurge in the Upper Great Plains: methods, models, and results. Impact Assess 14:419–433

Li C, Junttila O, Ernstsen A, Heino P, Palva ET (2003) Photoperiodic control of growth, cold acclimation and dormancy development in silver birch (*Betula pendula*) ecotypes. Physiol Plant 117:206–212

Li C, Viherä-Aarnio A, Puhakainen T, Junttila O, Heino P, Palva ET (2004) Ecotype-dependent control of growth, dormancy and freezing tolerance under seasonal changes in Betula pendula Roth. Trees-Struct Funct 17:127–132

Li C, Wu N, Liu S (2005) Development of freezing tolerance in different altitudinal ecotypes of *Salix paraplesia*. Biol Plant 49, 65–71

Liu Y, Koornneef M, Soppe WJJ (2007) The absence of histone H2B monoubiquitination in the *Arabidopsis* hub1 (rdo4) mutant reveals a role for chromatin remodeling in seed dormancy. Plant Cell 19:433–444

Li Z, Leighard GL, Abbott AG, Bielenberg DG (2009) Dormancy-associated MADS genes from the EVG locus of peach (*Prunus persica* (L.) Batsch) have distinct seasonal and photoperiodic expression patterns. J Exp Biol 60:3521–3530

Liu Y, Shi L, Ye N, Liu R, Jia W, Zhang J (2009) Nitric oxide-induced rapid decrease of abscisic acid concentration is required in breaking seed dormancy in *Arabidopsis*. New Phytol 183:1030–1042

Liu Y, Geyer R, van Zanten M, Carles A, Li Y, Hörold A, van Nocker S, Soppe WJJ (2011) Identification of the *Arabidopsis REDUCED DORMANCY 2* gene uncovers a role for the polymerase associated factor 1 complex in seed dormancy. PLoS ONE 6:e22241

Lopez-Molina L, Mongrand S, McLachlin DT, Chait BT, Chua NH (2002) ABI5 acts downstream of ABI3 to execute an ABA dependent growth arrest during germination. Plant J 32:317–328
Michaels SD, Amasino RM (1999) *FLOWERING LOCUS C* encodes a novel MADS domain protein that acts as a repressor of flowering. Plant Cell 11:949–956
Michaels SD, Ditta G, Gustafson-Brown C, Pelaz S, Yanofsky M, Amasino RM (2003) AGL24 acts as a promoter of flowering in *Arabidopsis* and is positively regulated by vernalization. Plant J 33:867–874
Mikami K, Takase H, Tabata T, Iwabuchi M (1989) Multiplicity of the DNA-binding protein HBP-1 specific to the conserved hexameric sequence ACGTCA in various plant gene promoters. FEBS Lett 256:67–70
Müller K, Tintelnot S, Leubner-Metzger G (2006) Endosperm-limited Brassicaceae seed germination: abscisic acid inhibits embryo-induced endosperm weakening of *Lepidium sativum* (cress) and endosperm rupture of cress and *Arabidopsis thaliana*. Plant Cell Physiol 47:864–877
Nakabayashi K, Bartsch M, Xiang Y, Miatton E, Pellengahr S, Yano R, Seo M, Soppe WJJ (2012). The time required for dormancy release in *Arabidopsis* is determined by DELAY OF GERMINATION1 protein levels in freshly harvested seeds. Plant Cell 24:2826–2838
Nambara E, Marion-Poll A (2003) ABA action and interactions in seeds. Trends Plant Sci 8:213–217
Nikolaeva MG (2004) On criteria to use in studies of seed evolution. Seed Sci Res 14:315–320
Oh E, Yamaguchi S, Kamiya Y, Bae G, Chung WI, Choi G (2006) Light activates the degradation of PIL5 protein to promote seed germination through gibberellin in *Arabidopsis*. Plant J 47:124–139
Olsen J (2010) Light and temperature sensing and signaling in induction of bud dormancy in woody plants. Plant Mol Biol 73:37–47
Olsen JE, Junttila O, Nilsen J, Eriksson ME, Martinussen I, Olsson O, Sandberg G, Moritz T (1997) Ectopic expression of oat phytochrome A in hybrid aspen changes critical daylength for growth and prevents cold acclimatization. Plant J 12:1339–1350
Parcy F, Valon C, Raynal M, Gaubier-Comella P, Delseny M, Giraudat J (1994) Regulation of gene expression programs during *Arabidopsis* seed development:Roles of the ABI3 locus and of endogenous abscisic acid. Plant Cell 6:1567–1582
Penfield S, Hall A (2009) A role for multiple circadian clock genes in the response to signals that break seed dormancy in *Arabidopsis*. Plant Cell 21:1722–1732
Penfield S, Josse EM, Kannangara R, Gilday AD, Halliday KJ, Graham IA (2005) Cold and light control seed germination through the bHLH transcription factor SPATULA. Curr Biol 15:1998–2006
Penfield S, King J (2009) Towards a systems biology approach to understanding seed dormancy and germination. Proc R Soc B Biol Sci 276:3561–3569
Pin PA, Nilsson O (2012) The multi-functional facet of FT in plant development. Plant Cell Environ 35:1742–1755
Pokhilko A, Fernandez AP, Edwards KD, Southern MM, Halliday KJ, Millar AJ (2012) The clock gene circuit in *Arabidopsis* includes a repressilator with additional feedback loops. Mol Syst Biol 8:574
Probert RJ (2000) The role of temperature in the regulation of seed dormancy and germination. In: Fenner M. (ed) Seeds: the ecology of regeneration in plant communities. CABI, Wallingford, pp 261–292
Puhakainen T, Li C, Boije-Malm M, Kangasjärvi J, Heino P, Palva ET (2004) Short-day potentiation of low temperature-induced gene expression of a C-repeat-binding factor-controlled gene during cold acclimation in silver birch. Plant Physiol 136:4299–4307
Pukacki P, Giertych M, Chatupka W (1980) Light filtering functions of bud scales in woody plants. Planta 150:132–133
Ratcliffe OJ, Bradley DJ, Coen ES (1999). Separation of shoot and floral identity in *Arabidopsis*. Development 126:1109–1120
Raz V, Bergervoet JHW, Koornneef M (2001) Sequential steps for developmental arrest in *Arabidopsis* seeds. Development 128:243–252

Rinne PLH, Welling A, Vahala J, Ripel L, Ruonala R, Kangasjärvi J, van der Schoot C (2011) Chilling of dormant buds hyperinduces FLOWERING LOCUS T and recruits GA-inducible 1,3-β-Glucanases to reopen signal conduits and release dormancy in Populus. Plant Cell 23:130–146

Rohde A, Bhalerao RP (2007) Plant dormancy in the perennial context. Trends Plant Sci 12:217–223

Rohde A, Prinsen E, De Rycke R, Engler G, Van Montagu M, Boerjan W (2002) PtABI3 impinges on the growth and differentiation of embryonic leaves during bud set in poplar. Plant Cell 14:1885–1901

Romeu JF, Monforte AJ, Sánchez G, Granell A, García-Brunton J, Badenes ML, Ríos G (2014) Quantitative trait loci affecting reproductive phenology in peach. BMC Plant Biol 14:52

Ruonala R, Rinne PLH, Baghour M, Moritz T, Tuominen H, Kangasjärvi J (2006) Transitions in the functioning of the shoot apical meristem in birch (*Betula pendula*) involve ethylene. Plant J 46:628–640

Ruonala R, Rinne PL, Kangasjärvi J, van der Schoot C (2008) CENL1 expression in the rib meristem affects stem elongation and the transition to dormancy in Populus. Plant Cell 20:59–74

Russell L, Larner V, Kurup S, Bougourd S, Holdsworth M (2000) The *Arabidopsis* COMATOSE locus regulates germination potential. Development 127:3759–3767

Ruttink T, Arend M, Morreel K, Storme V, Rombauts S, Fromm J, Bhalerao RP, Boerjan W, Rohde A (2007) A molecular timetable for apical bud formation and dormancy induction in poplar. Plant Cell 19:2370–2390

Santamaría ME, Hasbún R, Valera MJ, Meijón M, Valledor L, Rodríguez JL, Toorop PE, Cañal MJ, Rodríguez R (2009) Acetylated H4 histone and genomic DNA methylation patterns during bud set and bud burst in *Castanea sativa*. J Plant Physiol 166:1360–1390

Santamaría ME, Rodríguez R, Cañal MJ, Toorop PE (2011) Transcriptome analysis of chestnut (*Castanea sativa*) tree buds suggests a putative role for epigenetic control of bud dormancy. Ann Bot 108:485–498

Sasaki R, Yamane H, Ooka T, Jotatsu H, Kitamura Y, Akagi T, Tao R (2011). Functional and expressional analyses of *PmDAM* genes associated with endodormancy in Japanese apricot. Plant Physiol 157:485–497

Seo M, Hanada A, Kuwahara A, Endo A, Okamoto M, Yamauchi Y, North H, Marion-Poll A, Sun TP, Koshiba T, Kamiya Y, Yamaguchi S, Nambara E (2006) Regulation of hormone metabolism in *Arabidopsis* seeds:phytochrome regulation of abscisic acid metabolism and abscisic acid regulation of gibberellin metabolism. Plant J 48:354–366

Šírová J, Sedlářová M, Piterková J, Luhová L, Petřivalský M (2011) The role of nitric oxide in the germination of plant seeds and pollen. Plant Science 181:560–572

Smithberg MH, Weiser CJ (1968) Patterns of variation among climatic races of red-osier dogwood. Ecology 49:495–505

Steadman KJ, Bignell GP, Ellery AJ (2003) Field assessment of thermal after-ripening time for dormancy release prediction in *Lolium rigidum* seeds. Weed Res 43:458–465

Steward FC (1991) Plant physiology 10: a treatise: growth and development, vol 10. Academic Press, Inc., San Diego, California 92101, p 211

Tanino KK, Kalcsits L, Silim S, Kendall E, Gray GR (2010) Temperature-driven plasticity in growth cessation and dormancy development in deciduous woody plants: a working hypothesis suggesting how molecular and cellular function is affected by temperature during dormancy induction. Plant Mol Biol 73:49–65

To A, Valon C, Savino G, Guilleminot J, Devic M, Giraudat J, Parcy F (2006) A network of local and redundant gene regulation governs *Arabidopsis* seed maturation. Plant Cell 18:1642–1651

Toh S, Kamiya Y, Kawakami N, Nambara E, McCourt P, Tsuchiya Y (2012) Thermoinhibition uncovers a role for strigolactones in *Arabidopsis* seed germination. Plant Cell Physiol 53:107–117

Toyomasu T, Kawaide H, Mitsuhashi W, Inoue Y, Kamiya Y (1998) Phytochrome regulates gibberellin biosynthesis during germination of photoblastic lettuce seeds. Plant Physio 118:1517–1523

Wake CMF, Fennell A (2000) Morphological, physiological and dormancy responses of three Vitis genotypes to short photoperiod. Physiol Plant 109:203–210

Welling A, Moritz T, Palva ET, Junttila O (2002) Independent activation of cold acclimation by low temperature and short photoperiod in hybrid aspen. Plant Physiol 129:1633–1641

Xi W, Liu C, Hou X, Yu H (2010) *MOTHER OF FT AND TFL1* regulates seed germination through a negative feed back loop modulating ABA signaling in *Arabidopsis*. Plant Cell 22:1733–1748

Yamauchi Y, Ogawa M, Kuwahara A, Hanada A, Kamiya Y, Yamaguchi S (2004) Activation of gibberellin biosynthesis and response pathways by low temperature during imbibition of *Arabidopsis thaliana* seeds. Plant Cell 16:367–378

Yamaguchi S, Smith MW, Brown RGS, Kamiya Y, Sun TP (1998) Phytochrome regulation and differential expression of gibberellin 3 beta-hydroxylase genes in germinating *Arabidopsis* seeds. Plant Cell 10:2115–2126

Yanovsky MJ, Kay SA (2002) Molecular basis of seasonal time measurement in *Arabidopsis*. Nature 419:308–312

Zawaski C, Kadmiel M, Pickens J, Ma C, Strauss S, Busov V (2011) Repression of gibberellin biosynthesis or signaling produces striking alterations in poplar growth, morphology, and flowering. Planta 234:1285–1298

Chapter 15
Comparing Genetic Mechanisms of Bud Chilling Fulfillment and Seed Cold Stratification: A Role for Peach (*Prunus persica*)?

Douglas G. Bielenberg

Introduction

The requirement for exposure to chilling temperatures for release from dormancy and resumption of normal growth is characteristic of seeds (cold stratification) and buds (chilling requirement) of many plant species. Curiously, the potential for the same or a very similar mechanism to underlie both traits has not received much attention. Investigation of the chilling requirement of buds has been the greatest focus of horticulturalists dealing with fruit and nut trees where chilling requirement is major determinant of bloom time (Luedeling and Brown 2011). Fruit and nut tree species are perennial and generally clonally propagated by bud grafting, and therefore, seed biology is typically not a major cultural concern except in breeding programs (Loreti and Morini 2008). Literature has generally focused on vernalization as a model for the study of molecular and genetic regulation of the bud chilling requirement (Horvath et al. 2003; Horvath 2009). This reflects the earlier focus of the *Arabidopsis thaliana* community on understanding the control of flowering time, which subsequently produced some of the earliest insights into the molecular control of a chilling-triggered response (Michaels and Amasino 2000; Sung and Amasino 2006). However, comparing the physiological and developmental events of seed and bud dormancy (growth arrest of a vegetative meristem) to each other might be more informative than comparing them to the vegetative to reproductive meristem transition that occurs as the result of vernalization.

One impediment to comparing the genetic and molecular regulation of bud chilling requirement to either seed moist chilling requirement or vernalization is that the processes have been studied in different species, sometimes with quite different life histories; for example, such as the difference between *Arabidopsis thaliana* and woody perennials (Hanninen and Tanino 2011; Hemming and Trevaskis 2011).

D. G. Bielenberg (✉)
Department of Biological Sciences, Clemson University,
Clemson, SC 29634-0314 USA
e-mail: dbielen@clemson.edu

© Springer International Publishing Switzerland 2015

J. V. Anderson (ed.), *Advances in Plant Dormancy,* DOI 10.1007/978-3-319-14451-1_15

The evolutionary distance and adaptive histories of different species may introduce specific peculiarities. These factors are likely to obscure the fundamental regulatory processes of interest to researchers who wish to understand the core regulatory components that are essential and shared between the three chilling-related phenomena discussed above. Research in Arabidopsis has begun to address the genetic and molecular regulation associated with chilling requirement of moist seed and where these processes share similarities and differences to the well-described vernalization process (Holdsworth et al. 2008; Graeber et al. 2012; Penfield and Springthorpe 2012). Here, peach (*Prunus persica*) is proposed as a model that could be used in a similar manner to examine the genetic and regulatory similarities between bud chilling requirement and stratification of moist seed.

Peach is a Genetic Model System Within the Rosaceae with a Number of Traits that Make it a Highly Amenable Genetic and Molecular Subject

Among fruit trees, peach is one of the most amenable genetic systems for gene discovery and genomics (Shulaev et al. 2008). Peach is a self-fertile, diploid species with a relatively short juvenile period of 2–4 years. Thousands of well-characterized cultivars exist with significant variation in phenological traits such as chilling requirement and bloom date (Okie 1998; Bielenberg et al. 2009). Considerable genetic mapping resources are available for peach, which have been successfully used to localize numerous Mendellian and quantitative traits in the genome and, in some cases, even identify causal mutations responsible for trait variation (Monet and Bassi 2008; Falchi et al. 2013; Salazar et al. 2014). Finally, the peach genome is small (approx. 230 Mb), fully sequenced and well-annotated, which facilitates gene discovery from mapping projects (Verde et al. 2013; Jung and Main 2014).

Peach has the Most Extensive Literature Database Describing the Genetic Basis of Quantitative Bud chilling Requirement that Exists for Perennial Tree Species

Peach has been a model for studying the physiology and genetics of bud chilling requirement in fruit and nut trees for many decades. Phenological models of effective chilling temperatures and chilling accumulation were all developed in peach and later adapted for other species (Weinberger 1950; Richardson et al. 1974; Erez and Fishman 1998; Luedeling and Brown 2011). In recent years, a number of studies in peach (and other closely related *Prunus* sp.) have identified multiple QTLs for the genetic control of chilling requirement and subsequently bloom date in peach. Independent groups using diverse germplasm have identified at least three consistent, strong effect QTLs on LGs 1, 4 and 7, in addition to a number of other QTLs

with less consistent effects on chilling requirement and bloom date (Fan et al. 2010; Eduardo et al. 2011; Dirlewanger et al. 2012; Romeu et al. 2014). These QTL mapping efforts have been limited by the relatively low population numbers used in most peach mapping studies (100-400 individuals). However, the recent availability of high-density SNP mapping methods and genome-wide association studies using diverse germplasm collections are improving the resolution of mapping studies in peach (Falchi et al. 2013).

In at least one case, a very narrow, strong effect chilling requirement QTL co-localized with the previously described *evergrowing* non-dormant mutant (Bielenberg et al. 2008; Fan et al. 2010). This discovery led to the hypothesis that the *D*ormancy-*A*ssociated *M*ADS box genes (*DAM5* and *DAM6*) may have a role in repressing bud outgrowth during endodormancy. Subsequent experiments demonstrated that *DAM5* and *DAM6* were short-photoperiod induced and chilling repressed, consistent with their induction in the autumn and repression during winter (Li et al. 2009; Jiménez et al. 2010). Most interestingly, repression of *DAM5* and *DAM6* expression correlated with satisfaction of chilling requirement (measured by bud break competence) in field grown trees (Li et al. 2009). The same relationship was observed for time to bud break in trees grown under controlled chilling conditions (Jiménez et al. 2010).

Peach Seed Germinates in Response to Moist Chilling

Peach seeds have a requirement for moist stratification prior to germination (Mes 1959; Loreti and Morini 2008). Conservatively, up to 3 months of chilling exposure is recommended to maximize germination and seedling vigor (Loreti and Morini 2008). This stratification appears to be imposed by the seed coat, as seed coat removal will allow for radical and shoot growth by imbibed seeds without the need for chilling exposure (Mes 1959; Martinez-Gomez and Dicenta 2001). However, seedling shoots that have not received sufficient stratification (with or without seed coat removal) elongate briefly but then express stunted growth of their terminal axis, whereas roots appear unaffected. This stunted growth manifests as curled, distorted leaves, reduced internode elongation, which is described as either ‘dwarfing’ or ‘rosetting’ appearance (Loreti and Morini 2008). Interestingly, this ‘dwarfing/rosetting’ is only evident on the main axis of the seedling. Laterals that grow out below the ‘rosette’ elongate normally and do not seem to show adverse effects from insufficient chilling. Eventually, one of the subtending laterals takes over as the dominant apex of the seedling and normal (but delayed) plants can be recovered from these insufficiently stratified seeds. Therefore, peach seed seem to have two distinct processes that are regulated by chilling exposure, one is a seed coat-mediated inhibition of germination and the other is endogenous to the embryo shoot meristem (Seeley et al. 1998). These two distinct phenotypes have some implications for interpreting literature associated with stratification requirement of peach seed, as will be noted below.

The effect of chilling temperatures on imbibed seed germination and growth rates appears to be dosage dependent in nature as opposed to a threshold that must be crossed in order to initiate growth. As imbibed seeds are exposed to longer periods of chilling, time to radical protuberance decreases and rates of elongation of those radicals increase. The same effect is seen upon seedling shoot height growth (Martinez-Gomez and Dicenta 2001). This dosage-dependent chilling requirement on the speed of germination appears to be analogous to the effect of suboptimal to excessive chilling on buds, which results in a more rapid bud break response under growth-promoting conditions (Okie and Blackburn 2011a).

Significant variation between genotypes of peach for seed cold stratification times have been noted by breeders. Attempts to use stratification times as a marker for fulfillment of chilling requirement in mature trees led to the general observation that trees with low bud chill requirement produced seed with a reduced requirement for cold exposure prior to germination (Perez-Gonzalez 1997; Topp et al. 2008; Matias et al. 2011; Bruckner et al. 2012). Thus, there appears to be a linkage between the parental chilling requirement and temperature and duration of chilling exposure needed to promote germination in peach seeds (Perez-Gonzalez 1997; Bruckner et al. 2012). It should be noted that the material in these studies had a wide variation in germination rates, which may reflect the heterogenous nature of the genetic material (open pollinations). However, it may also be related to within-tree environmental variation either from canopy environmental heterogeneity or positional endogenous signals from the mother tree, as has shown to be the case for intra-canopy variation in bud chilling requirement (Okie and Blackburn 2011b).

What Evidence is There that Bud Chilling Requirement and Seed Stratification in Peach May Share a Molecular or Genetic Basis?

Suggestions that there are some mechanistic similarities between chilling requirement and seed stratification requirement in peach can be found in the limited number of studies that address seed dormancy. First, as mentioned above, the observed correspondence between germination rate (moist chilling requirement) of seed and the parental tree genotype suggests that the traits are genetically linked (Perez-Gonzalez 1990; Perez-Gonzalez 1997). The Blaker et al. (2013) study is the first to identify QTL for seed stratification requirement among peach QTLs and some of these QTL intervals overlap with those found in bud chilling requirement and bloom date studies (Blaker et al. 2013; Salazar et al. 2014). It is tempting to speculate that these shared QTL intervals are indicating the location of a common causal sequence between these two responses; at the very least, the potential for common causal genes has not been eliminated.

Two studies have examined parallel gene expression in stratifying seeds and chilling exposed buds and found a host of genes that have common expression

patterns which support the hypothesis of common gene regulatory events during chilling accumulation (Leida et al. 2012; Fu et al. 2014). Leida et al. (2012) examined the effect of different levels of stratification on expression of the 'dwarfing' growth inhibition phenotype in peach seeds and correlated this symptom with the expression of several genes also identified as differentially expressed during the endodormant to ecodormant transition in flower buds. This study specifically focused on the embryo shoot response to chilling exposure by removing the seed coat prior to stratification experiments. As would be expected, a number of genes involved in ABA signaling or metabolism were strongly regulated during the stratification period. Most intriguingly, however, was the dose-dependent repression of a subset of the *DAM* genes (*DAM1* and *DAM6*) during the stratification period. Decreased *DAM1* and *DAM6* expression were correlated with reduced 'dwarfing/rosetting' symptoms and increased height growth following stratification treatments. This observation is very similar to that presented in Jiménez et al. (2010) where a strong correlation between loss of *DAM5* and *DAM6* expression and increased bud break rate was observed. The expression pattern of *DAM5* observed by Leida et al. (2012) does not have the same correlation with shoot growth as that seen by Jiménez et al. (2010) for terminal bud break. Differential expression during embryo chilling may indicate an instance of differential functionalization of *DAM5* from *DAM6* as they appear to have arisen from a tandem duplication and were previously found to have very similar expression patterns in tissues of mature trees (Jiménez et al. 2009; Li et al. 2009; Jiménez et al. 2010).

Another study has demonstrated the similarity in expression of genes between endodormant buds and seeds during chilling accumulation (Fu et al. 2014). Fu et al. (2014) propose a role for stress responsive endoplasmic reticulum genes during the accumulation of chilling and release from dormancy. While the role of the featured genes in dormancy regulation remains to be determined, the examination of gene expression patterns in both buds and seeds highlighted a remarkable level of parallel expression dynamics of the selected genes during the two physiological events. Studies from these two groups demonstrate that peach is a system that lends itself to molecular and genetic comparisons between dormant buds and seeds (Leida et al. 2012; Fu et al. 2014).

Is There Evidence Against Mechanistic Similarities Between Bud Chilling Requirement and Seed Stratification Requirement?

The genetic study of Blaker et al. (2013) may also indicate that there is a distinct regulation of these two phenomena. Their mapping study identified multiple broad QTL in locations that do not overlap with QTL associated with regulating bud chilling requirement (Dirlewanger et al. 2012; Blaker et al. 2013; Romeu et al. 2014;

Zhebentyayeva et al. 2014). It must also be noted that the simple co-localization of QTLs for chilling requirement and seed germination mentioned in the previous section is by no means a guarantee that the same genes or gene networks are causally involved in the two processes. The QTLs in both the chilling requirement and seed germination studies are broad and encompass many potential candidate genes. It is possible that a refinement of the phenotyping for effect of chilling on different seed germination traits such as radical emergence versus shoot 'dwarfing/rosetting' may be informative in comparing the results of the germination study with QTLs observed in the bud chilling requirement literature.

Limitations of Peach as a Model for Comparative Investigations on the Molecular and Genetic Regulation of Chilling and Stratification Requirement

The first limitations are simply logistical. Time and expense of maintaining parent trees for generating experimental material is not insignificant and obtaining experimental material from trees can be laborious. Seeds must be extracted from the fruit immediately upon harvest, cleaned and removed from the pits (Loreti and Morini 2008). Careful choice of freestone germplasm can speed the removal from the fruit but 'cracking' the pits to remove the seeds must be done by hand. Secondly, discovery of genetic regulators of chilling phenomena will be primarily restricted to forward genetics approaches, since a juvenile period of 2-4 years makes mutant screens an impractical prospect. Additionally, there is not currently a repeatable transformation protocol for peach that will allow reverse genetic experiments (Abbott et al. 2008). Thirdly, the literature has a lack of standardized methods for seed dormancy experiments. Methods are not always explicit about the age and storage conditions of the seed used in published experiments. The moisture content and temperature of storage all have potential to modify subsequent experimental responses to stratification treatment (Holdsworth et al. 2008). Because seed is only produced once per year, storage conditions are highly relevant to experimental outcomes (Frisby et al. 1988). Finally, peach seed germination literature requires careful interpretation in order to formulate conclusions about the effect of stratification on seed germination and seedling growth. While some studies used radical emergence to score germination success, others have used shoot emergence from the soil (Mes 1959; Frisby et al. 1988; Martinez-Gomez and Dicenta 2001). These binary measures of stratification satisfaction are less common in the peach literature than methods that use radical or seedling vigor (growth rates) as a marker for stratification satisfaction (Martinez-Gomez and Dicenta 2001). As introduced above, there appear to be at least two different physiological processes that are impacted by moist stratification in peach seed, the radical emergence and the shoot dwarfing/rosetting symptom of insufficient chilling.

Summary

While working with trees is logistically challenging relative to annual model systems, peach is one of the more amenable woody perennial systems. The significant genetic, genomic and germplasm resources in peach make it a biological system with much promise for comparing the mechanisms of bud and seed chilling requirement in individuals with the exact same genetic background. This has the potential to trigger a new phase of comparative analysis for commonalities and differences between bud chilling requirement and seed moist chilling requirement for germination. The few studies to date provide some tantalizing evidence that there are mechanistic commonalities between these two phenomena such as the involvement of DAM genes.

References

Abbott AG, Arús P, Scorza R (2008) Genetic engineering and genomics. In: Layne DR, Bassi D (eds) The peach: botany, produciton and uses. Cambridge, CAB International, 85–105
Bielenberg DG, Wang Y, Li Z, Zhebentyayeva T, Fan S, Reighard GL, Scorza R, Abbott AG (2008) Sequencing and annotation of the evergrowing locus in peach [Prunus persica (L.) Batsch] reveals a cluster of six MADS-box transcription factors as candidate genes for regulation of terminal bud formation. Tree Genet Genomes 4(3):495–507
Bielenberg DG, Gasic K, Chaparro JX (2009) An introduction to peach (Prunus persica). In: Folta KM, Gardiner SE (eds) Genetics and genomics of rosaceae. New York, Springer, 223–234
Blaker KM, Chaparro JX, Beckman TG (2013) Identification of QTLs controlling seed dormancy in peach (Prunus persica). Tree Genet Genomes 9(3):659–668
Bruckner CH, Silva J, Cruz CD, Wagner A, Sanchez MAM (2012) Effect of the embryo genotype on the chilling requirement for overcoming peach seed dormancy. In: Girona J, Marsal J (eds) Vii international peach symposium, 195–201
Dirlewanger E, Quero-Garcia J, Le Dantec L, Lambert P, Ruiz D, Dondini L, Illa E, Quilot-Turion B, Audergon JM, Tartarini S, Letourmy P, Arus P (2012) Comparison of the genetic determinism of two key phenological traits, flowering and maturity dates, in three Prunus species: peach, apricot and sweet cherry. Heredity 109(5):280–292
Eduardo I, Pacheco I, Chietera G, Bassi D, Pozzi C, Vecchietti A, Rossini L (2011) QTL analysis of fruit quality traits in two peach intraspecific populations and importance of maturity date pleiotropic effect. Tree Genet Genomes 7(2):323–335
Erez A, Fishman S (1998) The dynamic model for chilling evaluation in peach buds. Act Horticulturae 465:507–510
Falchi R, Vendramin E, Zanon L, Scalabrin S, Cipriani G, Verde I, Vizzotto G, Morgante M (2013) Three distinct mutational mechanisms acting on a single gene underpin the origin of yellow flesh in peach. Plant J 76(2):175–187
Fan S, Bielenberg DG, Zhebentyayeva TN, Reighard GL, Okie WR, Holland D, Abbott AG (2010) Mapping quantitative trait loci associated with chilling requirement, heat requirement and bloom date in peach (Prunus persica). New Phytologist 185(4):917–930
Frisby JW, Seeley SD, Laborde JID (1988) Germination, emergence, and seedling growth of peach in relation to stratification time and temperature. Hortscience 23(3):825–825
Fu XL, Xiao W, Wang DL, Chen M, Tan QP, Li L, Chen XD, Gao DS (2014) Roles of endoplasmic reticulum stress and unfolded protein response associated genes in seed stratification and bud endodormancy during chilling accumulation in Prunus persica. Plos One 9(7):1–8

Graeber K, Nakabayashi K, Miatton E, Leubner-Metzger G, Soppe WJJ (2012) Molecular mechanisms of seed dormancy. Plant Cell Environ 35(10):1769–1786
Hanninen H, Tanino K (2011) Tree seasonality in a warming climate. Trends Plant Sci 16(8):412–416
Hemming MN, Trevaskis B (2011) Make hay when the sun shines: the role of MADS-box genes in temperature-dependant seasonal flowering responses. Plant Sci 180(3):447–453
Holdsworth MJ, Bentsink L, Soppe WJJ (2008) Molecular networks regulating Arabidopsis seed maturation, after-ripening, dormancy and germination. New Phytologist 179(1):33–54
Horvath D (2009) Common mechanisms regulate flowering and dormancy. Plant Sci 177(6):523–531
Horvath DP, Anderson JV, Chao WS, Foley ME (2003) Knowing when to grow: signals regulating bud dormancy. Trends Plant Sci 8(11):534–540
Jiménez S, Lawton-Rauh AL, Reighard GL, Abbott AG, Bielenberg DG (2009) Phylogenetic analysis and molecular evolution of the dormancy associated MADS-box genes from peach. Bmc Plant Biol 9(81):doi:10.1186/1471–2229-1189–1181
Jiménez S, Reighard GL, Bielenberg DG (2010) Gene expression of DAM5 and DAM6 is suppressed by chilling temperatures and inversely correlated with bud break rate. Plant Mol Biol 73(1–2):157–167
Jung S, Main D (2014) Genomics and bioinformatics resources for translational science in Rosaceae. Plant Biotechnol Rep 8(2):49–64
Leida C, Conejero A, Arbona V, Gomez-Cadenas A, Llacer G, Badenes ML, Rios G (2012) Chilling-dependent release of seed and bud dormancy in peach associates to common changes in gene expression. Plos One 7(5):1
Li Z, Reighard GL, Abbott AG, Bielenberg DG (2009) Dormancy-associated MADS genes from the EVG locus of peach [Prunus persica (L.) Batsch] have distinct seasonal and photoperiodic expression patterns. J Exp Bot doi:10.1093/jxb/erp195
Loreti F, Morini S (2008) Propagation techniques. In: Layne DR, Bassi D (eds) The peach: botany, production and uses. Cambridge, CAB International, 221–243
Luedeling E, Brown PH (2011) A global analysis of the comparability of winter chill models for fruit and nut trees. Int J Biometeorol 55(3):411–421
Martinez-Gomez P, Dicenta F (2001) Mechanisms of dormancy in seeds of peach (Prunus persica (L.) Batsch) cv. GF305. Scientia Hortic 91(1–2):51–58
Matias RGP, Bruckner CH, dos Santos CEM, Dias D, Silva DFP (2011) Selection of peach genotypes based on the chilling requirement for seed germination. Rev Brasileira De Fruticultura 33(1):571–576
Mes MG (1959) Gibberellic acid and the chilling requirements of peach seeds. Nature 184(4704):2034–2035
Michaels SD, Amasino RM (2000) Memories of winter: vernalization and the competence to flower. Plant Cell Environ 23(11):1145–1153
Monet R, Bassi D (2008) Classical genetics and breeding. In: Layne DR, Bassi D (eds) The peach: botany, production and uses. Cambridge, CAB International, 61–84
Okie WR. (1998) Handbook of peach and nectarine varieties: performance in the southeastern United States and index of names (SuDoc A 1.76:714). Byron, United States Department of Agriculture (Agricultural Research Service.)
Okie WR, Blackburn B (2011a) Increasing chilling reduces heat requirement for floral budbreak in peach. Hortscience 46(2):245–252
Okie WR, Blackburn B (2011b) Interactive effects of light and chilling on peach flower and leaf budbreak. Hortscience 46(7):1056–1062
Penfield S, Springthorpe V (2012) Understanding chilling responses in arabidopsis seeds and their contribution to life history. Philos Trans R Soc B-Biolo Sci 367(1586):291–297
Perez-Gonzalez S (1990) Relationship between parental blossom season and speed of seed germination in peach. Hortscience 25(8):958–960
Perez-Gonzalez S (1997) Genotypic differentiation in temperature requirements for stratification in subtropical peach seeds. Hortscience 32(6):1064–1068

Richardson EA, Seeley SD, Walker DR (1974) A model for estimating the completion of rest for redhaven and elberta peache trees. Hortscience 82:302–306

Romeu JF, Monforte AJ, Sanchez G, Granell A, Garcia-Brunton J, Badenes ML, Rios G (2014) Quantitative trait loci affecting reproductive phenology in peach. Bmc Plant Biol 14:52. doi:10.1186/1471-2229-14-52

Salazar JA, Ruiz D, Campoy JA, Sanchez-Perez R, Crisosto CH, Martinez-Garcia PJ, Blenda A, Jung S, Main D, Martinez-Gomez P, Rubio M (2014) Quantitative Trait Loci (QTL) and mendelian Trait Loci (MTL) analysis in prunus: a breeding perspective and beyond. Plant Mol Biol Rep 32(1):1–18

Seeley SD, Ayanoglu H, Frisby JW (1998) Peach seedling emergence and growth in response to isothermal and cycled stratification treatments reveal two dormancy components. J Am Soc Hortic Sci 123(5):776–780

Shulaev V, Korban SS, Sosinski B, Abbott AG, Aldwinckle HS, Folta KM, Iezzoni A, Main D, Arus P, Dandekar AM, Lewers K, Brown SK, Davis TM, Gardiner SE, Potter D, Veilleux RE (2008) Multiple models for rosaceae genomics. Plant Physiol doi:10.1104/pp.107.115618

Sung S, Amasino RM (2006) Molecular genetic studies of the memory of winter. J Exp Bot 57(13):3369–3377

Topp BL, Sherman WB, Raseira MCB (2008) Low-chill cultivar development. In: Layne DR, Bassi D (eds) The peach: botany, production and uses. Cambridge, CAB International, 106–138.

Verde I, Abbott AG, Scalabrin S, Jung S, Shu SQ, Marroni F, Zhebentyayeva T, Dettori MT, Grimwood J, Cattonaro F, Zuccolo A, Rossini L, Jenkins J, Vendramin E, Meisel LA, Decroocq V, Sosinski B, Prochnik S, Mitros T, Policriti A, Cipriani G, Dondini L, Ficklin S, Goodstein DM, Xuan PF, Del Fabbro C, Aramini V, Copetti D, Gonzalez S, Horner DS, Falchi R, Lucas S, Mica E, Maldonado J, Lazzari B, Bielenberg D, Pirona R, Miculan M, Barakat A, Testolin R, Stella A, Tartarini S, Tonutti P, Arus P, Orellana A, Wells C, Main D, Vizzotto G, Silva H, Salamini F, Schmutz J, Morgante M, Rokhsar DS, Int Peach Genome I (2013) The high-quality draft genome of peach (Prunus persica) identifies unique patterns of genetic diversity, domestication and genome evolution. Nature Genet 45(5):487-U447

Weinberger JH (1950) Chilling requirements of peach varieties. Proc Am Soc Hortic Sci 56:122–128

Zhebentyayeva TN, Fan SH, Chandra A, Bielenberg DG, Reighard GL, Okie WR, Abbott AG (2014) Dissection of chilling requirement and bloom date QTLs in peach using a whole genome sequencing of sibling trees from an F-2 mapping population. Tree Genet Genomes 10(1):35–51

Part V
Chemical Manipulation of Bud Dormancy

Chapter 16
Chemical Release of Endodormancy in Potato Involves Multiple Mechanisms

Michael Campbell

Introduction

Potato is the third largest agricultural commodity on a worldwide basis after wheat and rice. It is a vegetatively propagated crop that is stored as a modified stem, or tuber, initially in an endodormant state following harvest. Prolonged storage usually requires both controlled environmental conditions and the use of growth suppressants to prevent sprouting because cessation of endodormancy will result in shoot growth and degradation of tuber quality. The desire to maintain endodormancy, and suppress sprouting in stored potato tubers, can create challenges for breeding programs that require rapid dormancy release in order to increase planting cycles. Thus, there have been a number of approaches to shortening or terminating the endodormant state in potato tubers. A survey of 224 chemicals for the ability to repress endodormancy and induce premature sprouting demonstrated that ethylene chlorhydrin, thiocyanates, bromoethane (BE), and ethylene dichloride were highly effective at inducing growth (Denny 1926). Multiple methods to terminate dormancy in harvested potatoes has been previously reviewed (Bryan 1989).

It is important for programs that focus on potato breeding or seed tuber production to be able to terminate dormancy and shorten production time. The compound Rindite has shown some success in the commercial sector but the toxicity to both plant tissue and users is of concern. Additionally, the mode of action of Rindite in terminating potato tuber dormancy is unclear. Other compounds such as BE and cytokinins have been used to break potato tubers dormancy but widespread application of these compounds in the commercial sector has not been established due to toxicity or lack of an established procedure. The goal of this review is to discuss the possible modes of action for compounds that have shown some efficacy in

M. Campbell (✉)
School of Science, Penn State Erie, P1 Prischak Building, 4205 College Drive,
Erie, PA 16563, USA
e-mail: mac17@psu.edu

© Springer International Publishing Switzerland 2015

J. V. Anderson (ed.), *Advances in Plant Dormancy,* DOI 10.1007/978-3-319-14451-1_16

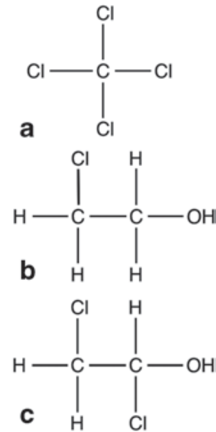


Fig. 16.1 The components of Rindite **a** carbon tetrachloride, **b** 2-chloroethanol, and **c** 1, 2-dichloroethanol

artificially terminating potato tuber dormancy in an attempt to suggest new avenues for breeders and growers to shorten breeding and production times.

Rindite and BE have been used to shorten dormancy in potato tubers but they are difficult to use due to their high toxicity. Phytohormones, such as cytokinin, induce cell division and growth in many plant tissues and in comparison with Rindite and BE they are less volatile and have a reduced toxicity. Application of kinetin and zeatin to potato tubers has been shown to result in the cessation of dormancy (Hemberg 1970). More recent experiments have shown that the response of dormant potato tubers to cytokinins increased with tuber age (Turnbull and Hake 1985), indicating that cytokinin application was not reliable as a means for breaking dormancy in freshly harvested tubers. Other studies have shown that dormant tubers were highly responsive to synthetic cytokinins including N-(2-chloro-4-pyridyl)-N′-phenylurea (CP) and 1-(α-ethylbenzyl)-3-nitroguanidine (NG) (Suttle 2008).

The differences in structure and chemical behavior between Rindite, BE, and synthetic cytokinins suggests a diverse mode of action for these compounds in terminating endodormancy in potato tubers. That diverse mode of action is supported in transcriptional studies of dormant potato tubers treated with these molecules (Fig. 16.1).

Rindite

Rindite, a mixture of 2-chloroethanol, 1, 2-dichloroethanol, and carbon tetrachloride (7:3:1), has been used to break endodormancy in potato tubers (Varga and Ferenczy 1956). Treatment of dormant tubers can occur immediately after harvest but to prevent increased tissue decay it was most effective following a 2-week postharvest incubation to allow for skin set (Kim et al. 1999). There is lack of experimental data that elucidates the mechanism of action for Rindite. Rindite is a highly toxic

compound that results in tissue stress but it is unclear whether dormancy release is a direct result of stress induction or a function of some other aspect of cellular and biochemical events triggered by Rindite. Thus, the mode of action for Rindite, as a promoter of dormancy cessation, is still unclear. 2-chloroethanol is metabolized in tobacco to chloroacetic acid via alcohol dehydrogenase and aldehyde dehydrogenase (Shang et al. 2001). The chlorinated alcohols associated with Rindite are probably metabolized by a similar mechanism that occurs for trichloroethanol. Metabolism of trichloroethanol results in glycosylation and then transport to the shoot (Mena-Benitez et al. 2008). How this metabolism relates to dormancy cessation is unclear but there is significant stress to tissues following Rindite treatments often including tissue damage.

Bromoethane (Fig. 16.2)

Application of BE vapor shortened endodormancy in potato tubers by over 40 % in multiple cultivars (Coleman 1983). The highly toxic nature of BE has resulted in limited use commercially as a method for dormancy termination. The use of BE did result in tuber damage particularly if it was applied prior to skin set. Application of BE to dormant mini-tubers results in a rapid increase in cellular respiration and an initial burst of ethylene followed by starch degradation and loss of tuber weight (Alexopoulos et al. 2009). Endodormant potatoes have elevated abscisic acid levels (ABA). Following exposure to BE, ABA levels increase but there is then a shift in tuber metabolism toward ABA catabolism (Destefano-Beltran et al. 2006a). The reduction of ABA following BE treatments is not the sole mechanism for dormancy cessation, since it has been shown that endogenous ABA levels are not necessarily indicative of dormancy status (Suttle et al. 2012). Analysis of RNA changes following BE treatments reveal transcript profiles that mimic those of nondormant tissues (Campbell et al. 2008). BE is a toxic compound that induces stress and results in ethylene release. However, studies that suppressed endogenous ethylene production during BE application demonstrate that BE does not result in dormancy cessation via ethylene signaling (Suttle 2009). The application of BE to nondormant tubers resulted in the loss of apical dominance due to initiation of programmed cell death and more rapid sprouting of lateral meristems (Teper-Bamnolker et al. 2012). There is some suggestion that BE termination of dormancy is specifically a function of a stress response (Alexopoulos et al. 2009), which is also a possible mechanism of action for the toxic mixture Rindite.

Fig. 16.2 Bromoethane (BE)

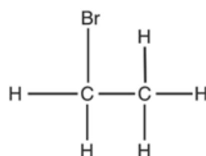


Table 16.1 A subset of transcripts induced by BE and natural dormancy cessation. (Data from Campbell et al. 2008)

Ribulose bisphosphate carboxylase small subunit
Putative protein
Cyclophilin
Cytochrome p450
Phenylcoumarin benzylic ether reductase
Nonspecific lipid transfer protein
2-Oxoglutarate dependent dioxygenase
Glucosyltransferase

Transcriptional profiling of tubers treated with BE demonstrated a down-regulation of many genes associated with ABA responses (Campbell et al. 2008). One day after exposure to BE transcripts encoding for proteins involved with pathogen response and stress were increased significantly. However, it is possible the initial stress of BE exposure, resulting in dormancy cessation, is a much more rapid response and release from the dormant state may occur in less than 24 h.

It has been shown that BE does not function via the induction of the stress hormone ethylene although there is induction of stress-related proteins (Suttle 2009). Microarray analysis of BE-treated potato tuber meristems and meristems allowed to undergo natural dormancy cessation revealed similar RNA expression patterns particularly after 8 days following BE exposure (Campbell et al. 2008). The RNA expression patters that were similar between BE and natural dormancy cessation resulted in transcript changes for proteins that are involved with phenylpropanoid biosynthesis, photosynthesis, and stress. (Table 16.1).

For example, induction of ribulose bisphoshate by BE indicates increased expression of the key enzyme for CO_2 fixation. Phenylcoumarin benzylic ether reductase is involved with the phenylpropanoid pathway and vascular development (Vander Mijnsbrugge et al. 2000). Cyclophilins are a large family of poorly described proteins in plants that putatively function as chaparones (Kumari et al. 2013). BE induced the expression of 2-oxoglutarate-dependent dioxygenase, which is a very large protein family involved with a diversity of biological processes including phytohormone biosynthesis, production of specific metabolites, methylation of DNA, and flavonoid production (Kawai et al. 2014). Induction of transcripts encoding for glutathione-S-transferase (GST) were among some of the earliest changes observed by BE treatment. GSTs are represented by a large family of proteins in plants exhibiting a diverse range of function including oxidative stress (Dixon et al. 2009; Noctor et al. 2012). The implication of oxidative stress in dormancy regulation is supported by application of hydrogen cyanamide (HC) to release dormancy in grape buds (Ophir et al. 2009; Or et al. 2002). HC indirectly results in oxidative stress due to in suppression catalase activity, which initiates dormancy cessation in grape buds.

Cytokinins (Fig. 16.3)

Application of cytokinins to endodormant potato tubers can result in sprouting and cessation of the dormant state but responses can be highly variable and use in breeding and seed tuber production is limited. The increased sensitivity of endodormant tubers to the synthetic cytokinins CP and NG was suggested to be due to a greater stability of these compounds in treated tissues, whereas zeatin and kinetin are rapidly metabolized resulting in a decreased response (Suttle 2001). Transcriptome analysis using RNA-seq has revealed that the cytokinin analog NG induces termination of dormancy within 4 days of exposure (Campbell et al. 2014). This termination is marked by the induction of transcripts associated with the initiation of CYCD3 homologs, which are regulators of the G1/S phase of the cell cycle (Dewitte and Murray 2003). High levels of cytokinins have been shown to increase CYCD3 expression in Arabidopsis and overexpression of *CYCD3* in transgenic plants maintained cell division in cultures without exogenously supplied cytokinin (Riou-Khamlichi et al. 1999). Thus, it appears that application of NG to dormant potato meristems terminates dormancy through a cytokinin response and induction of cell division through initiation of the cell cycle.

The RNA-seq of small RNAs from dormant and nondormant potato meristems resulted in three classes of miRNA comprising from 0.22 to 18.9% of the total sequences isolated. The largest percentage of small RNAs was composed of miR166. miR166 has been shown to regulate the activity and development of the shoot apical meristem (SAM) (Jung and Park 2007). There is an increase in the relative amount of miR166 in the total population of small RNAs sequenced from dormant

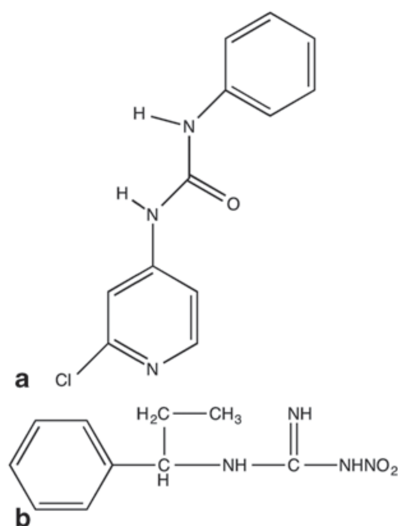


Fig. 16.3 Structures of synthetic cytokinins that result in dormancy cessation in potato **a** N-(2-chloro-4-pyridyl)-N′-phenylurea, **b** 1-(α-ethylbenzyl)-3-nitroguanidine)

Table 16.2 microRNAs found in potato tuber meristems. The above are the most prevalent sequences in each RNA samples (values given in percent of total RNA isolated). e-values for each sequence are found in column one and indicate relative similarity to miRNAs described in the miRBase database (http://www.mirbase.org)

miRNA	e-value	Dorm	Dorm	Dorm	Dorm	Average percent of total RNA	Std error	Gene targets
miR166f	0.001	9.24	11.93	18.63	21.59	17.38	2.48	WUSCHEL (WUS)-CLAVATA (CLV) pathway
miR166i	0.001	1.85	3.10	0.94	0.60	1.55	0.68	WUSCHEL (WUS)-CLAVATA (CLV) pathway
miR183	0.19	1.00	0.30	1.09	1.06	0.82	0.22	Unknown
miR159a	0.001	0.26	0.24	0.25	0.24	0.24	0.00	GAMYB-like1, GAMYB-like2(GA responsive element)
		Non-Dorm	Non-Dorm	Non-Dorm	Non-Dorm			
miR166f	0.001	3.45	2.72	11.35	7.73	7.27	2.17	WUSCHEL (WUS)-CLAVATA (CLV) pathway
miR166i	0.001	1.54	0.93	0.59	0.54	0.69	0.11	WUSCHEL (WUS)-CLAVATA (CLV) pathway
miR183	0.19	0.90	0.92	1.31	2.69	1.64	0.47	Unknown
miR159a	0.001	0.18	0.21	0.24	0.21	0.22	0.01	GAMYB-like1, GAMYB-like2(GA responsive element)

meristems (Table 16.2). This does suggest that regulation of stem cell activity in the apical meristem by miR166 may be a measure of dormancy status in potato tubers.

Additional miRNAs were found to vary between dormant and nondormant meristems including miR183 and miR159. The presence of small RNAs having identity to miR183, a gene associated with metazoan development (Wheeler et al. 2009), was not reported in the literature to be isolated from any plant species. Thus, it is not known whether the presence of the miR183 gene in potato represents a sequencing artifact or the first report of the miR183 gene in a plant species. The presence

of miR159 from potato meristems is not surprising. The gene miR159 has shown to be associated with modulation of gibberellin-regulated floral meristem development via repression of the GAMYB-like genes *MYB33* and *MYB65* (Achard et al. 2004; Allen et al. 2007; Millar and Gubler 2005), embryonic development (Li et al. 2013), seed development (Huang et al. 2013), growth and programmed cell death (Alonso-Peral et al. 2010). Presence of miR159 was relatively equivalent between dormant and nondormant meristems, suggesting that dormancy status did not alter miR159 activity.

Conclusions

Termination of endodormancy in potato tuber meristems using chemical treatments appears to involve at least two independent mechanisms. One method involves exposure to stress via compounds such as BE or Rindite. The stress treatment for dormancy release may function through the induction of apoptotic events (Teper-Bamnolker et al. 2012). The second method of dormancy release using chemicals involves exposure of potato meristems to synthetic cytokinins such as NG, which initiate cell division and transition through a G1/S-phase cell cycle block associated with the dormant state.

Examination of microRNAs following dormancy cessation demonstrates some shift in mRNA associated with meristem identity. The increase in the prevalence of miR166 should alter the expression of WUSCHEL/CLAVATA pathway, resulting in changes in meristem development (Zhu et al. 2011). The ability of NG to break endodormancy supports earlier work that cytokinins interact with the meristem identity pathway WUSCHEL/CLAVATA to control meristem growth (Gordon et al. 2009; Kurakawa et al. 2007; Leibfried et al. 2005; Sablowski 2009). It remains to be seen whether NG functions to terminate dormancy through miR166 alteration of the WUSCHEL/CLAVATA pathway.

The application and use of Rindite and BE as terminators of potato tuber dormancy is difficult due to the high toxicity of these compounds. The recent investigations showing the efficacy of cytokinin analogs as terminators of dormancy suggests not only an alternate mechanism of activation but perhaps a more environmentally and less toxic approach to manipulating potato tuber dormancy.

References

Achard P, Herr A, Baulcombe DC, Harberd NP (2004) Modulation of floral development by a gibberellin-regulated microRNA. Development 131:3357–3365

Alexopoulos AA, Aivalakis G, Akoumianakis KA, Passam HC (2009) Bromoethane induces dormancy breakage and metabolic changes in tubers derived from true potato seed. Postharvest Biol Technol 54:165–171

Allen RS, Li J, Stahle MI, Dubrou A, Gubler F, Millar AA (2007) Genetic analysis reveals functional redundancy and the major target genes of the Arabidopsis miR159 family. Proc Natl Acad Sci U S A 104:16371–16376

Alonso-Peral MM, Li J, Li Y, Allen RS, Schnippenkoetter W, Ohms S, White RG, Millar AA (2010) The microRNA159-regulated GAMYB-like genes inhibit growth and promote programmed cell death in Arabidopsis. Plant Physiol 154:757–771

Bryan J (1989) Breaking dormancy of potato tubers. International Potato Center, Lima Peru, p 12

Campbell MA, Segear E, Beers L, Knauber D, Suttle J (2008) Dormancy in potato tuber meristems: chemically induced cessation in dormancy matches the natural process based on transcript profiles. Funct Integr Genomics 8:317–328

Campbell MA, Suttle J, Douches DS, Buell CR (2014) Treatment of potato tubers with the synthetic cytokinin 1-(d-ethylbenzyl)-3-nitroguindine results in rapid termination of endodormancy and induction of transcripts associated with cell proliferation and growth. Funct Integr Genomics 14:789–799

Coleman WK (1983) An evaluation of bromoethane for breaking tuber dormancy in *Solanum tuberosum* L. Am Potato J 60:161–167

Denny FE (1926) Hastening the sprouting of dormant potato tubers. Am J Bot 13:118–125

Destefano-Beltran L, Knauber D, Huckle L, Suttle J (2006) Chemically forced dormancy termination mimics natural dormancy progression in potato tuber meristems by reducing ABA content and modifying expression of genes involved in regulating ABA synthesis and metabolism. J Exp Bot 57:2879–2886

Dewitte W, Murray JAH (2003) The plant cell cycle. Annu Rev Plant Biol 54:235–264

Dixon DP, Hawkins T, Hussey PJ, Edwards R (2009) Enzyme activities and subcellular localization of members of the Arabidopsis glutathione transferase superfamily. J Exp Bot 60:1207–1218

Gordon SP, Chickarmane VS, Ohno C, Meyerowitz EM (2009) Multiple feedback loops through cytokinin signaling control stem cell number within the Arabidopsis shoot meristem. Proc Natl Acad Sci U S A 106:16529–16534

Hemberg T (1970) The action of some cytokinins on the rest-period and the content of acid growth-inhibiting substances in potato. Physiol Plant 23:850–858

Huang DQ, Koh C, Feurtado JA, Tsang EWT, Cutler AJ (2013) MicroRNAs and their putative targets in Brassica napus seed maturation. BMC Genomics 14:140. doi:10.1186/1471-2164-14-140

Jung J-H, Park C-M (2007) MIR166/165 genes exhibit dynamic expression patterns in regulating shoot apical meristem and floral development in Arabidopsis. Planta 225:1327–1338

Kawai Y, Ono E, Mizutani M (2014) Evolution and diversity of the 2–oxoglutarate-dependent dioxygenase superfamily in plants. Plant J 78:328–343

Kim H, Jeon J, Choi K, Joung Y, Joung H (1999) Effects of rindite on breaking dormancy of potato microtubers. Am J Pot Res 76:5–8

Kumari S, Roy S, Singla-Pareek SL, Pareek A (2013) Cyclophilins: proteins in search of function. Plant Signal Behav 8. http://dx.doi.org/10.4161/psb.22734

Kurakawa T, Ueda N, Maekawa M, Kobayashi K, Kojima M, Nagato Y, Sakakibara H, Kyozuka J (2007) Direct control of shoot meristem activity by a cytokinin-activating enzyme. Nature 445:652–655

Leibfried A, To JPC, Busch W, Stehling S, Kehle A, Demar M, Kieber JJ, Lohmann JU (2005) WUSCHEL controls meristem function by direct regulation of cytokinin-inducible response regulators. Nature 438:1172–1175

Li WF, Zhang SG, Han SY, Wu T, Zhang JH, Qi LW (2013) Regulation of LaMYB33 by miR159 during maintenance of embryogenic potential and somatic embryo maturation in *Larix kaempferi* (Lamb.) Carr. Plant Cell Tissue Organ Cult 113:131–136

Mena-Benitez GL, Gandia-Herrero F, Graham S, Larson TR, McQueen-Mason SJ, French CE, Rylott EL, Bruce NC (2008) Engineering a catabolic pathway in plants for the degradation of 1,2-Dichloroethane. Plant Physiol 147:1192–1198

Millar AA, Gubler F (2005) The arabidopsis GAMYB-Like Genes, MYB33 and MYB65, are microRNA-regulated genes that redundantly facilitate anther development. Plant Cell Online 17:705–721

Noctor G, Mhamdi A, Chaouch S, Han YI, Neukermans J, Marquez-Garcia B, Queval G, Foyer CH (2012) Glutathione in plants: an integrated overview. Plant Cell Environ 35:454–484

Ophir R, Pang X, Halaly T, Venkateswari J, Lavee S, Galbraith D, Or E (2009) Gene-expression profiling of grape bud response to two alternative dormancy-release stimuli expose possible links between impaired mitochondrial activity, hypoxia, ethylene-ABA interplay and cell enlargement. Plant Mol Biol 71:403–423

Or E, Vilozny I, Fennell A, Eyal Y, Ogrodovitch A (2002) Dormancy in grape buds: isolation and characterization of catalase cDNA and analysis of its expression following chemical induction of bud dormancy release. Plant Sci 162:121–130

Riou-Khamlichi C, Huntley R, Jacqmard A, Murray JAH (1999) Cytokinin activation of arabidopsis cell division through a D-Type cyclin. Science 283:1541–1544

Sablowski R (2009) Cytokinin and WUSCHEL tie the knot around plant stem cells. Proc Natl Acad Sci U S A 106:16016–16017

Shang TQ, Doty SL, Wilson AM, Howald WN, Gordon MP (2001) Trichloroethylene oxidative metabolism in plants: the trichloroethanol pathway. Phytochemistry 58:1055–1065

Suttle J (2001) Dormancy-related changes in cytokinin efficacy and metabolism in potato tubers during postharvest storage. Plant Growth Regul 35:199–206

Suttle J (2008) Effects of synthetic phenylurea and nitroguanidine cytokinins on dormancy break and sprout growth in russet burbank minitubers. Am J Pot Res 85:121–128

Suttle J (2009) Ethylene is not involved in hormone- and bromoethane-induced dormancy break in russet burbank minitubers. Am J Pot Res 86:278–285

Suttle JC, Abrams SR, De Stefano-Beltrán L, Huckle LL (2012) Chemical inhibition of potato ABA-8’-hydroxylase activity alters in vitro and in vivo ABA metabolism and endogenous ABA levels but does not affect potato microtuber dormancy duration. J Exp Bot 63(15):5717–5725. doi:10.1093/jxb/ers146.

Teper-Bamnolker P, Buskila Y, Lopesco Y, Ben-Dor S, Saad I, Holdengreber V, Belausov E, Zemach H, Ori N, Lers A, Eshel D (2012) Release of apical dominance in potato tuber is accompanied by programmed cell death in the apical bud meristem. Plant Physiol 158:2053–2067

Turnbull C, Hake D (1985) The control of bud dormancy in potato. Planta 162:359–365

Vander Mijnsbrugge K, Beeckman H, De Rycke R, Van Montagu M, Engler G, Boerjan W (2000) Phenylcoumaran benzylic ether reductase, a prominent poplar xylem protein, is strongly associated with phenylpropanoid biosynthesis in lignifying cells. Planta 211:502–509

Varga MB, Ferenczy L (1956) Effect of ‘Rindite’ on the development of the groth-substances in potato tubers. Nature 178:1075

Wheeler BM, Heimberg AM, Moy VN, Sperling EA, Holstein TW, Heber S, Peterson KJ (2009) The deep evolution of metazoan microRNAs. Evol Dev 11:50–68

Zhu H, Hu F, Wang R, Zhou X, Sze S-H, Liou Lisa W, Barefoot A, Dickman M, Zhang X (2011) Arabidopsis argonaute10 specifically sequesters miR166/165 to regulate shoot apical meristem development. Cell 145:242–256

Chapter 17
Exploring Alternative Bud Break Enhancing Product in 'Zesy003' (Gold9) by Painting Application

Gustavo Hernández and Rob Craig

Introduction

Hydrogen cyanamide (HC) has been used extensively in the kiwifruit industry as a bud break enhancing tool to moderate seasonal fluctuations in fruit production and maximize productivity. As a secondary effect, this substance reduces unwanted lateral flowers, which reduces thinning costs and increases the average size of the fruit produced (Erez 1995; Mc Pherson et al. 2001; Hernandez and Craig 2010). Exclusion of HC from Annex 1 of the Directive 91/414/EEC now prevents the use of this substance for bud break enhancement in Europe and has resulted in concerns by other kiwifruit growers around the world where future use may be restricted.

In the cultivar 'Hayward', HC application results in a 50–100% increase in flower loadings and cropping levels, a major reduction of lateral flowers, and condensing and advancement of flowering. Hayward producers would be under significant financial pressure if these results are not achieved. HC has also produced excellent results in the cultivar 'Zesy002' (Gold3), increasing flower loading up to 100–150%. The New Zealand industry is also currently cropping approximately 150 ha of the cultivar 'Zesy003' (Gold9) variety. This variety has shown very high flower loadings, high levels of lateral flowers and very variable maturity development. This research investigates the ability of HC and Armobreak™ to condense flowering and, possibly, maturity development.

Zespri International Limited has funded research for orchard management operations and discovery of bud break enhancers that could serve as substitutes for HC. These trials have achieved interesting results over the years, although these results do not reach the current level of production achieved by HC, especially in years with low winter chill.

G. Hernández (✉) · R. Craig
Punchbowl Ltd., 646 Glenbrook Rd, R D 4, Pukekohe 2679, New Zealand
e-mail: gustavo@punchbowl.co.nz

© Springer International Publishing Switzerland 2015

J. V. Anderson (ed.), *Advances in Plant Dormancy,* DOI 10.1007/978-3-319-14451-1_17

Materials and Methods

Trial Design

An orchard block of *Actinidia chinensis* Planch 'Zesy003' (Gold9) kiwifruit vines in the Auckland Region of New Zealand was allocated for this trial. The vines were mature ex-Hayward and 3 years from grafting, trained on opposing female pergola structures (bay dimensions of 5.0 × 5.5 m) and managed using standard commercial practice for kiwifruit to achieve 44 t/ha.

The bud break enhancing treatments were applied by painting individual canes to the point of complete wetness. Vines were selected for cane uniformity with either 1 or 2 groups of suitable canes per vine and both vines and canes were randomly allocated to product and treatment timing. There were five treatment dates approximately 7 days apart starting 48 days before natural bud break (DBNB); natural bud break was on September 11th, 2013. The treatment products as specified below were applied to 20 individual plant/cane replicates per treatment time giving a total of 100 canes per product and 100 untreated canes (Controls). Two litres of diluted treatment product was required per 20 canes. The dilution of each product was prepared as follows:

- HC: 180 ml Hi Cane® and 6 ml Driftstop® with water up to 3 L of mixture. This is equivalent to an application rate of 6 % Hi Cane® and 0.2 % Driftstop®.
- AB: 60 ml Armobreak™ and 450 ml Break-N™ with water up to 3 L of mixture. This is equivalent to an application rate of 2 % Armobreak™ + 15 % Break-N™.

Monitoring and Data Analysis

Analysis of winter chill was determined using temperature records obtained from the Pukekohe Plant and Food Research station 6.5 km away from the experimental site.

Treated canes were assessed weekly during bud break and every 3 days during flowering. Buds were considered to have reached bud break when they formed a dome shape as defined by Brundell (1975). Each flower was considered to be open when the petals permitted easy access by bees.

Raw data were tested for normality, variance homogeneity, and outliers. When one of the assumptions of the ANOVA test was violated, the raw data were transformed in the most suitable way to accomplish the requirements. Data were then analysed using the ANOVA one-way procedure of SPSS (SPSS Inc., Chicago, Massachusetts). Post hoc comparisons were made using Duncan's test at 95 % level of significance.

This study analyses earliness by determining the average date of bud break and flowering of each repetition (cane), and the standard deviation (SD) of this average was used to assess the speed in the progression of this event. Approximately two SD units at each side of the mean date will comprise 95 % of the buds breaking or

flowers opening, so it is also a good estimate of the length of the flowering process; slower bud break and flowering progression is indicated by a higher SD of the mean date.

Bud break performance is determined by the percentage of winter buds (WB) that have shown bud break (%BB). The flower loading performance is determined by the average number of king flower inflorescences (KFl) per shoot (KFl/shoot), the percentage of shoots that carry at least one inflorescence (%floral shoots). This determines the production load or number of inflorescences per winter bud (KFl/WB). Finally, the incidence of lateral flowers, which commonly is considered to be unfavourable in kiwifruit production, can be determined by the percentage of inflorescences with at least one side flower (%laterals).

Fruit maturity is analysed before harvest, following the Zespri Quality Manual indications for maturity testing, equipment and calibration (Quality Manuals ZILNZ/HM/9 December 2013 and ZIL/NZ/UCCE/24 February 2014) and Harvest Clearance Standards (ZIL/NZ/HCSLS/9 December 2013). Zespri fruit maturity protocols specify de-greening time before standard cool storage according to the five percentile greenest fruit in the colour distribution. Protocols would have a longer de-greening time the greener the 5 % of the distribution tail is, and the de-greening time required at higher temperature compared to standard cool storage leads to less storage potential of the fruit. With these criteria the interest is not only to achieve a lower colour score, but also more homogeneous colour distribution with shorter tails on the greener side. For example, Protocol A fruit would need more de-greening time than Protocol B fruit, and Protocol B fruit more de-greening than Protocol C and so on; Protocol N fruit does not need de-greening time.

Results

Winter Chill

The winter chill of 2013 in the Pukekohe area was 279 h below 7 °C with a mean winter temperature of 11.4 °C, which is relatively low compared to the area's 19-year mean of 388 h and mean winter temperature of 11.0 °C (Table 17.1). Thus, 2013 was deemed to be a below average winter chill year.

Table 17.1 Winter chill summary: Average temperature (°C), cumulative hours below 7 °C for Pukekohe Research Station Meteorological Station

	May	June	July	August	Winter
Hours below 7 °C	36	78	137	28	279
(°C) Mean temperature 2013	12.8	10.9	10.2	11.6	11.4
Hours below 7 °C	41	107	141	100	388
(°C) Average mean temperature 1994–2013	13.0	10.7	10.0	10.4	11.0

Hydrogen Cyanamide Application Response and Timing

Gold9 is a very floral variety and, as shown in Table 17.2, the behaviour of HC on Gold9 differs compared to other varieties (Hernandez and Craig 2010; Hernandez et al. 2014). Based on KFl/WB, there is little difference between the control and the HC mean of all application dates; however, there are two HC application dates at 48 and 27 DBNB that are significantly higher in KFl/WB (Table 17.3). There was no identifiable time of application trend in KFl/WB across the application dates. In contrast, there was a trend in KFl/shoot across application dates where the latter two application dates (27 and 22 DBNB) show significantly lower KFl/shoot than the first application date. These later two dates also show significantly higher %BB (Table 17.3).

BB duration, as measured by the SD in date (Table 17.3), is significantly reduced in the latter two applications compared to the first two dates and the control. In flowering duration a trend of more condensed flowering in the two latter applications was noticeable, although it was not statistically significant. Looking at the bud break and flowering progression (Fig. 17.1) of the monitored canes, these two latter applications can be noticed by the increased slope of the progression curves compared to all other treatment curves, which would indicate a faster progression of both events. There were no significant differences identified in lateral flower production or % floral shoots (Table 17.3).

Performance of Armobreak™

The untreated Gold9 variety produces adequate flower load. The commercially significant variables are duration of flowering (SD flowering date) and %lateral flowers. The larger the SD on flowering date the more spread the flowering, which may decrease bee and artificial pollination efficiency and potentially increase fruit maturity variability—factors that can affect rejection rate at harvest (Goodwin et al. 2013).

As Table 17.3 indicates, treatment with AB produced similar flower loading levels, as measured by KFl/WB, to the Control in the first three application dates, but was significantly better than the Control in the last two application dates. When comparing the product means across all application dates, HC, AB and the Control performed at a similar level. For both HC and AB, the last two application dates had significantly higher %BB. There is a trend of lower KFl/shoot production with HC in latter application dates, but treatment with AB did not show this same trend.

The incidence of lateral flowers was very low in this study, at levels that were commercially insignificant. Consequently, there were no changes in trends for lateral flower loadings across any of the product treatments (Table 17.3).

The first four treatment dates with HC and AB advanced bud break and flowering by a similar number of days for each of the treatment dates. The last application date had a mean flowering date similar to the Control (Table 17.3). The last two

Table 17.2 Analysis of variance (ANOVA) of the average date of bud break (BB), flowering (Fl) for the canes painted with hydrogen cyanamide (HC) and Armobreak™ (AB) compared to the Control (CO) in the kiwifruit variety Gold9. Percentage of bud break and number of inflorescences (KFl) emerged per winter bud (WB) and per shoot. Percentage of inflorescences with lateral flowers and percentage of shoots with at least one inflorescence (%floral shoots). Standard deviation (SD) of the mean date of bud break and mean date of flowering for the population of buds belonging to the factor value indicated

Factor	Factor value	Mean BB date		SD (days)		Mean Fl date		SD (days)		BB (%)		KFl/WB		KFl/shoot		Lateral flowers (%)		Floral shoots (%)	
Product	CO	21-Sep	b	5.6	a	29-Oct	c	3.0	b	52%	c	1.91	a	3.6	a	2%	a	95%	a
	HC	13-Sep	a	5.4	a	28-Oct	b	2.9	b	69%	a	2.03	a	3.0	b	2%	a	97%	a
	AB	14-Sep	a	5.6	a	26-Oct	a	2.4	a	58%	b	2.14	a	3.6	a	3%	a	95%	a
Sig.		<0.001		N.S.		<0.001		<0.001		<0.001		N.S.		<0.001		N.S.		N.S.	
Treatment Date (DBNB)	48	11-Sep	a	7.5	c	24-Oct	a	2.9	b	58%	b	2.15	ab	3.59	a	3%	ab	95%	a
	42	16-Sep	b	6.1	b	27-Oct	b	3.2	b	49%	c	1.72	c	3.34	ab	5%	a	93%	a
	36	16-Sep	b	5.4	b	28-Oct	b	2.9	b	57%	b	1.83	bc	3.14	b	3%	ab	95%	a
	27	18-Sep	b	4.6	a	29-Oct	c	2.5	a	70%	a	2.43	a	3.55	a	1%	b	97%	a
	22	21-Sep	c	4.3	a	31-Oct	d	2.4	a	65%	a	2.00	bc	3.16	b	2%	b	96%	a
Sig.		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.05		<0.05		N.S.	
Interaction Sig.		<0.001		<0.001		<0.001		<0.01		<0.001		<0.001		<0.001		<0.01		N.S.	

Means within the same column, variety and factor with the same letter are not significantly different at $p = 0.05$ according to Duncan's test

Table 17.3 Average date of bud break (BB), flowering (Fl) and standard deviation (SD) for the canes treated with hydrogen cyanamide (HC) and Armobreak™ (AB) compared to the control (CO) in the kiwifruit variety Gold9 for the indicated treatment dates (in days before natural bud break or DBNB). Percentage of bud break and number of inflorescences (KFl) emerged per winter bud (WB) and per shoot. Percentage of inflorescences with lateral flowers and percentage of shoots with at least one inflorescence (%floral shoots)

Product	Date treat (DBNB)	Mean BB date		SD (days)		Mean Fl date		SD (days)		BB (%)		KFl/WB		KFl/shoot		Lateral flowers (%)		Floral shoots (%)	
CO		21-Sep	e	5.6	cb	29-Oct	dc	3.0	ed	52%	f	1.91	bc	3.55	bc	2%	bcd	95%	ab
HC	48	04-Sep	a	7.7	fe	22-Oct	a	3.1	e	71%	c	2.66	a	3.73	ab	2%	bcd	99%	a
	42	14-Sep	dc	7.0	ed	28-Oct	c	3.8	f	43%	g	1.30	d	3.01	cd	9%	a	97%	a
	36	12-Sep	c	4.9	ba	28-Oct	c	2.8	edc	62%	de	1.87	c	2.99	cd	1%	cd	97%	a
	27	16-Sep	d	3.8	a	30-Oct	d	2.5	dc	90%	a	2.50	a	2.75	de	1%	cd	97%	a
	22	20-Sep	e	3.7	a	01-Nov	e	2.4	cb	81%	b	1.83	cd	2.26	e	1%	cd	94%	ab
AB	48	09-Sep	b	8.4	f	21-Oct	a	2.9	edc	52%	f	1.80	cd	3.41	bc	5%	ab	94%	ab
	42	15-Sep	dc	6.2	dc	26-Oct	b	2.7	edc	49%	fg	1.69	cd	3.25	bcd	5%	bc	89%	b
	36	14-Sep	dc	5.6	cb	25-Oct	b	2.7	edc	56%	ef	1.92	bc	3.30	bcd	4%	bcd	94%	ab
	27	16-Sep	d	4.0	a	29-Oct	dc	1.9	ba	67%	cd	2.88	a	4.28	a	0%	cd	98%	a
	22	20-Sep	e	4.0	a	31-Oct	ed	1.8	a	67%	cd	2.42	ab	3.62	bc	0%	d	98%	a

Means within the same column and variety with the same letter are not significantly different at $p=0.05$ according to Duncan's test

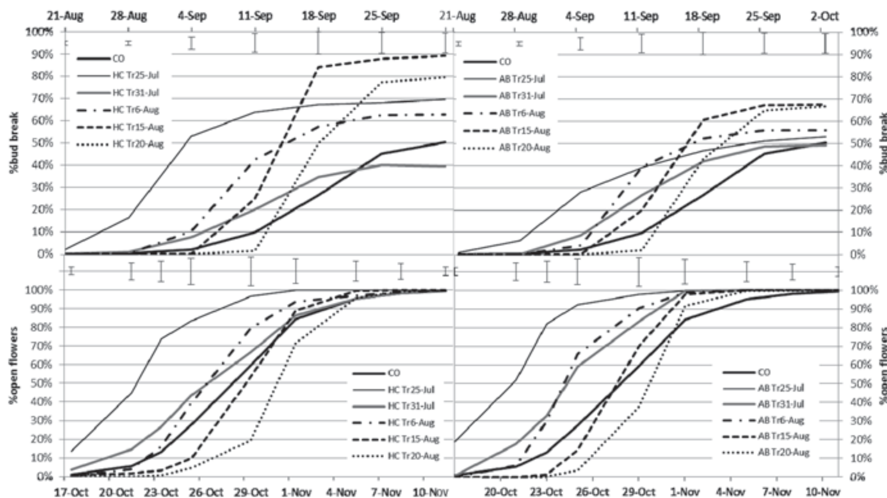


Fig. 17.1 Bud break (*upper graphs*) and flowering (*lower graphs*) progression of Gold9 treated canes at the indicated treatment dates (Tr) by painting with hydrogen cyanamide (HC, *left*) and with Armobreak™ (AB, *right*) compared with the untreated control (CO). *Vertical bars* show the least significant difference (LSD) between any treatment*date at a significance $p<0.05$

application dates for AB had significantly shorter flowering than the Control and bud break and flowering curve progression had a steeper slope in these two treatments when compared with all other application dates and control (Fig. 17.1).

Fruit Maturity Results

Table 17.4 shows the results of the maturity test performed on 24th of April, only for the canes treated 27 DBNB. HC induced a significantly lower colour score and less variability in fruit flesh colour (Fig. 17.2), resulting in a Protocol B maturity classification, which is defined as Zespri fruit needing less de-greening. However,

Table 17.4 Maturity monitoring results of fruit sampled on April 24th, 2014 from 'Zesy003' (Gold9) canes treated with hydrogen cyanamide (HC), Armobreak™ (AB) treated 27 days before natural bud break, and control (CO)

Treatment	Fresh weight (g/fruit)		Dry matter (%)		Equatorial SSC (Brix)		Firmness (kgf)		Average hue	
CO	109.9	a	18.5	a	8.0	a	5.63	b	105.9	b
HC	108.3	a	18.4	a	8.4	a	5.84	a	105.1	a
AB	106.4	a	18.3	a	8.2	a	5.75	ab	105.9	b
Sig	N.S.		N.S.		N.S.		$0.05<p<0.1$		<0.05	

SSC: Soluble solid content of the fruit juice, measured with optical refractometer
N.S.: Nonstatistically significant

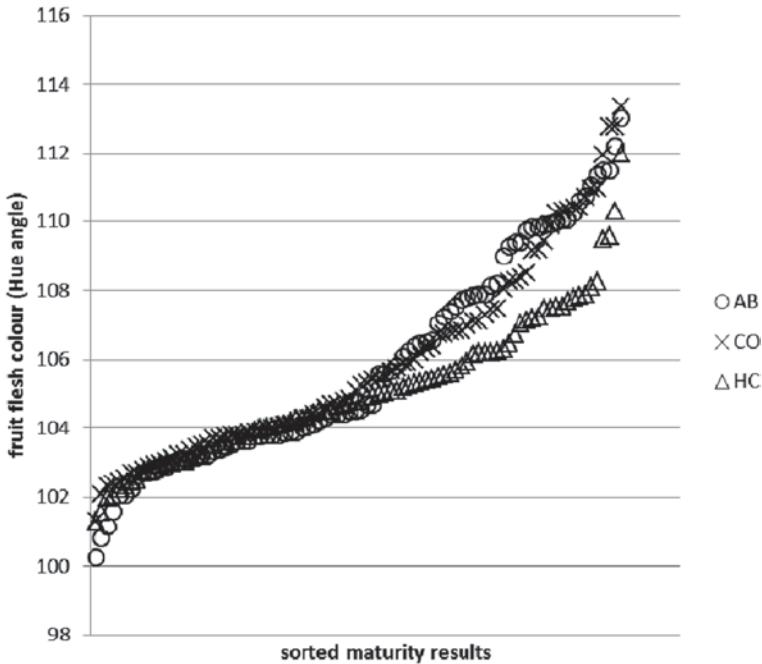


Fig. 17.2 Preharvest fruit flesh Hue angle distribution in Gold9 treated 27 days before natural bud break with hydrogen cyanamide (HC), Armobreak™ (AB) and control (CO)

Armobreak™ and the control remained Protocol A maturity classification, which is defined as Zespri fruit needing more de-greening time because of the longer tail in the higher Hue value distribution than Protocol B, before standard cool storage.

Discussion

Hydrogen Cyanamide Response and Application Timing

Averaging all application dates, flower loadings for the Gold9 variety were unaffected by HC application, although this was expected due to its high natural level of flower production. Gold 9 did show a trend in reduction of flowering duration in the two latter application dates, which could potentially increase bee pollination efficacy and reduce the variability in fruit maturity at harvest (Goodwin et al. 2013).

Because Gold9 inherently produces flower loadings in excess of the variety's production capability, there is no need for a flower load response from HC. Thus, the priorities are condensing flowering duration and possibly improving pollination, reducing maturity variability, and reducing lateral flower loadings to minimize thinning costs and increase fruit production efficiency.

In this study, the levels of lateral flowers observed were 2% or less for most of the treatment dates (including controls), and 9% for one application timing. This background level is very low for this variety (Gold9) compared with natural bud break in other commercial field observations, and this lack of variation has not allowed any knowledge development for this variable.

HC late application seemed to condense flowering, even though delaying the HC treatment seems to reduce flower production.

Maturity results indicate that treatment of canes with HC induced less flesh colour variability and resulted in a significantly lower colour score, while all other maturity variables were similar. These results suggest that HC treatment would allow for an earlier harvest and better storage, since the colour variability is reduced, and the de-greening time needed would be shorter.

Armobreak™ Performance

The Gold9 variety seems to be relatively winter chill insensitive and naturally produces flower loadings in excess of this variety's inherent productivity capability in most years. The opportunity for these bud break enhancing products is to condense the flowering period in the Gold9 variety.

AB treatments produced shorter flowering duration than both HC and the Control, with this effect more pronounced in the last two application dates at 27 and 22 DBNB. This effect should aid bee pollination and would potentially reduce maturity variability at harvest; however, maturity results indicate that the flesh colour variability did not decrease with AB in the 27 DBNB treatment date. This would force the fruit to undergo more time of de-greening at a higher temperature than the standard cool storage, waiting for the 5% greenest fruit to reach an acceptable colour, and potentially reducing economic returns in the market.

Conclusions and Further Research

Flowering in the variety Gold9 can be condensed with the use of Hi-Cane® or Armobreak™ between 27 and 22 DBNB. The use of Hi-Cane® at 27 DBNB has helped to reduce the Colour Score and its variability at preharvest. Further research is required to understand the nature of the Hi-Cane® and Armobreak™ response at a physiological and genetic expression level on Gold9 and in late applications from 20 to 30 DBNB. It would also be useful to repeat this trial in a colder winter to study the influence of winter chilling on Gold9 WB dormancy, natural bud break date, and effect of these products in these different conditions.

Acknowledgements This work was co-funded by Zespri International Limited (Project PC1448).

References

Brundell D (1975) Flower development of the Chinese gooseberry (*Actinidia chinensis* Planch.) I. Development of the flowering shoot. N Z J Bot 13:473–483

Council Directive of 15 July 1991 concerning the placing of plant protection products on the market (91/414/EEC) Official Journal L 230, 19/08/1991 P. 0001–0032

Erez A (1995) Means to compensate for insufficient chilling to improve bloom and leafing. Acta Hort 395:81–95

Goodwin RM, McBrydie HM, Taylor MA (2013) Wind and honey bee pollination of kiwifruit (Actinidia chinensis 'HORT16A'). N Z J Bot 51(3):229–240

Hernandez G, Craig R (2010) Effects of Alternatives to Hydrogen Cyanamide on Commercial Kiwifruit Production. Acta Hort 913:357–363

Hernandez G, Craig R, Tanner D (2014) Assessment of alternative bud break enhancers for commercial Kiwifruit production of 'Zesy002' (Gold3). Plant Dormancy Symposium, Auckland (New Zealand) 4–7 November 2013. In press

Mc Pherson HG Richardson AC Snelgar WP Currie MB (2001) Effects of hydrogen cyanamide on bud break and flowering in kiwifruit (*Actinidia deliciosa* 'Hayward'). N Z J Crop Hortic Sci 29:277–285

Chapter 18
Assessment of Alternative Bud Break Enhancers for Commercial Kiwifruit Production of 'Zesy002' (Gold3)

Gustavo Hernández, Rob Craig and David Tanner

Introduction

Hydrogen cyanamide (HC) has been used extensively in the kiwifruit industry as a bud break enhancing tool and to moderate seasonal fluctuations in fruit production. As a secondary effect, HC reduces unwanted lateral flowers, which reduces thinning costs and increases the average size of the fruit produced (Erez 1995; Mc Pherson et al. 2001; Hernandez and Craig 2010). The current restriction on the use of HC for dormancy interruption in Kiwifruit in the European Union, after being excluded from Annex 1 of the Directive 91/414/EEC, leads to a concern by growers that its future use will be restricted in New Zealand. Additionally, in the 2011/12 season, the Gold3 variety has been found, in some instances, to react erratically to the use of HC.

The New Zealand kiwifruit industry has harvested approximately 400 ha of the variety *Actinidia chinensis* Planch. 'Zesy002' (Gold3) in 2013. Additionally, the Zespri Gold kiwifruit *Actinidia chinensis* Planch. 'Hort16A', which is more sensitive to *Pseudomonas syringae* pv. *actinidiae* (Psa), is being substituted by Gold3, and this variety has become widespread in the New Zealand kiwifruit industry. There is, therefore, an obvious need to better understand the Gold3 variety's behaviour under HC application, and an aim of this chapter is to find and understand alternative products to HC for use in breaking dormancy.

The cane-painting technique consists of applying the product mixture onto the canes with a painting brush until achieving complete wetness. This technique adequately covers the objective of identifying the products that may induce positive

G. Hernández (✉) · R. Craig
Punchbowl Ltd., 646 Glenbrook Rd, RD 4, Pukekohe 2679, New Zealand
e-mail: gustavo@punchbowl.co.nz

D. Tanner
Zespri International Limited, 4043, Mt Maunganui 3149, New Zealand

© Springer International Publishing Switzerland 2015

J. V. Anderson (ed.), *Advances in Plant Dormancy,* DOI 10.1007/978-3-319-14451-1_18

effects by ensuring that the winter buds are in full contact with the product. Because of the flexibility of the location and the reduced size of the experiment compared to any spraying technique, these trials were easily applied over a number of time points to understand the progression of the plant responsiveness to several application dates. This technique may be equivalent to an air-blast spray application with a volume rate of around 1000 l/ha (Bill Snelgar, pers comm). However, the results of this cane-painting trial need to be verified in conditions more similar to the standard air-blast spray that are applied on commercial fields, in order to provide agronomic recommendations to the growers.

Material and Methods

Trial Design

Two orchard blocks of Gold3 kiwifruit vines were allocated for this trial. All vines were mature ex-Hayward (*Actinidia deliciosa var. deliciosa* 'Hayward') and 2 years from grafting, trained on opposing female pergola structures (bay dimensions of 5.0 m × 5.5 m) and managed using standard commercial practice for Gold3 kiwifruit. The bud break enhancing treatments were applied by painting individual canes to the point of complete wetness. Vines were selected for cane uniformity with either 10 or 20 suitable canes per vine and both vines and canes were randomly allocated to product and timing treatments. All treatment products were applied to either one or two canes per treatment product on each vine. There were five treatment dates approximately 7 days apart starting 45 days from natural bud break. In total there were nine treatment products (but only treatments with commercially available products are presented) and an untreated Control, consisting of 20 individual plant/cane replicates per treatment timing to give a total of 200 canes per application timing and 1000 canes in total. Two litres of diluted treatment product was required per 20 canes.

The dilution of the commercial products was prepared as follows:

- HC: 180 ml Hi Cane® and 6 ml Driftstop® with the balance in water (3 L of mixture). This is equivalent to an application of 6 % Hi Cane and 0.2 % Driftstop®.
- AB: 60 ml Armobreak™ and 450 ml Break-N™ with the balance in water (3 L of mixture). This is equivalent to an application of 2 % Armobreak™ + 15 % Break-N™.
- ER: 180 ml Erger® and 270 ml Active Erger® with the balance in water (3 l of mixture). This is equivalent to an application of 6 % Erger® + 9 % Active Erger®.

The treatments were applied 45, 39, 33, 25 and 17 days before (calculated) natural bud break (DBNB); 8 September 2012 was the date of natural bud break of the Control. On the 6th and 7th of August 2012, the male canes were painted with HC, 33 DBNB of the female Control.

Monitoring and Data Analysis

In order to monitor the influence of environmental factors, the hourly air temperature (in °C), relative humidity (%) and rainfall (mm) were recorded from an agro-meteorological station located approximately 600 m from the experimental vines. The cumulative Richardson Chill Units (Richardson et al. 1974) and the cumulative hours below 7 °C were calculated.

These canes were assessed weekly during bud break and every 3 days during flowering. Buds were considered to have reached bud break when they formed a dome shape as defined by Brundell (1975). Each flower was considered to be open when the petals permitted easy access to bees.

Raw data were tested for normality, variance homogeneity and outliers. When one of the assumptions of the ANOVA test was violated, the raw data were transformed in the most suitable way to accomplish the requirements. Data were then analysed using the ANOVA one-way procedure of SPSS (SPSS Inc., Chicago, Massachusetts). Post hoc comparisons were made using Duncan's test at 95 % level of significance.

Results

Winter Chill

The winter chill of 2012 was above the average of the last 18 years (Table 18.1). Natural bud break (defined as 10 % bud break) occurred on 8 September2012 for the Control in these relatively favourable conditions for bud break.

Hydrogen Cyanamide Application Timing

As shown in Table 18.2, treatment with HC resulted in good to an exceptional number of King Flowers (inflorescences) per winter bud (KFl/WB). These results indi-

Table 18.1 Winter chill summary: Average temperature (°C), cumulative hours below 7 °C and cumulative Richardson Chill Units (RCU) (Richardson et al. 1974) for Pukekohe research station meteorological station

	May	June	July	August	Winter
RCU 2012	222	348	404	341	*1315*
Hours below 7 °C	82	134	167	62	*445*
(°C) Mean temperature 2012	11.9	10.3	10.0	11.0	*10.8*
Average RCU 1994–2012	139	297	396	363	*1195*
Hours below 7 °C	41	108	141	104	*394*
(°C) Average mean temperature 1994–2012	13.0	10.7	10.0	10.3	*11.0*

Table 18.2 Performance of hydrogen cyanamide: Average date of bud break (BB), flowering (Fl) and standard deviation (SD) of these two for the five application timings with hydrogen cyanamide (HC) in the kiwifruit variety 'Gold3'. Percentage of bud break and number of inflorescences (KFI) emerged per winter bud (WB) and per shoot. Percentage of inflorescences with lateral flowers

Treatment	Application date	Mean BB		SD (days)	Mean Fl		SD (days)	BB (%)		KFI/WB		KFI/Shoot		Lateral flowers (%)		Deformed (%)	
CO	–	19-Sep	cde	6.3	2-Nov	cd	3.3	57	c	1.47	d	2.59	e	19	b	2.2	abc
HC	25/Jul	2-Sep	a	2.2	27-Oct	a	1.3	72	b	3.34	a	4.65	a	42	a	0.9	a
	31/Jul	10-Sep	b	3.9	29-Oct	b	1.4	60	c	2.51	b	4.14	ab	35	ab	1.7	a
	6/Aug	11-Sep	b	3.6	1-Nov	c	1.4	75	ab	2.51	b	3.36	b	23	bc	2.8	a
	14/Aug	16-Sep	bc	2.9	2-Nov	cd	2.5	79	a	1.95	c	2.47	bc	20	c	3.4	a
	22/Aug	21-Sep	c	4.2	4-Nov	d	2.6	74	ab	1.43	c	1.97	c	18	c	2.1	a
Sig.		<0.001		–	<0.001		–	<0.05		<0.001		<0.001		<0.05		N.S.	

Means within the same column with the same letter are not significantly different according to Duncan's test ($p = 0.05$)

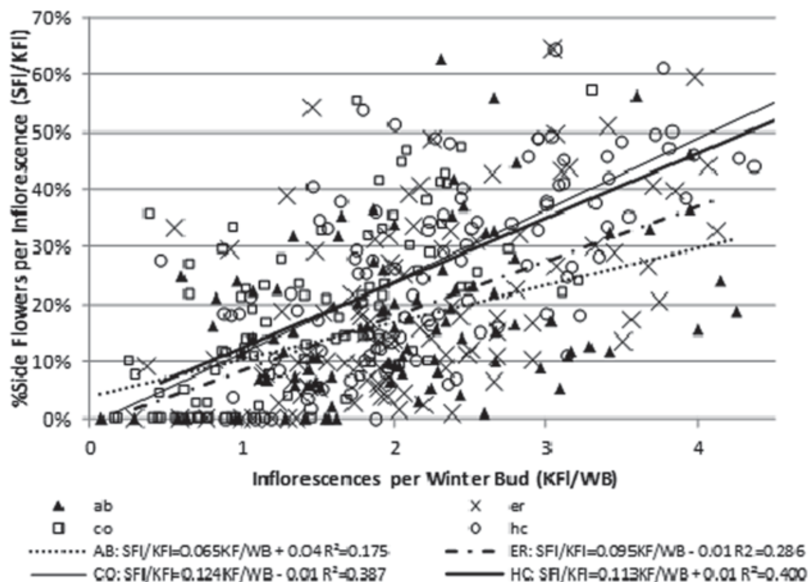


Fig. 18.1 Relationship between king flowers per winter bud (KFl/WB) and % lateral flowers (SFl/KFl)

cate good performance through the first four application dates, which ranged from 3.34 KFl/WB at the 25th of July (45 DBNB) application to 1.95 KFl/Wb at the 14th of August (25 DBNB) application date. HC and the control have a significant correlation between KFl/WB and the percentage of inflorescences with lateral flowers (SFl/KFl) and, as shown in Fig. 18.1, the percentage of inflorescences with lateral flowers increases with increasing KFl/WB. The SFl/KFl, in response to HC treatment, ranged from 18.3 % at the latest application to 41.9 % at the first application date.

Product Performance Comparison

The results for timing of bud break and flowering, %bud break (%BB), KFl/WB and SFl/KFl are presented in detail in Table 18.3. All of the successful products show an optimum application timing window of 3 or 4 weeks. Due to the existence of this optimum application timing window, the best three application dates for each product, based on KFl/WB, have been selected, combined and summarized in Table 18.3a. Employing this procedure is recognizing that retaining the nonoptimal application dates in the presentation would bias the comparison between the products. Subsequent discussion, unless otherwise indicated, will be restricted to the results presented in Table 18.3a. All of the results for each treatment date are shown in Table 18.3b.

Table 18.3. Performance of three best dates (Table 18.3a, upper table) and all dates of application (Table 18.3b, lower table): Average date of bud break (BB), flowering (Fl) and standard deviation (SD) of these dates for the three best application timings with hydrogen cyanamide (HC), Armobreak™ (AB) and Erger® (ER) compared to the Control (CO) in the kiwifruit variety 'Gold3'. Percentage of bud break and number of inflorescences (KFl) emerged per winter bud (WB) and per shoot. Percentage of inflorescences with lateral flowers

Treatment	Average best treatments date (DBNB)	Mean BB date		SD (days)	Mean F1 date		SD (days)	BB (%)		KF1/WB		KF1/Shoot		Lateral flowers	
CO	–	19-Sep	c	6.3	2-Nov	bc	3.3	56.7%	b	1.47	c	2.59	c	19%	b
HC	39	7-Sep	a	3.2	29-Oct	a	1.3	69.2%	a	2.79	a	4.05	a	33%	a
AB	34	16-Sep	b	4.4	2-Nov	b	2.2	65.4%	a	2.37	b	3.63	ab	20%	b
ER	25	17-Sep	bc	4.3	3-Nov	c	2.3	64.9%	a	2.27	b	3.52	b	18%	b
Sig.		<0.001		<0.001	<0.001		<0.001	<0.05		<0.001		<0.001		<0.05	
Treatment	Treatments date (DBNB)	Mean BB		SD (days)	Mean FL		SD (days)	BB (%)		KFL/WB		KFL/Shoot		Lateral Flowers(%)	
CO	–	20-Sep	ghij	6.3	2-Nov	def	3.3	56.1	defg	1.4	d	2.6	gh	18.3	efgh
HC	45	2-Sep	a	2.2	27-Oct	a	1.3	72.4	bc	3.34	a	4.65	ab	41.9	a
	39	10-Sep	bc	3.9	29-Oct	b	1.4	60.4	defg	2.51	b	4.14	abcde	35.2	abc
	33	11-Sep	bcde	3.6	1-Nov	cd	1.4	74.9	ab	2.51	b	3.36	defg	22.9	cdefg
	25	16-Sep	cdefgh	2.9	2-Nov	def	2.5	79.4	a	1.95	cd	2.47	gh	19.9	defgh
	17	21-Sep	hij	4.2	4-Nov	ef	2.6	73.8	ab	1.43	d	1.97	h	18.3	efgh
AB	45	16-Sep	defgh	5.2	31-Oct	cd	3.0	57.6	efg	1.98	cd	3.44	cdefg	26.8	bcde
	39	20-Sep	ghij	7.4	2-Nov	def	3.0	56.2	efg	1.64	cd	2.8	fgh	19.3	efgh
	33	16-Sep	cdefgh	5.1	2-Nov	def	2.3	64.7	cdef	2.51	b	3.86	bcde	22.2	cdefg
	25	17-Sep	efghi	3.0	4-Nov	f	1.4	73.8	ab	2.64	b	3.59	cdef	11.4	fgh
	17	24-Sep	j	1.7	8-Nov	h	1.0	74.1	ab	1.47	d	1.98	h	6.5	h

Table 18.3. (continued)

Treatment	Average best treatments date (DBNB)	Mean BB date		SD (days)	Mean F1 date		SD (days)	BB (%)		KF1/WB		KF1/Shoot		Lateral flowers	
ER	45	16-Sep	defgh	7.8	1-Nov	cd	4.3	54.1	g	1.76	cd	3.19	efg	22.6	cdefg
	39	18-Sep	fghi	6.0	2-Nov	def	2.7	55.7	fg	1.84	cd	3.29	defg	25.0	bcdef
	33	15-Sep	cdefg	5.7	1-Nov	def	2.8	59.0	defg	2.54	b	4.18	abcd	32.8	abcd
	25	16-Sep	defghi	5.2	3-Nov	ef	2.2	67.3	bcd	2.53	b	3.77	bcde	12.7	fgh
	17	22-Sep	ij	2.0	7-Nov	gh	1.8	68.1	bcd	1.76	cd	2.62	fgh	9.2	gh
Sig.		<0.001		<0.001	<0.001		<0.001	<0.001		<0.001		<0.001		<0.001	

Means within the same column with the same letter are not significantly different according to Duncan’s test ($p = 0.05$)

From a commercial perspective, the most significant variables are firstly KFl/WB as a measure of potential crop load, then % lateral flowers indicates a potential cost of thinning, and the standard deviation (SD) on flowering date would indicate the spread or spontaneity of flowering. The larger the SD on flowering date, the more spread the flowering is, which potentially could create pollination problems and flow-on effects of variable maturity at harvest.

Flower Loading and Bud Break

KFl/WB is a direct measure of crop load potential and is the most important measure of bud break enhancement performance. For KFl/WB, HC performed significantly better for enhancing bud break than AB and ER with 0.4–0.5 more KFl/WB. This second group in turn performed significantly better than the Control (natural bud break) with 0.8–0.9 higher KFl/WB. While the trend in %BB performance was similar to that for KFl/WB, there were no significant differences observed between the three treatment products (HC, AB, ER).

Lateral and Deformed Flowers

Increasing levels of lateral flowers means increased variability in fruit size and increasing cost of production in the form of fruit thinning costs. In this trial, we have observed a consistent correlation between the KFl/WB and the SFl/KFl, as shown in Fig. 18.1. Although all treatments have this correlation, HC and the control have an especially high correlation, whereas ER and AB have a lower level of correlation (however still highly significant, $p < 0.01$). This indicates significant increases in thinning costs correlated to the success in flowering performance.

Bud Break and Flowering Progression

The timing of mean bud break and flowering, as presented in Table 18.3a, may affect the earliness of harvest maturity. Additionally, the more compact and spontaneous the flowering the more successful bee pollination performance tends to be. In this work, the SD, as presented in Table 18.3b, is a measure of the spread in date of bud break and flowering; the higher the SD, the greater the spread.

HC has generated significantly earlier bud break and flowering than all other treatments. Bud break in plants treated with AB and ER were 4–8 days later than HC, but only 2–4 days later in flowering. These second tier products were slightly ahead of the Control at bud break but there was no significant difference in flowering date.

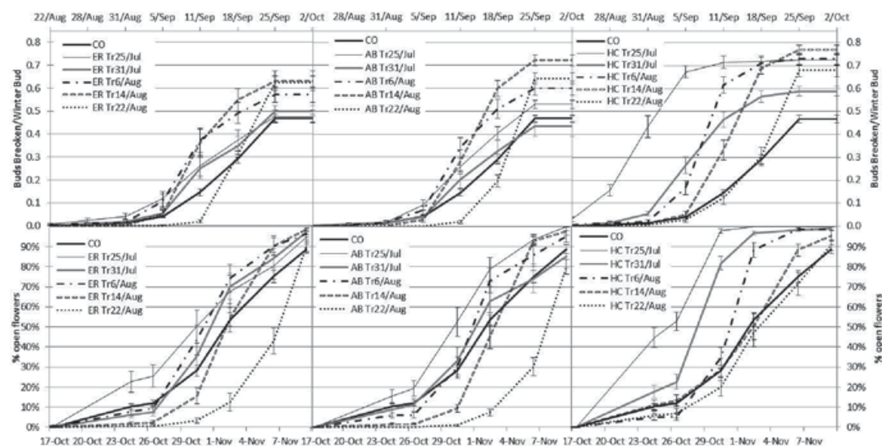


Fig. 18.2 Progression of bud break (*upper graphs*) and flowering (*lower graphs*) of each product—hydrogen cyanamide (HC), Armobreak™ (AB), Erger® (ER)—compared to the control (CO) at the indicated treatment dates (Tr). *Vertical bars* indicate the standard error

All treatment products had a trend of earlier bud break and flowering with earlier dates of application of the product (see Table 18.3b). For HC, the difference in bud break timing between the first and last application date was 19 days; however, this difference is reduced to 8 days for flowering. The difference between the average date of bud break based on the first and last application dates was 8 days for canes treated with AB and 6 days for the canes treated with ER, and at flowering this difference held at 8 days for both products.

HC treatment produced the most compact bud break and flowering, as measured by the SD, ahead of treatment with AB and ER, which in turn produced more compact bud break and flowering than the Controls (see Table 18.3b). Additionally, HC treatment produced a trend in the spread of flowering, where the earlier the application the more compact the flowering. This is opposite to the trend observed with AB and ER, where the spread in both bud break and flowering is reduced the later the application date. Detailed graphs of the progression of bud break and flowering over time for all product treatments and application timings are provided in Fig. 18.2.

Discussion

HC is the industry standard bud break enhancer and is employed with the 'Hayward' variety to increase flower loadings, reduce spread of flowering and reduce the incidence of lateral flowers. In the 'Hort16A' variety its purpose is to increase flower loadings and to reduce maturity variability at harvest. The 'Gold3' variety, in its short life span, has demonstrated erratic bud break dynamics under natural bud break conditions and also, in some cases, when treated with HC.

While recognizing that 2012 was a high winter chill year, it seems 'Gold3' had a relatively wide HC application timing window for optimal bud break performance ranging from July 25th (45 DBNB) until mid-August (25 DBNB). Because the highest KFl/WB for HC treatment occurred at the first application date, it is probable that this investigation did not explore the earliest possible HC application timing to achieve successful bud break enhancement.

Treatment with AB and ER showed promising levels of performance with KFl/WB at a mean 83 % of the performance level of HC (Table 18.2a), but with lower levels of lateral flowers. These products need further investigation with commercial application technology. With 'Gold3' being such a large fruited variety, a moderate flower loading of approximately 2.0 KFl/WB and 30 winter buds per square meter can achieve a yield of 15,000 Class 1 trays or 50 t per hectare. Hence, AB and ER may also have real commercial potential.

Alternative Bud Break Enhancers to HC Require Further Studies

AB and ER seem to promote bud break and flowering in a very different way than HC does. Genetic expression in kiwifruit vines treated with HC (Walton et al. 2009; Wu et al. 2012; Wood et al. 2013) identified expression of genes responding to a sublethal stress (H_2O_2-mediated signalling cascade), disruption of the mitochondrial electron transport chain (mETC) and down-regulation of the genes *Ade_DRM1_1G* and *SVP-like* (which are also inversely correlated with growth potential of the bud). However, to date, there is a lack of knowledge on the physiological impact of the alternatives to hydrogen cyanamide at a cellular level. Thus, further studies would be needed to identify nontoxic compounds that are able to trigger similar physiological effects to hydrogen cyanamide against the existing growth inhibitors in the winter bud.

Our Current Knowledge About These Alternative Bud Break Enhancers

Armobreak™ is a super-penetrating surfactant that increases the assimilation of the nitrogen and calcium contained in Break-N™, the product designed to be used with Armobreak™ to promote kiwifruit bud break (International Agro Additive Specialities, B.V.). The super-penetrant Armobreak™ seems to enhance the mild bud break effect of nitrogen- and calcium-based fertilizer in other species (Erez et al. 2008). It has also been studied as a way to decrease the amount of HC needed in a mixed bud break enhancement treatment.

Erger® and Active Erger® are nitrogen-based fertilizers with a high content of calcium. According to various sources (Panceri and Santos 2007; Erez et al. 2008; Hawerroth et al. 2010; Pereira et al. 2010), nitrogen-based fertilizers with a calcium content stimulate bud development in several species of fruit trees, but only when a

certain percentage of the chilling requirement has been met. This would agree with the results obtained for the third and fourth treatment dates of AB and ER as shown in Table 18.3b, optimal dates for AB and ER, contrasting with the efficacy of the early treatments of HC. However, the result of the last treatment date differs in that both ER and AB treatments stimulated a more complete %BB but failed to increase the KFl/WB over that of the control. This last treatment date may be too late to stimulate the development of more floral buds, the fate of which may have already been determined. The decreasing trend of appearance of side flowers in the inflorescences in the last two AB and ER treatment dates is also in accordance with the idea of these treatments not being able to stimulate the development of floral buds.

Polito and Grant (1984) observed that floral primordial remain undifferentiated during winter until shortly before bud break, when the primordia become trilobed as bracts and lateral flower primordia are initiated. According to Snowball (1996), flower evocation could occur immediately prior to flower initiation. In this study, all treatments obtained significantly more KFl/WB than the control. These results may involve a very narrow window of flower evocation just before bud break, and the decrease in efficacy of these treatments at the last treatment date may be caused by the fate determination of the primordia.

This study was conducted with a paint brush application technique, which gives very good coverage of all winter buds with minimal run-off. The adjacent commercial 'Gold3' blocks, that had received the same orchard management over the preceding 2 years, were sprayed on 9 August with industry standard air-blast sprayer technology. Observational studies showed some inconsistency in performance of these vines within the experimental area, particularly in %BB. It is important that further work be conducted to observe the transferability of these results into industry standard orchard spray systems. Also, this inconsistency suggests techniques that achieve better air-blast spray coverage, such as higher water rates, may be beneficial.

Conclusions

- Hydrogen cyanamide has a broad application window of at least 3 weeks, ranging from 45 or more days down to 25 days prior to natural bud break, on the 'Gold3' variety.
- AB and ER have potential as effective commercial bud break enhancers on the 'Gold3' variety.
- Further work will be required to demonstrate that the outcomes of this study are transferable into commercial air-blast spray technology systems and has potential in years with lower winter chill.

Acknowledgements This research project was funded by Zespri International Limited. We also want to thank the collaboration of Mandeep Pannu and Ngaire-Grace Skelton.

Reference

Brundell D (1975) Flower development of the Chinese gooseberry (*Actinidia chinensis* Planch.) I. Development of the flowering shoot. N Z J Bot 13:473–483

Council Directive of 15 July 1991 concerning the placing of plant protection products on the market (91/414/EEC) (19/08/1991) Official Journal L 230:0001–0032

Erez A (1995) Means to compensate for insufficient chilling to improve bloom and leafing. Acta Hortic 395:81–95

Erez A, Yablowitz Z, Aronovitz A, Hadar A (2008) Dormancy breaking chemicals; efficiency with reduced phytotoxicity. Acta Hortic 772:105–112

Hawerroth FJ, Petri JL, Leite GB (2010) Erger and calcium nitrate concentration for budbreak induction in apple trees. Acta Hortic 872:239–244

Hernandez G, Craig R (2010) Effects of alternatives to hydrogen cyanamide on commercial kiwifruit production. Acta Hortic 913:357–363

Mc Pherson HG Richardson AC Snelgar WP Currie MB (2001) Effects of hydrogen cyanamide on budbreak and flowering in kiwifruit (*Actinidia deliciosa* ‘Hayward’). N Z J Crop Hortic Sci 29:277–285

Panceri CP, Santos HP (2007) Evaluation of alternative products for breaking dormancy in grapevine. VIII international symposium of temperate zone fruits in the tropics and subtropics, Florianópolis. Program and abstracts, document 207:146

Pereira N, Oliveira CM, Mota M, Sousa RM (2010) Evaluation of five dormancy breaking agents to induce synchronized flowering in ‘Rocha’ pear. 11th international pear symposium. Acta Hortic 909:423–428

Polito VS, Grant JA (1984) Initiation and development of pistillate flowers in *Actinidia chinensis*. Scientia Hortic 22(4):365–371. doi:10.1016/S0304-4238(84)80008-4

Richardson EA, Seeley SD, Walker DR (1974) A model for estimating the completion of rest for Redhaven and Elberta peach trees. HortScience 9(4):331–332

Snowball AM (1996) The timing of flower evocation in kiwifruit. J Hortic Sci 71:335–347

Walton EF, Wu R-M, Richardson AC, Davy M, Hellens RP et al (2009) A rapid transcriptional activation is induced by the dormancy-breaking chemical hydrogen cyanamide in kiwifruit (Actinidia deliciosa) buds. J Exp Bot 60:3835–3848. doi:10.1093/jxb/erp231

Wood M, Rae GM, Wu R-M, Walton EF, Xue B et al (2013) Actinidia DRM1—An intrinsically disordered protein whose mrna expression is inversely correlated with spring budbreak in kiwifruit. PLoS ONE 8(3):e57354. doi:101371/journal.pone.0057354

Wu R-M, Walton EF, Richardson AC, Wood M, Hellens RP, Varkonyi-Gasic E (2012) Conservation and divergence of four kiwifruit SVP-like MADS-box genes suggest distinct roles in kiwifruit bud dormancy and flowering. J Exp Bot 63:797–807. doi:10.1093/jxb/err304

Chapter 19
Effect of BLUPRINS® Application on Bud Release from Dormancy in Kiwifruit, Cherry, and Table Grape

V. Ziosi, A. Di Nardo, A. Fontana, F. Vitali and G. Costa

Introduction

In temperate regions, buds of woody plants enter a dormancy phase in winter, whereby metabolic and respiratory activities are reduced and growth is suspended. This physiological reaction enables plants to tolerate prolonged periods of adverse conditions such as freezing temperatures in their native habitats (Vegis 1964). Dormancy is naturally released after an adequate period of exposure to cold temperatures. In warm-winter areas, where plants undergo chilling deficiency, reduced and uneven bud breaking is the major cause of low productivity in orchards (Arora et al. 2003). In these regions, the application of chemicals such as hydrogen cyanamide, the most successful dormancy-breaking agent for deciduous fruit crops, has been a widespread practice to trigger bud growth resumption. Since hydrogen cyanamide has been proven toxic for humans, it was taken or is about to be taken off many markets, leaving very few options for the growers. Moreover, hydrogen cyanamide can be phytotoxic under specific environmental conditions, posing serious risk to crops like stone-fruit species (Walton et al. 2009). Therefore, finding safe and effective bud breaking products is critical for maintaining economic production.

BLUPRINS® is an innovative dormancy-breaking agent by Biolchim SpA containing amino acids and polysaccharides, which, in combination with its activator BLUACT, delivers inorganic and organic nitrogen, organic carbon, and calcium. This study tests the efficacy of BLUPRINS® as an alternative to hydrogen cyanamide on kiwifruit, cherry, and table grape.

V. Ziosi (✉) · A. Di Nardo · A. Fontana · F. Vitali
Biolchim S.p.A., via San Carlo 2130, 40059 Medicina, Bologna, Italy
e-mail: ziosi@biolchim.it

G. Costa
Dipartimento di Colture Arboree, Università di Bologna, via Fanin 46, 40127 Bologna, Italy

© Springer International Publishing Switzerland 2015

J. V. Anderson (ed.), *Advances in Plant Dormancy,* DOI 10.1007/978-3-319-14451-1_19

Materials and Methods

Trial on Kiwifruit

A trial was performed on an *Actinidia chinensis* var. Gold3 orchard located in Pukekohe, New Zealand, in 2013. Vines with uniform canes were selected, and one cane per vine was assigned to the hydrogen cyanamide (Hi Cane®, Nufarm, New Zealand) treatment, to the BLUPRINS® (Biolchim, Italy) treatment, or to the untreated control (mock treatment with water only). Each product was applied on 20 different canes on each time point, for a total of 100 canes per treatment. Bud breaking agents were applied by cane painting to the point of complete wetness to ensure a homogeneous distribution of the product along the cane and an optimal covering of the buds' surfaces. BLUPRINS® was applied at a rate of 7% in mixture with its nutritional activator BLUACT at 20%. BLUACT is a fertilizer containing 15% N and 7% CaO designed to support bud growth after resumption from dormancy.

In order to evaluate the efficacy of the products at different time points, treatments were performed 57, 51, 45, 36, and 31 days before expected natural bud break (DBNB), with 20 replicates per treatments per time point. Time points were chosen in late winter, after plant exposure to winter chill but before any bud growth or development could occur, and within the typical application time window of hydrogen cyanamide. Natural bud break was determined when 10% of buds were broken on untreated canes. In order to calculate the average bud break and flowering dates (Table 19.1), buds were considered to have reached bud break when they formed a dome shape as described in Brundell (1975), while flowers were considered open when the petals allowed the entrance of a bee. Vines and canes were randomly allocated to the products and application time points. The average number of king inflorescences per winter bud (KF/WB) was calculated as an indicator of the potential crop load. The percentage of lateral flowers (% LAT FL) as an indicator of the variability in fruit size and need for thinning.

Environmental factors (air temperature, humidity, and rainfall) were monitored throughout the trial period. Winter (May–August) average temperature in Pukekohe was 11.4 °C in 2013, with 300 cumulative hours below 7 °C.

Differences between treatments were evaluated for each factor by one-way ANOVA followed by Duncan's test (Table 19.1). Data were transformed whenever needed to fulfil the prerequisites for ANOVA. Analyses were performed with the software SPSS (SPSS Inc., Chicago, USA).

Trials on Cherry

Plants of *Prunus avium* (L.) variety Ferrovia grafted onto *P. mahaleb* located in Trani (Apulia, Italy) were treated with BLUPRINS® 45 or 30 before expected natural bud break for two consecutive seasons (2011 and 2012). In season 2011, BLUPRINS® was tested at 6, 8 and 10% on each treatment time point, whereas

Table 19.1 Effect of BLUPRINS® (BP) and hydrogen cyanamide (HC) treatment at different time points on production traits of *A. chinensis* var. Gold3 as compared to untreated control (UTC). Product (*Prod*); treatment date in days before natural bud break (Treat time (DBNB)); average bud break date (Av. BB date) and its standard deviation (SD of BB); average bloom date (Av. Bloom date), and its standard deviation (SD of Bloom); number of king flower inflorescences per winter bud KF/WB; percentage of floral shoots (% FL ST); percentage of lateral flowers (% LAT FL). Different letters within the same column indicate significant differences within a column (according to one-way ANOVA followed by Duncan's test, $P < 0.05$)

Prod	Treat time (DBNB)	Av. BB date		SD of BB (days)		Av. Bloom date		SD of Bloom (days)		KF/WB		% FL ST		% LAT FL	
UTC	–	01 Oct	h	5.2	dc	08 Nov	g	2.5	dc	1.37	e	86	c	9	f
HC	57	07 Sept	a	6.5	ed	25 Oct	a	2.9	ed	2.02	d	98	a	32	a
	51	11 Sept	b	4.4	cb	29 Oct	b	2.6	cde	2.38	cd	99	a	15	def
	45	14 Sept	cb	4.0	cba	30 Oct	b	1.8	ba	2.92	ab	99	a	28	abc
	36	18 Sept	ed	3.5	ab	03 Nov	c	1.4	a	3.38	a	100	a	22	bcd
	31	22 Sept	fg	2.9	a	05 Nov	d	1.5	ba	3.06	ab	99	a	12	def
HC	–	14 Sept	–	4.2	–	30 Oct	–	2	–	2.75	–	99	–	22	–
BP	57	28 Sept	h	5.8	d	08 Nov	gf	3.1	ed	1.44	e	84	c	10	ef
	51	16 Sept	dc	7.8	e	28 Oct	b	3.6	j	2.24	cd	97	a	31	ab
	45	24 Sep	g	7.5	e	02 Nov	c	3.2	je	1.54	e	93	ac	19	cde
	36	20 Sept	fe	4.3	cb	02 Nov	c	1.5	ba	3.27	a	100	a	21	cd
	31	24 Sept	g	4.2	cba	05 Nov	d	2.2	cb	2.59	bc	98	a	12	def
BP	–	22 Sept	–	5.9	–	02 Nov	–	2.7	–	2.22		94	–	19	–

Table 19.2 Hours below 7 °C accumulated at the moment of the treatments at the location where the trials on *P. avium* were performed (Trani, Italy). DBNB: days before natural bud break

	Winter 2011	Winter 2012
45 DBNB	562	976
30 DBNB	586	1031

in 2012 concentrations were reduced to 4 and 6 %. An evaluation of the 2011 results suggested that treatments with concentrations of 8 and 10 % would make the product application economically disadvantageous. For each treatment, two plants with seven uniform branches carrying each 80–90 buds were selected. Trees were 7 years of age in 2011 and rows of cherry trees were alternated by rows of olive trees with a planting distance of 8 × 8 m. BLUPRINS® was always mixed with its activator BLUACT at a concentration of 16 % and applied with a knapsack sprayer. Previous trials showed that spraying ensured a uniform and complete distribution of the product on cherry trees, allowing also for a faster application in comparison with the painting technique. Winter temperatures were particularly mild in 2011; the cumulative hours below 7 °C at the moment of the treatment were enough to satisfy the plants' chilling requirements in both years (Table 19.2).

Trial on Table Grape

Three treatments with bud break enhancers were performed on grapevines (*Vitis vinifera*) var. Victoria located in Sicily, Italy. Groups of five homogeneous plants were selected and each was treated either with a mixture containing BLUPRINS® at 4 % and BLUACT at 20 %, or with hydrogen cyanamide at 4 %, or with water only (control plants). Treatments were carried out 60 days before the expected natural bud break on a first set of plants and 45 days before the expected natural bud break on a second set of plants (on Dec 24 or Jan 8, respectively). Treatments were performed with a knapsack sprayer.

Results

Trial on Kiwifruit (Table 19.1)

Bud Break and Flowering Development Both BLUPRINS®- and HC-treated kiwifruit plants flowered on average significantly earlier than the untreated control (UTC) plants. Only BLUPRINS® applicationat 57 DBNB did not trigger a significantly earlier flowering compared to UTC. BLUPRINS®'s maximum effect on bud break enhancement was reached with the treatment at 51 DBNB, which caused bud break 15 days before the UTC. HC's maximum effect was reached instead with

the application at 57 DBNB, which prompted flowering 24 days before the UTC. BLUPRINS® and HC had a similar effect on bud break when applied at 51 and 45 DBNB, respectively. The lowest standard deviations (SD) both for bud break and for bloom date were achieved with late applications of the products (36–31 DBNB).

Potential Crop Load Both BLUPRINS® and HC enhanced flower loading (KF/WB) over UTC, giving the highest values in late applications (45 DBNB onwards for HC, 36 DBND for BLUPRINS®). BLUPRINS® application at 51 DBNB produced 2.24 KF/WB, which is comparable to the result obtained with HC (2.38 KF/WB).

Lateral Flowers BLUPRINS®-treated canes showed a significantly lower average number of lateral flowers than HC-treated canes (19 vs. 28 %). For both products, the highest percentage of lateral flowers occurred for the application time point in which the earliest average bud break occurred (57 DBNB for HC and 51 for BLUPRINS®).

Trials on Cherry

In both years, BLUPRINS® stimulated an earlier bud break in comparison with untreated plants (Figs. 19.1 and 19.2). In 2011, the product was most effective at the latest application and highest dose (10 % 30 DBNB, Fig. 19.1a), while early treatments (45 DBNB) were most effective in 2012 (Fig. 19.1b). Treatments at 6 % caused bud break anticipation of only one day in 2011 (30 DBND), while treatments at 8 % anticipated flowering of two (45 DBNB) or three (30 DBNB) days. Full bloom was reached at the same date for all treatments and control in 2011 (Fig. 19.1a). In 2012, BLUPRINS® application at doses as low as 4 % caused flowering anticipation of 3 days (45 DBNB). Later applications were not effective in 2012.

Trial on Table Grape

All treatments advanced bud break compared to the untreated control (Fig. 19.3). BLUPRINS®-treated buds showed a more uniform development as compared to hydrogen cyanamide-treated buds. In fact, in the hydrogen cyanamide treatment bud development showed a marked asynchrony: on the assessment day, more than 20 % of the buds had visible inflorescences while 13 % were still dormant (Fig. 19.3). Of the BLUPRINS® treatments, the one conducted 60 days before the expected natural bud break (BLUPRINS 60) exhibited a higher uniformity in bud development than the BLUPRINS® treatment at 45 days before natural bud break. Moreover, all buds resumed growth in BLUPRINS®-treated plants, leaving no "blind" buds (buds that do not burst), while both the untreated control plants and the hydrogen cyanamide-treated plants had more than 10 % dormant buds on the assessment date.

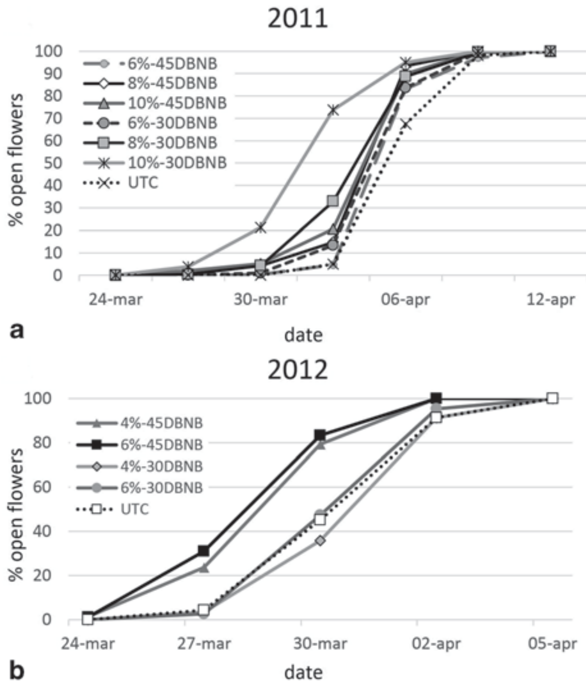


Fig. 19.1 Progression of flowering of *P. avium* var. Ferrovia following the application of 6, 8, or 10 % BLUPRINS® at 30 or 45 days before natural break (30 DBNB, 45 DBNB) during spring 2011 (**a**) and 2012 (**b**), respectively. Points in each treatment represent the mean percentage of open flowers on 14 canes. On each cane, 90 flower buds were counted on average

Fig. 19.2 Branches of *P. avium* var. Ferrovia treated with BLUPRINS® at 10 % 30 days before natural break (DBNB) (**a** and **c**) compared to untreated control branches (**b** and **d**). **a** and **b** March 30, 2011; **c** and **d** April 2, 2011

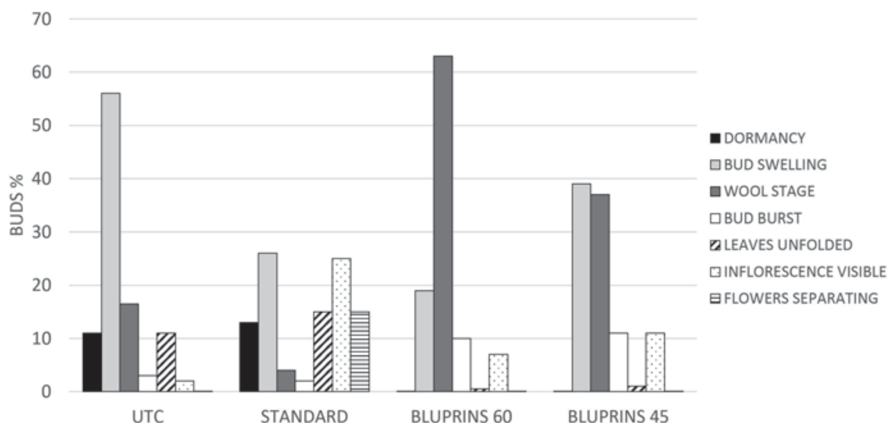


Fig. 19.3 Distribution of untreated control (UTC), hydrogen cyanamide (standard), and BLUPRINS®-treated *V. vinifera* buds into their phenological stages on February 18. BLUPRINS^{W} was applied either 60 (BLUPRINS 60) or 45 (BLUPRINS 45) days before the expected natural bud break

Discussion

The possible loss of hydrogen cyanamide as a bud break enhancer is a threat to achieving stable high-quality produce in many crops. Moreover, the expected rise of global temperatures up to 4.8 °C by the end of the century (IPCC 2013) will spread the problem of bud break induction to an increasing number of crops and world areas. The need to find novel bud breaking agents is therefore very high.

On kiwifruit var. Gold3, BLUPRINS®, an amino acid- and polysaccharide-based product for bud break enhancement, effectively advanced bud break and bloom date over the control. When applied close to the date of natural bud break, BLUPRINS helped maintaining a limited spread of flowering (SD of flowering date), which is important to achieve successful insect pollination. BLUPRINS® also increased the number of king flowers per winter bud (KF/WB), thus enhancing the potential crop load. The effect of BLUPRINS® on the potential crop load was not as large as HCs. A difference in effectiveness is common when comparing products based on natural ingredients (like BLUPRINS®), with synthetic chemicals (such as HC). The increase of lateral flowers was similar for BLUPRINS® and HC, indicating that thinning costs would be similar for BLUPRINS®.

On sweet cherry, BLUPRINS® application caused an anticipation of bud break at all dosages and timings tested, apart from the lowest dose on the latest application in 2012. Moreover, flowering was more concentrated in time on BLUPRINS®-treated plants. Synchronous flowering may facilitate uniform pollination and consequently fruit set, thus reducing the number of fruit pickings at harvest. It is possible that the difference in performance between the two application dates in 2011 is due to the higher temperatures that occurred during the second treatment (average daytime temperature 7.5 °C vs. 4.4 °C during the first treatment).

Similarly, BLUPRINS® anticipated bud break on table grape over control plants, although less efficiently than HC. On the contrary, BLUPRINS® improved uniformity of bud development better than HC, which promoted an irregular bud development. Furthermore, BLUPRINS® reduced the number of "blind" buds compared to HC treatment, thus increasing the potential yield.

BLUPRINS® showed an efficacy as a bud breaking agent on kiwifruit var. Gold3, sweet cherry, and table grape and could, therefore, be considered an alternative to hydrogen cyanamide on these crops. In addition, preliminary results indicate that BLUPRINS® could be successfully employed also on pear, apple, apricot, and peach. Due to its mode of action, on physiological processes, BLUPRINS® does not induce phytotoxicity even on the sensitive stone fruit crops.

References

Arora R, Rowland LJ, Tanino K (2003) Induction and release of bud dormancy in woody perennials: a science comes of age. HortScience 38(5):911–921

Brundell D (1975) Flower development of the Chinese gooseberry (*Actinidia chinensis* Planch.). N Z J Bot 13(3):485–496

Intergovernmental Panel on Climate Change (2013) Climate Change 2013—The Physical Science bases

Vegis A (1964) Dormancy in higher plants. Annu Rev Plant Physiol 15(1):185–224

Walton EF, Wu R, Richardson AC, Davy M, Hellens RP, Thodey K, Janssen BJ, Gleave AP, Rae GM, Wood M, Schaffer RJ (2009) A rapid transcriptional activation is induced by the dormancy-breaking chemical hydrogen cyanamide in kiwifruit (*Actinidia deliciosa*) buds. J Exp Bot 60(13):3835–48

Index

A
Abiotic stress response, 58, 209
Abscisic acid (ABA), 5, 37, 90, 129, 188, 201, 224, 239, 271
  phytohormone, 178
Actinidia spp., 92
  Actinidia chinensis, 138, 280, 289
Apical dominance, 76, 83, 117, 188, 199, 225, 271
Apple, 77, 80, 108, 109, 110, 111, 112, 113, 115, 116, 127, 162, 167, 200
  woody species, 80
Apricot, 92, 114, 124, 130, 138, 140, 147, 148, 159, 160, 162, 164, 166, 200
Arabidopsis, 6, 11, 12, 14, 15, 20, 21, 22, 23, 37, 39, 57, 59, 61–63, 77, 78, 87, 95, 114, 124, 140, 164, 166, 201, 208, 211, 213, 224, 228, 236, 240, 241, 244, 245, 258
  Arabidopsis thaliana, 52, 61, 62, 112, 127, 140, 257
  clock system, 53, 54
ArmobreakTM, 279, 286, 287, 298

B
Biolchim SpA, 301
BLUPRINS®, 302, 304–308
Bromoethane, 192, 269, 275
Bud, 150, 171, 172, 182, 187, 197, 226, 290
  activation, 188, 189
  arrest, 58, 75, 80, 82, 83, 94, 224, 243
  break, 83, 92, 111, 115, 117, 128, 147, 165, 188, 259, 261, 279, 280, 282, 287, 289, 290, 293, 296, 299, 308
  breaking agent, 302, 307, 308
  burst, 57, 65, 79, 115, 124, 130, 153, 239, 245
  dormancy, 63, 79, 84, 91, 92, 96, 97, 107, 108, 109, 112, 114–117, 123, 129, 130, 138, 147, 154, 171, 200, 225, 235, 245, 257
  outgrowth, 117, 149, 154, 155, 190, 199, 225, 226, 229, 259

C
Castanea, 56
Cherry, 82, 124, 304, 307, 308
Chilling, 4, 57, 63–65, 76, 77, 80–83, 91, 92, 96, 97, 114, 117, 125, 126, 129, 130, 150, 159, 165, 169, 236, 259, 299
  requirements, 83, 125, 126, 128, 139, 153, 159, 164, 166
Circadian clock, 52, 54, 56–62, 64, 85, 93, 114, 212, 249
Cis-acting elements, 62, 225, 227, 229, 230
Climatic change, 111, 123
Cold acclimation, 60, 62, 64, 65, 169, 173, 178, 201
Cold hardiness (CH), 169
Comparative transcriptome analysis, 230
Cytokinin, 20, 129, 188, 189, 211, 225, 270, 273

D
DELAY OF GERMINATION 1 (DOG1), 240, 245
DELLA, 21, 40, 42, 212
Dormancy, 3, 4, 6, 12, 23, 35, 37, 40–42, 52, 57
  cycling, 36, 37, 38, 41, 44, 114
  genes, 126, 127
  release, 76, 77, 83, 89, 92, 96, 114, 117, 153, 154, 188, 190, 192, 243, 275

© Springer International Publishing Switzerland 2015
J. V. Anderson (ed.), *Advances in Plant Dormancy,* DOI 10.1007/978-3-319-14451-1

DORMANCY ASSOCIATED MADS-BOX (DAM), 63, 91, 109, 115, 127, 160, 244
Dormex®\t See Hydrogen cynamide, 279

E
Endodormancy, 61, 63, 75, 76, 91, 107, 117, 125, 128, 137–141, 169, 173, 175, 198, 200, 210, 236, 259
Endodormancy markers, 187, 188, 199
Environmental signal, 10, 35, 36, 37, 40, 64, 239
Epigenetic, 24, 54, 63, 65, 77, 89, 95, 128, 160, 236
 regulation, 54, 63, 65, 94, 96, 97, 131, 245, 249
Erger®, 298
Ethylene, 15, 16, 17, 23, 188, 192, 201, 213, 243, 271, 272
Eucalyptus, 55, 56, 62

F
Flower development, 116, 123, 124, 125, 159
Flowering, 3, 58, 63, 83, 87, 112–114, 123, 124, 199, 204, 208, 239, 279, 280, 282, 287, 297, 307
Freezing tolerance, 62, 65, 117, 129, 242

G
Gene expression, 4, 38, 53, 54, 63, 64, 113, 139, 143, 211, 227, 240, 245
Gene regulatory networks, 178, 243
Genetic, 5, 10, 21, 37, 77, 91, 108, 109, 110, 126, 128, 139, 228, 235, 257, 298
Genomic, 5, 65, 91, 108, 111, 116
Germination, 3, 4, 11, 13, 15, 20, 35, 39, 224, 227, 260
Gibberellins, 44, 129, 188, 224
Gold3, 279, 289, 290, 298, 299, 302
Gold9, 279, 282, 287
Grapevines, 169, 170, 171, 175, 176, 178

H
Heat shock, 22
Hi Cane®, 280, 290, 302
Hormones, 11, 23, 39, 90, 129, 189, 225, 236, 249
Hormone signaling, 40, 116, 208
Hydrogen cyanamide, 96, 178, 272, 279, 289, 299, 301, 302, 307

I
Invasive weed, 197, 200

J
Jasmonate, 18, 23

K
Kiwifruit, 92, 114, 140, 279, 280, 289, 298
 trial on, 302, 307

L
Leafy spurge (Euphorbia esula), 76, 137, 197, 236
Life cycle, 3, 4, 35, 41, 80, 246
Low temperatures, 61, 82, 85, 108, 109, 113, 123, 169, 170, 172, 175, 176, 178, 182

M
MADS-box, 63, 91, 109, 113, 131, 139, 147, 163, 209, 245
MADS domain, 244
Malus, 112, 127
Meta-analysis, 207, 208, 213, 214
Molecular control, 257

P
Painting canes, 280, 290
Peach, 63, 76, 83, 91, 92, 93, 109, 111, 116, 124, 137, 138, 140, 141, 160
Peach DAMs, 93, 96
Pears, 80, 92, 108, 117
Perennial, 52, 56, 75, 79, 92, 108, 115, 123, 137, 169, 200, 202, 236, 250, 257
Perennial trees, 197, 213
Photoperiod, 57, 58, 60, 76, 79, 81, 93, 94, 108, 113, 127, 164, 176, 198, 200–203, 208, 209, 236, 239, 240, 242, 246, 249
Physiological age, 191, 193
Phytochrome, 15, 58, 113, 211, 226
Pipfruits, 108, 115, 116
Plasmodesmata., 114, 129, 130, 131
Populus, 56, 58–65, 112, 115, 124, 127, 200
Potato dormancy, 138
Potato tuber, 82, 178, 188, 189, 190, 269, 270, 272
Prunus, 62, 123, 124, 162

Q
Quantitative trait locus, 10, 58, 109, 138, 160

R
Rindite, 192, 269, 270, 271, 275
Rosaceae, 108, 114, 148, 159, 160, 162, 166

S
Seasonal stress, 75
Seed, 3, 4, 5, 6, 11, 13, 17, 18, 20, 37
  dormancy, 3, 4, 6, 11, 20, 40, 41, 129, 179, 236
  germination, 3, 6, 11, 12, 14–17, 18, 19, 21, 22, 41, 226, 243
Simple sequence repeat, 111, 112, 162
SOC1, 85, 86, 141, 202, 244
Soil seed bank, 37, 40, 41
Sprouting, 81, 169, 178, 187, 188, 191, 193, 271
Stratification, 89, 236, 257, 259–262
Stress, 11, 18, 96, 178, 271, 272
Strigolactone, 19, 20

T
Table grape, 308
Temperate fruit, 92, 107, 108, 123–125, 169
Temperature, 3, 4, 6, 15, 17, 19, 22, 36, 42, 64, 77, 83, 113, 125, 173
Thermoinhibition, 4, 6, 10, 12, 17
Transcription, 12, 20, 54, 62, 64, 113, 208
Transcriptional analysis, 270, 272
Transcription factors, 18, 40, 113, 116, 140, 207, 243

V
Vascular development, 272
Vernalization and floral induction, 77, 78, 85, 86

W
Winter dormancy, 58, 60, 64, 76, 80, 81

Z
Zesy002 *See* Gold3, 279
Zesy003 *See* Gold9, 279