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Sustainable Agriculture Reviews

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Agroecology Scaling Up for Food Sovereignty and Resiliency

Miguel A. Altieri and C.I. Nicholls

Abstract The Green Revolution not only failed to ensure safe and abundant food production for all people, but it was launched under the assumptions that abundant water and cheap energy to fuel modern agriculture would always be available and that climate would be stable and not change. In some of the major grain production areas the rate of increase in cereal yields is declining as actual crop yields approach a ceiling for maximal yield potential. Due to lack of ecological regulation mechanisms, monocultures are heavily dependent on pesticides. In the past 50 years the use of pesticides has increased dramatically worldwide and now amounts to some 2.6 million tons of pesticides per year with an annual value in the global market of more than US$ 25 billion. Today there are about one billion hungry people in the planet, but hunger is caused by poverty and inequality, not scarcity due to lack of production. The world already produces enough food to feed nine to ten billion people, the population peak expected by 2050. There is no doubt that humanity needs an alternative agricultural development paradigm, one that encourages more ecologically, biodiverse, resilient, sustainable and socially just forms of agriculture. The basis for such new systems are the myriad of ecologically based agricultural styles developed by at least 75% of the 1.5 billion smallholders, family farmers and indigenous people on 350 million small farms which account for no less than 50% of the global agricultural output for domestic consumption.

This position paper draws from material used in the paper “It is possible to feed the world by scaling up agroecology” written by Miguel A Altieri for the Ecumenical Advocacy Alliance, May 2012.

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As an applied science, agroecology uses ecological concepts and principles for the design and management of sustainable agroecosystems where external inputs are replaced by natural processes such as natural soil fertility and biological control. The global south has the agroecological potential to produce enough food on a global per capita basis to sustain the current human population, and potentially an even larger population, without increasing the agricultural land base.

Keywords Agroecology • Organic farming • Food security • Industrial agriculture • World hunger • Peasant agriculture

1 Why Industrial Agriculture Is No Longer Viable?

The Green Revolution, the symbol of agricultural intensification not only failed to ensure safe and abundant food production for all people, but it was launched under the assumptions that abundant water and cheap energy to fuel modern agriculture would always be available and that climate would be stable and not change. Agrochemicals, fuel-based mechanization and irrigation operations, the heart of industrial agriculture, are derived entirely from dwindling and ever more expensive fossil fuels. Climate extremes are becoming more frequent and violent and threaten genetically homogeneous modern monocultures now covering 80% of the 1,500 million hectares of global arable land. Moreover industrial agriculture contributes with about 25–30% of greenhouse gas (GHG) emissions, further altering weather patterns thus compromising the world's capacity to produce food in the future.

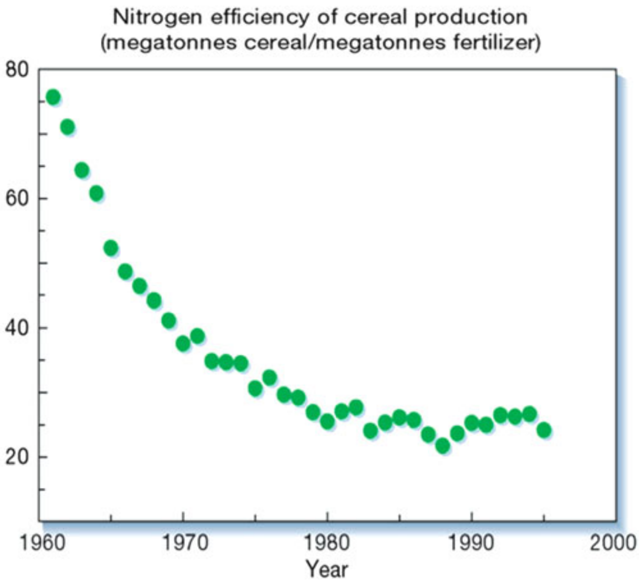

Fig. 1 The law of diminishing returns: more inputs, less yields

1.1 The Ecological Footprint of Industrial Agriculture

In some of the major grain production areas of the world, the rate of increase in cereal yields is declining as actual crop yields approach a ceiling for maximal yield potential (Fig. 1). When the petroleum dependence and the ecological footprint of industrial agriculture are accounted for, serious questions emerge about the social, economic and environmental sustainability of modern agricultural strategies. Intensification of agriculture via the use of high-yielding crop varieties, fertilization, irrigation and pesticides impact heavily on natural resources with serious health and environmental implications. It has been estimated that the external costs of UK agriculture, to be at least 1.5–2 billion pounds each year. Using a similar framework of analysis the external costs in the US amount to nearly 13 billion pounds per year, arising from damage to water resources, soils, air, wildlife and biodiversity, and harm to human health. Additional annual costs of USD 3.7 billion arise from agency costs associated with programs to address these problems or encourage a transition towards more sustainable systems. The US pride about cheap food, is an illusion: consumers pay for food well beyond the grocery store.

http://www.agron.iastate.edu/courses/agron515/eatearth.pdf

Due to lack of ecological regulation mechanisms, monocultures are heavily dependent on pesticides. In the past 50 years the use of pesticides has increased dramatically worldwide and now amounts to some 2.6 million tons of pesticides per year with an annual value in the global market of more than US$25 billion. In the

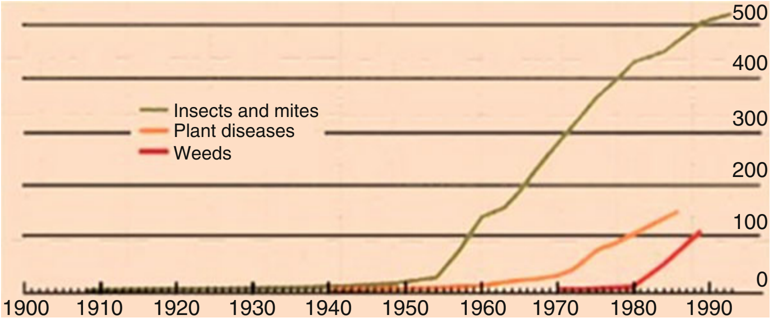

Fig. 2 The rapid development of resistance to pesticides by insects, pathogens and weeds

US alone, 324 million kg of 600 different types of pesticides are used annually with indirect environmental (impacts on wildlife, pollinators, natural enemies, fisheries, water quality, etc.) and social costs (human poisoning and illnesses) reaching about $8 billion each year. On top of this, 540 species of arthropods have developed resistance against more than 1,000 different types of pesticides, which have been rendered useless to control such pests chemically (Fig. 2).

http://ipm.ncsu.edu/safety/factsheets/resistan.pdf

Although there are many unanswered questions regarding the impact of the release of transgenic plants into the environment which already occupy >180 million hectares worldwide, it is expected that biotech crops will exacerbate the problems of conventional agriculture and, by promoting monoculture, will also undermine ecological methods of farming. Transgenic crops developed for pest control emphasize the use of a single control mechanism, which has proven to fail over and over again with insects, pathogens and weeds. Thus transgenic crops are likely to increase the use of pesticides as a result of accelerated evolution of 'super weeds' and resistant insect pest strains. Transgenic crops also affect soil fauna potentially upsetting key soil processes such as nutrient cycling. Unwanted gene flow from transgenic crops may compromise via genetic pollution crop biodiversity (i.e. maize) in centers of origin and domestication and therefore affect the associated systems of agricultural knowledge and practice along with the millenary ecological and evolutionary processes involved.

http://www.colby.edu/biology/BI402B/Altieri%202000.pdf

1.2 Agribusiness and World Hunger

Today there are about one billion hungry people in the planet, but hunger is caused by poverty (1/3 of the planet's population makes less than $2 a day) and inequality (lack of access to land, seeds, etc.), not scarcity due to lack of production. The world already produces enough food to feed nine to ten billion people, the population peak expected by 2050. The bulk of industrially produced grain crops goes to biofuels and confined animals. Therefore the call to double food production by 2050 only applies if we continue to prioritize the growing population of livestock and automobiles over hungry people. Overly simplistic analyses in support of industrialized agriculture cite high yields and calculations of total food supply to illustrate its potential to alleviate hunger. However, it has been long understood that yields are a necessary but not sufficient condition to meeting people's food needs (Lappe et al. 1998). Seventy eight percent of all malnourished children under five who live in the Third World are in countries with food surpluses. There is already an abundant supply of food even while hunger grows worldwide. It is not supply that is the crucial factor, but distribution – whether people have sufficient "entitlements" through land, income, or support networks to secure a healthy diet. Rather than helping, too much food can actually add to hunger by undercutting prices and destroying the economic viability of local agricultural systems. Farmers are not able to sell their produce in a way that allows them to cover costs, and so food may rot in the fields while people go hungry (Holt Gimenez and Patel 2009).

In addition roughly one-third of food produced for human consumption is wasted globally, which amounts to about 1.3 billion tons per year, enough to feed the entire African continent. Most of this food is wasted by consumers in Europe and North-America is 95–115 kg/year/per capita while this figure in Sub-Saharan Africa and South/Southeast Asia is only 6–11 kg/year.

http://www.fao.org/fileadmin/user_upload/ags/publications/GFL_web.pdf

1.3 The Concentration of Global Food Production

Solutions to hunger and food supply need to take into account distribution of food and access to income, land, seeds and other resources. Industrial agriculture has accelerated land and resource concentration in the hands of a few undermining the possibility of addressing the root causes of hunger (Lappe et al. 1998). The concentration of global food production under the control of a few transnational corporations, bolstered by free trade agreements, structural adjustment policies, and subsidies for the overproduction of crop commodities, has created North-South food trade imbalances and import dependencies that underlie a growing food insecurity in many countries. Production of cash crop exports in exchange for food imports and the expansion of biofuels can undermine food self-sufficiency and threaten local ecosystems. This situation is aggravated by food insecure governments including China, Saudi Arabia and South Korea that rely on imports to feed their people which are snatching up vast areas of farmland (>80 millions hectares already transacted) abroad for their own offshore food production. Food corporations and private investors, hungry for profits in the midst of the deepening financial crisis, see investment in foreign farmland as an important new source of revenue from the production of biomass.

http://www.grain.org/bulletin_board/tags/221-land grabbing

2 Peasant Agriculture: The Basis for the New Twenty-first Century Agriculture

There is no doubt that humanity needs an alternative agricultural development paradigm, one that encourages more ecologically, biodiverse, resilient, sustainable and socially just forms of agriculture. The basis for such new systems are the myriad of ecologically based agricultural styles developed by at least 75% of the 1.5 billion smallholders, family farmers and indigenous people on 350 million small farms which account for no less than 50% of the global agricultural output for domestic consumption (ETC 2009). Most of the food consumed today in the world is derived from 5,000 domesticated crop species and 1.9 million peasant-bred plant varieties mostly grown without agrochemicals (ETC 2009). Industrial agriculture threatens this crop diversity through the replacement of native varieties with hybrid strains and the contamination of crop and wild species from the introduction of genetically modified organisms. As the global food supply relies on a diminishing variety of crops, it becomes vulnerable to pest outbreaks, the breeding of superbugs, and climate disruptions.

In Brazil there are about 4.8 million traditional family farmers (about 85% of the total number of farmers) that occupy 30% of the total agricultural land of the country. Such family farms control about 33% of the area sown to maize, 61% of that under beans, and 64% of that planted to cassava, thus producing 84% of the total cassava and 67% of all beans. Smallholder farmers in India possessing on average 2 ha of land each, make up about 78% of the country's farmers while owning only 33% of the land, but responsible for 41% of national grain production. Their contribution to both household food security and to farm outputs is thus disproportionately high (Via Campesina 2010).

The majority of the world's peasant farmers tend small diversified farming systems which offer promising models for promoting biodiversity, conserving natural resources, sustaining yield without agrochemicals, providing ecological services and remarkable lessons about resiliency in the face of continuous environmental and economic change. For these reasons most agroecologists acknowledge that traditional agroecosytems have the potential to bring solutions to many uncertainties facing humanity in a peak oil era of global climate change and financial crisis (Altieri 2004; Toledo and Barrera- Bassols 2009). Undoubtedly, the ensemble of traditional crop management practices used by many resource-poor farmers which fit well to local conditions and can lead to the conservation and regeneration of the natural resource base represents a rich resource for modern workers seeking to create novel agroecosystems well adapted to the local agroecological and socioeconomic circumstances of smallholders.

Peasant practices and techniques tend to be knowledge-intensive rather than input-intensive, but clearly not all are effective or applicable, therefore modifications and adaptations may be necessary and this is where **agroecology** has played a key role in revitalizing the productivity of small farming systems (Altieri et al. 1998). Since the 1980s thousands of projects launched by non-governmental organisations (NGO), farmers organizations and some University and research centers reaching hundreds of thousands of farmers, have applied general agroecological principles to customize agricultural technologies to local needs and circumstances, improving yields while conserving natural resources and biodiversity. The conventional technology transfer model breaks down in peasant regions as it is top down and based on a magic-bullet technology transfer approach incapable of understanding that new agroecological systems require peoples' participation and need to be tailored and adapted in a site-specific way to highly variable and diverse farm conditions (Uphoff 2002).

3 How Is the International Community Reacting?

The solutions for smallholder agriculture advocated by big bilateral donors, governments and the initiatives of private foundations have tended to center around the promotion of synthetic fertilizers and pesticides, which are costly for farmers and often resource depleting. This drive for a new 'Green Revolution' as exemplified by the Alliance for a Green Revolution in Africa (AGRA) has tended to sideline more sustainable, farmer led approaches. Others [(CGIAR 2012, recent sustainable intensification report of FAO- (http://www.fao.org/agriculture/crops/core-themes/theme/spi/scpi-home/framework/sustainable-intensification-in-fao/en/), latest report of the expert Montpellier Panel - (https://workspace.imperial.ac.uk/africanagriculturaldevelopment/Public/Montpellier%20Panel%20Report%202012.pdf)] have tried to co-opt agroecology by stating that it is an option that can be practiced along with other approaches such as transgenic crops, conservation farming, microdosing of fertilisers and herbicides, and integrated pest management. Of course in this way the term agroecology would be rendered meaningless, like sustainable agriculture, a concept devoid of meaning, and divorced from the reality of farmers, the politics of food and of the environment. As a science however, agroecology provides the productive basis for rural movements that promote food sovereignty and confront head on the root causes that perpetuate hunger, therefore it cannot be appropriated by conventional institutions. Agroecology does not need to be combined with other approaches. Without the need of hybrids and external agrochemical inputs, it has consistently proven capable of sustainably increasing productivity and has far greater potential for fighting hunger, particularly during economic and climatically uncertain times, which in many areas are becoming the norm (Altieri et al. 2011b).

Despite these co-opting attempts, the realization of the contribution of peasant agriculture to food security in the midst of scenarios of climate change, economic and energy crisis led to the concepts of food sovereignty and agroecology to gain much worldwide attention in the last two decades. Two recent major international reports (IAASTD 2009; de Schutter 2010) state that in order to feed nine billion people in 2050, we urgently need to adopt the most efficient farming systems and recommend for a fundamental shift towards agroecology as a way to boost food production and improve the situation of the poorest. Both reports based on broad consultations with scientists and extensive literature reviews contend that small-scale farmers can double food production within 10 years in critical regions by using agroecological methods already available. The future food challenge should be met using environmentally friendly and socially equitable technologies and methods, in a world with a shrinking arable land base (which is also being diverted to produce biofuels), with less and more expensive petroleum, increasingly limited supplies of water and nitrogen, and within a scenario of a rapidly changing climate, social unrest and economic uncertainty (Godfray et al. 2010). The only agricultural systems that will be able to confront future challenges are agroecological systems that exhibit high levels of diversity, integration, efficiency, resiliency and productivity (Holt Gimenez and Patel 2009).

4 What Are Agroecological Production Systems?

As an applied science, agroecology uses ecological concepts and principles for the design and management of sustainable agroecosystems where external inputs are replaced by natural processes such as natural soil fertility and biological control (Altieri 1995). Agroecology takes greater advantage of natural processes and beneficial on-farm interactions in order to reduce off-farm input use and to improve the efficiency of farming systems. Agroecological principles used in the design and management of agroecosystems (Table 1) enhances the

Table 1 Agroecological principles for the design of biodiverse, energy efficient, resource-conserving and resilient farming systems

Enhance the recycling of biomass, with a view to optimizing organic matter decomposition and nutrient cycling over time
Strengthen the "immune system" of agricultural systems through enhancement of functional biodiversity – natural enemies, antagonists, etc.
Provide the most favorable soil conditions for plant growth, particularly by managing organic matter and by enhancing soil biological activity
Minimize losses of energy, water, nutrients and genetic resources by enhancing conservation and regeneration of soil and water resources and agrobiodiversity
Diversify species and genetic resources in the agroecosystem over time and space at the field and landscape level
Enhance beneficial biological interactions and synergies among the components of agrobiodiversity, thereby promoting key ecological processes and services.

functional biodiversity of agroecosystems which is integral to the maintenance of immune, metabolic and regulatory processes key for agroecosystem function (Gliessman 1998).

Agroecological principles take different technological forms depending on the biophysical and socioeconomic circumstances of each farmer or region. A key principle of agroecology is the diversification of farming systems promoting mixtures of crop varieties, intercropping systems, agroforestry systems, livestock integration, etc. which potentiate the positive effects of biodiversity on productivity derived from the increasing effects of complementarity between plant-animal species translated in better use of sunlight, water, soil resources and natural regulation of pest populations. Promoted diversification schemes (Box 1) are multi-functional as their adoption usually means favorable changes in various components of the farming systems at the same time (Gliessman 1998). In other words they function as an “ecological turntable” by activating key processes such as recycling, biological control, antagonisms, allelopathy, etc., essential for the sustainability and productivity of agroecosystems. Agroecological systems are not intensive in the use of capital, labor, or chemical inputs, but rather rely on the efficiency of biological processes such as photosynthesis, nitrogen fixation, solubilization of soil phosphorus, and the enhancement of biological activity above and below ground. The “inputs” of the system are the natural processes themselves, this is why agroecology is referred to as an “agriculture of processes”.

When designed and managed with agroecological principles, farming systems exhibit attributes of diversity, productivity, resilience and efficiency. Agroecological initiatives aim at transforming industrial agriculture partly by transitioning the existing food systems away from fossil fuel-based production largely for agroexport

Box 1 Temporal and Spatial Designs of Diversified Farming Systems and Their Main Agroecological Effects (Altieri 1995; Gliessman 1998)

Crop Rotations: Temporal diversity in the form of cereal-legume sequences. Nutrients are conserved and provided from one season to the next, and the life cycles of insect pests, diseases, and weeds are interrupted.

Polycultures: Cropping systems in which two or more crop species are planted within certain spatial proximity result in biological complementarities that improve nutrient use efficiency and pest regulation thus enhancing crop yield stability.

Agroforestry Systems: Trees grown together with annual crops in addition to modifying the microclimate, maintain and improve soil fertility as some trees contribute to nitrogen fixation and nutrient uptake from deep soil horizons while their litter helps replenish soil nutrients, maintain organic matter, and support complex soil food webs.

Cover Crops and Mulching: The use of pure or mixed stands of grass-legumes e.g., under fruit trees can reduce erosion and provide nutrients to the soil and enhance biological control of pests. Flattening cover crop mixtures on the soil surface in conservation farming is a strategy to reduce soil erosion and lower fluctuations in soil moisture and temperature, improve soil quality, and enhance weed suppression resulting in better crop performance.

Crop- livestock mixtures: High biomass output and optimal nutrient recycling can be achieved through crop- animal integration. Animal production that integrates fodder shrubs planted at high densities, intercropped with improved, highly-productive pastures and timber trees all combined in a system that can be directly grazed by livestock enhances total productivity without need of external inputs.

crops and biofuels towards an alternative agricultural paradigm that encourages local/national food production by small and family farmers based on local innovation, resources and solar energy. This implies access of peasants to land, seeds, water, credit and local markets, partly through the creation of supportive economic policies, financial incentives, market opportunities and agroecological technologies (Vía Campesina 2010). Agroecological systems are deeply rooted in the ecological rationale of traditional small-scale agriculture, representing long established examples of successful agricultural systems characterized by a tremendous diversity of domesticated crop and animal species maintained and enhanced by ingenuous soil, water and biodiversity management regimes, nourished by complex traditional knowledge systems (Koohafkan and Altieri 2010).

5 How Does Agroecology Differ from Other Alternative Agricultural Approaches?

Organic agriculture is practiced in almost all countries of the world, and its share of agricultural land and farms is growing, reaching a certified area of more than 30 million hectares globally. Organic farming is a production system that sustains agricultural productivity by avoiding or largely excluding synthetic fertilizers and pesticides. FIBL scientists in Central Europe conducted a 21-year study of the agronomic and ecological performance of organic, and conventional farming systems. They found crop yields to be 20% lower in the organic systems, although input of fertilizer and energy was reduced by 31–53% and pesticide input by 98%. Researchers concluded that the enhanced soil fertility and higher biodiversity found in organic plots rendered these systems less dependent on external inputs. When practiced based on agroecological principles organic practices buildup of soil organic matter and soil biota, – minimize pest, disease and weed damage, conserve soil, water, and biodiversity resources, promote long-term agricultural productivity with produce of optimal nutritional value and quality. http://www.fibl.org/en.html

Organic farming systems managed as monocultures that are in turn dependent on external biological and/or botanical (i.e. organic) inputs are not based on agroecological principles. This 'input substitution' approach essentially follows the same paradigm of conventional farming: that is, overcoming the limiting factor but this time with biological or organic inputs. Many of these "alternative inputs" have become commodified, therefore farmers continue to be dependent on input suppliers, cooperative or corporate (Rosset and Altieri 1997). Agroecologists argue that organic farming systems that do not challenge the monoculture nature of plantations and rely on external inputs as well as on foreign and expensive certification seals, or fair-trade systems destined only for agro-export, offer little to small farmers who in turn become dependent on external inputs and foreign and volatile markets. By keeping farmers dependent on an input substitution approach, organic agriculture's fine-tuning of input use does little to move farmers toward the productive redesign of agricultural ecosystems that would move them away from dependence on external inputs. Niche (organic and/or fair trade) markets for the rich in the North exhibit the same problems of any agro-export scheme that does not prioritize food sovereignty (defined here as 'the right of people to produce, distribute and consume healthy food in and near their territory in ecologically sustainable manner'), often perpetuating dependence and at times hunger (Altieri 2010).

6 Assessing the Performance of Agroecological Projects

There are many competing visions on how to achieve new models of a biodiverse, resilient, productive and resource efficient agriculture that humanity desperately needs in the immediate future. Conservation (no or minimum tillage) agriculture,

Box 2 Requirements of Agroecologically Based Agricultural Systems (Koohafkan et al. 2011). GHG: greenhouse gases

1. Use of local and improved crop varieties and livestock breeds so as to enhance genetic diversity and enhance adaptation to changing biotic and environmental conditions.
2. Avoid the unnecessary use of agrochemical and other technologies that adversely impact on the environment and on human health (e.g. heavy machineries, transgenic crops, etc.)
3. Efficient use of resources (nutrients, water, energy, etc.), reduced use of non-renewable energy and reduced farmer dependence on external inputs
4. Harness agroecological principals and processes such as nutrient cycling, biological nitrogen fixation, allelopathy, biological control via promotion of diversified farming systems and harnessing functional biodiversity
5. Making productive use of human capital in the form of traditional and modern scientific knowledge and skills to innovate and the use of social capital through recognition of cultural identity, participatory methods and farmer networks to enhance solidarity and exchange of innovations and technologies to resolve problems
6. Reduce the ecological footprint of production, distribution and consumption practices, thereby minimizing GHG emissions and soil and water pollution
7. Promoting practices that enhance clean water availability, carbon sequestration, and conservation of biodiversity, soil and water conservation, etc.
8. Enhanced adaptive capacity based on the premise that the key to coping with rapid and unforeseeable change is to strengthen the ability to adequately respond to change to sustain a balance between long-term adaptability and short-term efficiency
9. Strengthen adaptive capacity and resilience of the farming system by maintaining agroecosystem diversity, which not only allows various responses to change, but also ensures key functions on the farm
10. Recognition and dynamic conservation of agricultural heritage systems that allows social cohesion and a sense of pride and promote a sense of belonging and reduce migration

sustainable intensification production, transgenic crops, organic agriculture and agroecological systems are some of the proposed approaches, each claiming to serve as the durable foundation for a sustainable food production strategy. Although goals of all approaches may be similar, technologies proposed (high versus low input) methodologies (farmer-led versus market driven, top down versus bottom-up) and scales (large scale monocultures versus biodiverse small farms) are quite different and often antagonistic. However when one examines the basic attributes that a sustainable production system should exhibit (Box 2), agroecological approaches certainly meet most of these attributes and requirements (Altieri 2002;

Table 2 A set of guiding questions to assess if proposed agricultural systems are contributing to sustainable livelihoods (Koohafkan et al. 2011)

1. Are they reducing poverty?
2. Are they based on rights and social equity?
3. Do they reduce social exclusion, particularly for women, minorities and indigenous people?
4. Do they protect access and rights to land, water and other natural resources?
5. Do they favor the redistribution (rather than the concentration) of productive resources?
6. Do they substantially increase food production and contribute to household food security and improved nutrition?
7. Do they enhance families' water access and availability?
8. Do they regenerate and conserve soil, and increase (maintain) soil fertility?
9. Do they reduce soil loss/degradation and enhance soil regeneration and conservation?
10. Do practices maintain or enhance organic matter and the biological life and biodiversity of the soil?
11. Do they prevent pest and disease outbreaks?
12. Do they conserve and encourage agrobiodiversity?
13. Do they reduce greenhouse gas emissions?
14. Do they increase income opportunities and employment?
15. Do they reduce variation in agricultural production under climatic stress conditions?
16. Do they enhance farm diversification and resilience?
17. Do they reduce investment costs and farmers dependence on external inputs?
18. Do they increase the degree and effectiveness of farmer organizations?
19. Do they increase human capital formation?
20. Do they contribute to local/regional food sovereignty?

Gliessman 1998; UK Food Group 2010; Parrot and Mardsen 2002; Uphoff 2002). Similarly by applying the set of questions listed in Table 2 to assess the potential of agricultural interventions in addressing pressing social, economic and ecological concerns, it is clear that most existing agroecological projects confirm that proposed management practices are contributing to sustainable livelihoods by improving the natural, human, social, physical and financial capital of target rural communities (Koohafkan et al. 2011).

In order for an agricultural strategy to fit within the sustainability criteria, it must contain the basic requirements of a viable and durable agricultural system capable of confronting the challenges of the twenty-first century while carrying out its productive goals within certain limits in terms of environmental impact, land degradation levels, input and energy use, GHG emissions, etc. As depicted in Fig. 3 threshold indicators may be defined that are site or region specific, thus their values will change according to prevailing environmental and socio- economic conditions. In the same region, threshold value ranges may be the same for an intensive large scale system and a low-input small scale system as yields would be measured per unit of GHG emitted, per unit of energy or water used, per unit of N leached, etc. Without a doubt most monoculture based systems will surpass the threshold levels and therefore will not be considered sustainable and unfit for food provisioning in an ecologically and socially sound manner (Koohafkan et al. 2011).

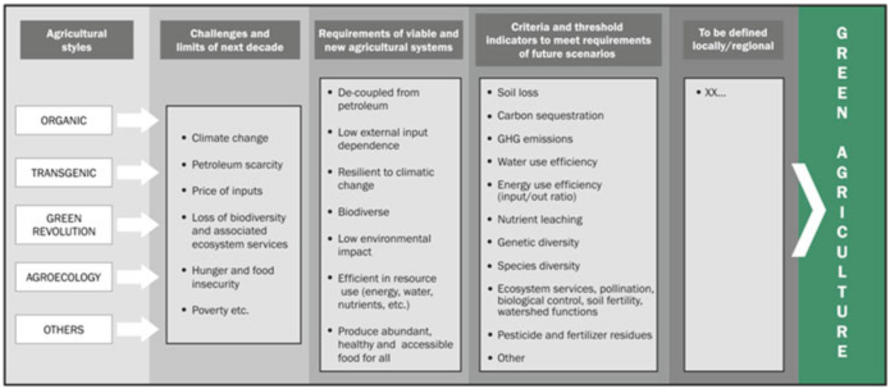

Fig. 3 The basic requirements of a viable and durable agricultural system capable of confronting the challenges of the twenty-first century while carrying out its productive goals within certain thresholds established locally or regionally (Koohafkan et al. 2011)

7 The Spread and Productive/Food Security Potential of Agroecological Systems

The first global assessment of agroecologically based projects and/or initiatives throughout the developing world was conducted by Pretty et al. (2003) who documented clear increases in food production over some 29 million hectares, with nearly nine million households benefiting from increased food diversity and security. Promoted sustainable agriculture practices led to 50–100% increases in per hectare cereal production (about 1.71 Mg per year per household – an increase of 73%) in rain-fed areas typical of small farmers living in marginal environments; that is an area of about 3.58 million hectares, cultivated by about 4.42 million farmers. In 14 projects where root crops were main staples (potato, sweet potato and cassava), the 146,000 farms on 542,000 ha increased household food production by 17 t per year (increase of 150%). Such yield enhancements are a true breakthrough for achieving food security among farmers isolated from mainstream agricultural institutions. A re-examination of the data in 2010, the analysis demonstrates the extent to which 286 interventions in 57 "poor countries" covering 37 million hectares (3% of the cultivated area in developing countries) have increased productivity on 12.6 million farms while improving ecosystem services. The average crop yield increase was 79%.
http://www.bis.gov.uk/assets/foresight/docs/food-and-farming/11-546-future-of-food-and-farming-report.pdf

8 Africa

There is a growing body of evidence emerging from Africa demonstrating that agroecological approaches can be highly effective in boosting production, incomes, food security and resilience to climate change and empowering communities (Christian Aid

2011). For example the UK Government's Foresight Global Food and Farming project conducted an analysis of 40 projects and programs in 20 African countries where sustainable crop intensification was promoted during the 1990s–2000s. The cases included crop improvements, agroforestry and soil conservation, conservation agriculture, integrated pest management, horticulture, livestock and fodder crops, aquaculture and novel policies and partnerships. By early 2010, these projects had documented benefits for 10.39 million farmers and their families and improvements on approximately 12.75 million hectares. Food outputs by sustainable intensification via the use of new and improved varieties was significant as crop yields rose on average by 2.13-fold (Pretty et al. 2011). Most households substantially improved food production and household food security. In 95% of the projects where yield increases were the aim, cereal yields improved by 50–100%. Total farm food production increased in all. The additional positive impacts on natural, social and human capital are also helping to build the assets base so as to sustain these improvements in the future (Action Aid 2010).

Although some of the reported yield gains reported in the study depended on farmers having access to improved seeds, fertilizers and other inputs (which more than often is not the case) food outputs improved mainly by diversification with a range of new crops, livestock or fish that added to the existing staples or vegetables already being cultivated. These new system enterprises or components included: aquaculture for fish raising; small patches of land used for raised beds and vegetable cultivation; rehabilitation of formerly degraded land; fodder grasses and shrubs that provide food for livestock (and increase milk productivity); raising of chickens and zero-grazed sheep and goats; new crops or trees brought into rotations with maize or sorghum, adoption of short- maturing varieties (e.g. sweet potato and cassava) that permit the cultivation of two crops per year instead of one (Pretty et al. 2011).

Another meta analysis conducted by UNEP–UNCTAD (2008) assessing 114 cases in Africa revealed that a conversion of farms to organic methods increased agricultural productivity by 116%. In Kenya, maize yields increased by 71% and bean yields by 158%. Moreover, increased diversity in food crops available to farmers resulted in more varied diets and thus improved nutrition. Also the natural capital of farms (soil fertility, levels of agrobiodiversity, etc.) increased with time after conversion.

One of the most successful diversification strategies has been the promotion of tree-based agriculture. Agroforestry of maize associated with fast growing and N-fixing shrubs (e.g. Calliandra and Tephrosia) has spread among tens of thousands of farmers in Cameroon, Malawi, Tanzania, Mozambique, Zambia and Niger resulting in a maize production of 8 t compared with 5 t obtained under monoculture (Garrity 2010).

Another agroforestry system in Africa is one dominated by Faidherbia trees which improve crop yields, protect crops from dry winds and the land from water erosion. In the Zinder Regions of Niger, there are now about 4.8 million hectares of Faidherbia-dominated agroecosystems. The foliage and pods from the trees also provide much-needed fodder for cattle and goats during the long Sahelian dry seasons. Encouraged by the experience in Niger, about 500,000 farmers in Malawi and the southern highlands of Tanzania maintain Faidherbia trees in their maize fields (Reij and Smaling 2008).

In southern Africa, Conservation Agriculture (CA) is an important innovation based on three agroecological practices: minimum soil disturbance, permanent soil cover and crop rotations. These systems have spread in Madagascar, Zimbabwe, Tanzania and other countries reaching no less than 50,000 farmers who have dramatically increased their maize yields to 3–4 MT/ha while conventional yields average between 0.5 and 0.7 MT/ha. Improved maize yields increase the amount of food available at the household level, but also increase income levels (Owenya et al. 2011).

9 Asia

Pretty and Hine (2009) evaluated 16 agroecological projects/initiatives spread across eight Asian countries and found that some 2.86 million households have substantially improved total food production on 4.93 million hectares, resulting in greatly improved household food security. Proportional yield increases are greatest in rainfed systems, but irrigated systems have seen small cereal yield increases combined with added production from additional productive system components (such as fish in rice, vegetables on dykes) (Action Aid 2010).

The System of Rice Intensification (SRI) is an agro- ecological methodology for increasing the productivity of irrigated rice by changing the management of plants, soil, water and nutrients (Stoop et al. 2002). It has spread throughout China, Indonesia, Cambodia and Vienam reaching more than a million hectares with average yield increases of 20–30%. The benefits of SRI, which have been demonstrated in over 40 countries include: increased yield at times >50%, up to 90% reduction in required seed, up to 50% savings in water. SRI principles and practices have also been adapted for rainfed rice as well as for other crops such as wheat, sugarcane and teff, among others, with yield increases and associated economic benefits.

(http://sri.ciifad.cornell.edu/countries/cambodia/camcedacimpact03.pdf)

On what probably can be considered the largest study undertaken on sustainable agriculture in Asia, Bachmann et al. (2009) examined the work of MASIPAG, a network of small- scale farmers, farmers' organizations, scientists and non-governmental organizations (NGOs). By comparing 280 full organic farmers, 280 in conversion to organic agriculture and 280 conventional farmers, these researchers found that food security is significantly higher for organic farmers. Results of the study summarized in Table 3 show good outcomes particularly for the poorest in rural areas. Full organic farmers eat a more diverse, nutritious and secure diet. Reported health outcomes are also substantially better for the organic group. The study reveals that the full organic farmers have considerably higher on-farm diversity, growing on average 50% more crops than conventional farmers, better soil fertility, less soil erosion, increased tolerance of crops to pests and diseases, and better farm management skills. The group also has, on average, higher net incomes.

Table 3 Main findings of the MASIPAG study on farmers practicing farmer-led sustainable agriculture (Bachmann et al. 2009)

More food secure: 88% of organic farmers find their food security better or much better than in 2000 compared to only 44% of conventional farmers. Of conventional farmers, 18% are worse off. Only 2% of full organic farmers are worse off
Eating an increasingly diverse diet: Organic farmers eat 68% more vegetables, 56% more fruit, 55% more protein rich staples and 40% more meat than in 2000. This is an increase between 2 and 3.7 times higher than for conventional farmers
Producing a more diverse range of crops: Organic farmers on average grow 50% more crop types than conventional farmers
Experiencing better health outcomes: In the full organic group 85% rate their health today better or much better than in 2000. In the reference group, only 32% rate it positively, while 56% see no change and 13% report worse health

10 Latin America

Since the early 1980s rural producers in partnership with NGOs and other organizations, have promoted and implemented alternative, agroecological featuring resource-conserving yet highly productive systems, such as polycultures, agroforestry, and the integration of crops and livestock (Altieri 2009).

An analysis of several agroecological field projects in operation during the 1990s (these initiatives now involve almost 100,000 farming families/units and cover more than 120,000 ha of land) showed that traditional crop and animal combinations can often be adapted to increase productivity when the biological structuring of the farm is improved and labor and local resources are efficiently used (Altieri 1999). In fact, most agroecological technologies promoted by NGOs improve traditional agricultural yields increasing output per area of marginal land from 400–600 to 2,000–2,500 kg ha^{-1} enhancing also the general agrobiodiversity and its associated positive effects on food security and environmental integrity. Some projects emphasizing green manures and other organic management techniques can increase maize yields from 1 to 1.5 t ha^{-1} (a typical highland peasant yield) to 3–4 t ha^{-1}.

An IFAD (2004) study which covered a total of 12 farmer organizations that comprise about 5,150 farmers and close to 9,800 ha showed that small farmers who shifted to organic agricultural production in all cases obtained higher net revenues relative to their previous situation. Many of these farmers produce coffee and cacao under very complex and biodiverse agroforestry systems.

In the states of Parana and Santa Catarina, Brazil thousands of hillside family using cover crops minimize soil erosion and weed growth and exhibit positive effects on soil physical, chemical and biological properties (Petersen et al. 1999). This is how an innovative organic minimum tillage system emerged. By using cover crop mixtures including legumes and grasses mulch biomass can reach 8,000 kg/ha and a mulch thickness of 10 cm leading to 75% or more inhibition of weed emergence. Maize yields have risen from 3 to 5 t ha^{-1} and soybeans from 2.8 to 4.7 t ha^{-1} without using herbicides or chemical fertilizers (Altieri et al. 2011a).

In Cuba, it is estimated that agroecological practices are used in 46–72% of the peasant farms producing over 70% of the domestic food production, e.g. 67% of roots and tubers, 94% of small livestock, 73% of rice, 80% of fruits and most of the honey, beans, cocoa, maize, tobacco, milk and meat production (Funes et al. 2002; Machin et al. 2010; Rosset et al. 2011). Small farmers using agroecological methods obtain yields per hectare sufficient to feed about 15–20 people per year with energy efficiencies of no less than 10:1 (Funes-Monzote 2009). Another study conducted by Funes-Monzote et al. (2009) shows that small farmers using integrated crop-livestock farming systems were able to achieve a three-fold increase in milk production per unit of forage area (3.6 t/ha/year) as well as a seven-fold increase in energy efficiency. Energy output (21.3 GJ/ha/year) was tripled and protein output doubled (141.5 kg/ha/year) via diversification strategies of specialized livestock farms.

Perhaps the most widespread agroecological effort in Latin America promoted by NGOs and peasant organizations is the rescuing of traditional or local crop varieties (variedades criollas), their in-situ conservation via community seed banks and their exchange through hundreds of seed fairs (ferias de semillas) notoriously in Mexico, Guatemala, Nicaragua, Peru, Bolivia, Ecuador and Brasil. For example in Nicaragua the project Semillas de Identidad which involves more than 35,000 families on 14,000 ha have already recuperated and conserved 129 local varieties of maize and 144 of beans. http://www.swissaid.org.co/kolumbien/global/pdf/campa_a_28.05.08.pdf

In Brasil, the Bionatur Network for Agro-ecological Seeds (Rede Bionatur de Sementes Agroecológicas) is one of the strategic tools that the Landless peasant movement (MST) has launched for the participatory breeding of seeds adapted to agroecological management and their dissemination among hundreds of thousands of peasants.

An increasing number of indigenous groups or cabildos in the Andean and MesoAmerican countries have adopted agroecology as a fundamental strategy for the conservation of their germplasm and the management of agriculture in their autonomous territory. These efforts are tied to their struggle to preserve their land and cultural identity. The Mesoamerican indigenous population includes about 12 million people. In Mexico, the peasant sector that still uses indigenous languages controls an area estimated at 28 million hectares.

11 Agroecology and Resiliency to Climatic Extremes

Of key importance for the future of agriculture are results from observations of agricultural performance after extreme climatic events which reveal that resiliency to climate disasters is closely linked to the level of on-farm biodiversity, a major feature of agroecological systems (Altieri and Koohafkan 2008). A survey conducted in Central American hillsides after Hurricane Mitch showed that farmers using diversification practices such as cover crops, intercropping and agroforestry suffered less damage than their conventional monoculture neighbors. The study revealed that diversified plots had 20–40% more topsoil, greater soil moisture and less erosion and experienced lower economic losses than their conventional neighbors (Holt-Gimenez 2000). Similarly in Sotonusco, Chiapas, coffee systems exhibiting high levels of vegetational complexity and plant diversity suffered less damage from Hurricane Stan than more simplified coffee systems (Philpott et al. 2008). In the case of coffee, the more shaded systems have also been shown to protect crops from decreasing precipitation and reduced soil water availability because the overstory tree cover is able to reduce soil evaporation and increase soil water infiltration (Lin 2007). Forty days after Hurricane Ike hit Cuba in 2008, researchers conducted a farm survey in the Provinces of Holguin and Las Tunas and found that diversified farms exhibited losses of 50% compared to 90 or 100% in neighboring monocultures. Likewise agroecologically managed farms showed a faster productive recovery (80–90%) 40 days after the hurricane than monoculture farms (Rosset et al. 2011).

Diversified farming systems such as agroforestry, silvopastoral and polycultural systems provide a variety of examples on how complex agroecosystems are able to adapt and resist the effects of drought. Intercrops of sorghum and peanut, millet and peanut, and sorghum and millet exhibited greater yield stability and less productivity declines during a drought than in the case of monocultures (Natarajan and Willey 1996). In 2009 the valle del Cauca in Colombia experienced the driest year in a 40 year record. Intensive silvopastoral systems for livestock production combining fodder shrubs planted at high densities under trees and palms with improved pastures, not only provided environmental goods and services for livestock producers but also greater resilience to drought (Murgueitio et al. 2011).

12 Scaling Up Agroecological Innovations

The cases reported above show that in Africa, Asia and Latin America there are many NGO and farmer led initiatives promoting agroecological projects that have demonstrated a positive impact on the livelihoods of small farming communities in various countries (Altieri et al. 2011b). Agroecological production is particularly well suited for smallholder farmers, who comprise the majority of the rural poor. Resource-poor farmers using agroecological systems are less dependent on external resources and experience higher and more stable yields enhancing food security. Some of these farmers, who may devote part of their production for certified organic export production without sacrificing food security, exhibit significantly higher incomes than their conventional counterparts. Agroecological management makes conversion to organic production fairly easy, involving little risk and requires few, if any, fixed investments.


With so many proven on-farm social, productive and ecological benefits, the relatively limited adoption and dissemination of agroecological innovations begs two questions: (1) If agroecological systems are so profitable and efficient, why have they not been more widely disseminated and adopted? and (2) and how can agroecology be multiplied and scaled up? There are a number of constraints that discourage adoption and dissemination of agroecological practices thus impeding its widespread adoption. Barriers range from technical issues such as lack of information by farmers and extension agents to policy distortions, market failure, lack of land tenure and infrastructural problems. In order to further spread agroecology among farmers it is essential to overcome part or all of these constraints. Major reforms must be made in policies, institutions, and research and development agendas to make sure that agroecological alternatives are massively adopted, made equitably and broadly accessible, and multiplied so that their full benefit for sustainable food security can be realized. Farmers must have higher access to local-regional markets, government support such as credit, seeds and agroecological technologies. It should also be recognized that a major constraint to the spread of agroecology has been that powerful economic and institutional interests have backed research and development for the conventional agroindustrial approach, while research and development for agroecology and sustainable approaches has in most countries been largely ignored or even ostracized (Altieri 2002).

In Latin America, a key factor in the expansion of localized agroecology efforts in several isolated rural areas was the Campesino a Campesino-CAC movement which uses a "peasant pedagogic method" that focuses on sharing experiences, strengthening local research and problem-solving capacities in a horizontal process of exchange of ideas and innovations among farmers (Holt–Gimenez 2006). It was via the CAC method that soil conservation practices were introduced in Honduras, and hillside farmers adopting the various techniques tripled or quadrupled their yields from 400 kg/ha to 1,200–1,600 kg. This tripling in per-hectare grain production ensured that the 1,200 families that initially participated in the program have ample grain supplies for the ensuing year. The adoption of velvet bean (*Mucuna pruriens*) which can fix up to 150 kg of nitrogen per ha as well as produce 35 tones of organic matter per year, helped tripled maize yields to 2,500 kg/ha. Labor requirements for weeding were cut by 75% and herbicides eliminated entirely.

In the early 1990s organized social rural movements such as the Via Campesina, the Landless Workers Movement (MST) and others massively adopted agroecology as a banner of their technological approach to achieve food sovereignty. What constitutes the soul of the Cuban agroecological revolution was the adoption via the CAC process of agroecological methods by 110,000 family farmers associated with the Asociacion Nacional de Agricultores Pequenos (ANAP) who in less than a decade, controlling less than 35% of the land produce over 70% of the domestic food production, e.g. 67% of roots and tubers, 94% of small livestock, 73% of rice and 80% of fruits (Rosset et al. 2011).

Successful scaling up of agroecology depends heavily on human capital enhancement and community empowerment through training and participatory methods that seriously take into account the needs, aspirations and circumstances of smallholders. In addition to the CAC process there are other initiatives to scale up agroecology which involve capacity building emphasizing training, farmer field schools, on-farm demonstrations, farmer to farmer exchanges, field visits and other marketing and policy initiatives.

12.1 Non-Governmental Organisations (NGO) Led Initiatives

Since the early 1980s, hundreds of agroecologically- based projects have been promoted by NGOs and church based groups throughout the developing world, which incorporate elements of both traditional knowledge and modern agricultural science. A variety of projects exist featuring resource-conserving yet highly productive systems, such as polycultures, agroforestry, soil conservation, water harvesting, biological pest control and the integration of crops and livestock, etc. Approaches to train farmers on agroecological methods and disseminate best practices include a great variety: field days, on-farm demonstrations, training of trainers, farmers cross-visits, etc. Much of the spread of cover cropping based conservation agriculture in southern Africa reaching >50,000 farmers has been attained via one or more these methods.

12.2 Inter-Organization Collaboration

One of the best examples of this approach are the Farmer Field School (FFS) which consist of a group- based learning process used by a number of governments, NGOs and international agencies collaborating in the promotion of agroecological method. The most successful FFS was promoted by the FAO Intercountry Programme for the Development and Application of Integrated Pest Control in Rice in South and South-East Asia launched in 1980. Farmers carried out experiential learning activities that helped them understand the ecology of their rice fields via simple experiments, regular field observations and group analysis. Thousands of farmers reported substantial and consistent reductions in pesticide use and in many cases there was also convincing increases in yield attributable to the effect of training. IPM Farmer Field School programs, at various levels of development, are being conducted in over 30 countries worldwide. http://www.fao.org/docrep/006/ad487e/ad487e02.htm

12.3 Developing Local Markets

There are thousands of initiatives throughout the world aimed at closing the circuits of production and consumption via development of local farmers markets and community supported agriculture. One of the most exciting examples is REDE ECOVIDA in southern Brasil, which consists of a space of articulation between organized family farmers, supportive NGOs and consumers whose objective is to promote agroecological alternatives and develop solidarious markets that tighten the circle between local producers and consumers, ensuring local food security and that the generated wealth remains in the community (van der Ploeg 2009). Presently Ecovida encompasses 180 municipalities and approximately 2,400 families of farmers (around 12,000 persons) organized in 270 groups, associations and cooperatives. They also include 30 NGOs and 10 ecological consumers' cooperatives. All kinds of agriculture products are cultivated and sold by the Ecovida members, including vegetables, cereals, fruits, juice, fruit-jelly, honey, milk, eggs and meat reaching thousands of consumers.

http://www.ifoam.org/about_ifoam/standards/pgs_projects/pgs_projects/15649.php

12.4 Government Policies

Governments can launch policies that support and protect small farmers. The *Ministerio do Desenvolvimento Rural* (MDA) in Brasil has played a major role in supporting education and research projects, but most importantly has created important instruments for family farmers to have access to know-how, credit, markets, etc.

One examples is the public purchasing programme *Programa de Aquisiçao de Alimentos* (PAA) created in 2003. The program addresses the issue of lack of market access for the products of a large number of family farms. Family farms are therefore unable to reach their full earning potential. In the scope of four program lines, farmers are given a purchase guarantee for specific quantities at specific prices making the operations of thousands of small farms more economically viable.

http://www.rural21.com/uploads/media/rural_2011_4_36-39_01.pdf

12.5 Political Advocacy and Action

With or without government support, major global peasant rural movements (such as the Via Campesina) have already initiated an agroecological revolution and have launched a strategy followed by millions of farmers to strengthen and promote agroecological models of food provision in the framework of food sovereignty. No less than 30% of the ten million hectare territory controlled by the MST in Brasil is under agroecological management. Thousands of MST members have received agroecological theoretical and practical training on the many MST institutes such as the Latin American School of Agroecology established in an MST settlement in Lapa, state of Parana.

In addition to promoting capacity building and agroecological innovations on the ground, rural movements advocate for a more radical transformation of agriculture, one guided by the notion that ecological change in agriculture cannot be promoted without comparable changes in the social, political, cultural and economic arenas. The organized peasant and indigenous based agrarian movements (i.e. the Via Campesina) consider that only by changing the export-led, free-trade based, industrial agriculture model of large farms can the downward spiral of poverty, low wages, rural-urban migration, hunger and environmental degradation be halted. Most oppose the out-of-control trade liberalization as they consider it the main mechanism driving farmers off their land and the principal obstacle to local economic development and food sovereignty. These movements embrace the concept of food sovereignty, which constitutes an alternative to the current mainstream thinking on food production. The concept behind food sovereignty contrasts the neo-liberal approach that believes that international trade will solve the world's food problem. Instead, it focuses on local autonomy, local markets and community action for access and control of land, water, agrobiodiversity, etc., which are of central importance for communities to be able to produce food locally (via Campesina 2010).

13 The Way Forward

Thousands of projects throughout Africa, Asia and Latin America show convincingly that agroecology provides the scientific, technological and methodological basis to assist small holder farmers enhance crop production in a sustainable and

Table 4 Global comparison of yields of organic versus conventional production using an average yield ratio (organic: non-organic). 1,0: org. = conventional <1,0: conventional higher than organic. >1,0: organic higher than conventional

	(A) World			(B) Developed countries			(C) Developing countries		
Food category	*N.*	Av.	S.E.	*N.*	Av.	S.E.	*N.*	Av.	S.E.
Grain products	171	1.312	0.06	69	0.928	0.02	102	1.573	0.09
Starchy roots	25	1.686	0.27	14	0.891	0.04	11	2.697	0.46
Sugars and sweeteners	2	1.005	0.02	2	1.005	0.02			
Legumes (pulses)	9	1.522	0.55	7	0.816	0.07	2	3.995	1.68
Oil crops and veg. oils	15	1.078	0.07	13	0.991	0.05	2	1.645	0.00
Vegetables	37	1.064	0.10	31	0.876	0.03	6	2.038	0.44
Fruits, excl. wine	7	2.080	0.43	2	0.955	0.04	5	2.530	0.46
All plant foods	266	1.325	0.05	138	0.914	0.02	128	1.736	0.09
Meat and offal	8	0.988	0.03	8	0.988	0.03			
Milk, excl. butter	18	1.434	0.24	13	0.949	0.04	5	2.694	0.57
Eggs	1	1.060		1	1.060				
All animal foods	27	1.288	0.16	22	0.968	0.02	5	2.694	0.57
All plant and animal foods	293	1.321	0.05	160	0.922	0.01	133	1.802	0.09

resilient manner thus allowing them to provide for current and future food needs. Agroecological methods produce more food on less land, using less energy, less water while enhancing the natural resource base, providing ecological services and lowering outputs of greenhouse gases. Researchers at the University of Michigan compared yields of organic versus conventional production for a global dataset of 293 examples and estimated the average yield ratio (organic: non-organic) of different food categories for the developed and the developing world. For most food categories, the average yield ratio was slightly <1.0 for studies in the developed world and >1.0 for studies in the developing world (Table 4). This means that the global south has the agroecological potential to produce enough food on a global per capita basis to sustain the current human population, and potentially an even larger population, without increasing the agricultural land base. The reason why the potential resides in the South and not in the North, is because in developing countries still resides a large peasant-indigenous population, with a rich traditional agricultural knowledge and a broad genetic diversity which conforms the basis of resilient diversified agroecosystems. http://www.organicvalley.coop/fileadmin/pdf/organics_can_feed_world.pdf

The evidence is overwhelming, so the question is what else is needed to convince policy makers and funders to take a brave stand and bid on agroecology? The issue seems to be political or ideological rather than evidence or science based.

No matter what data is presented, governments and donors influenced by big interests marginalize agroecological approaches focusing on quick-fix, external

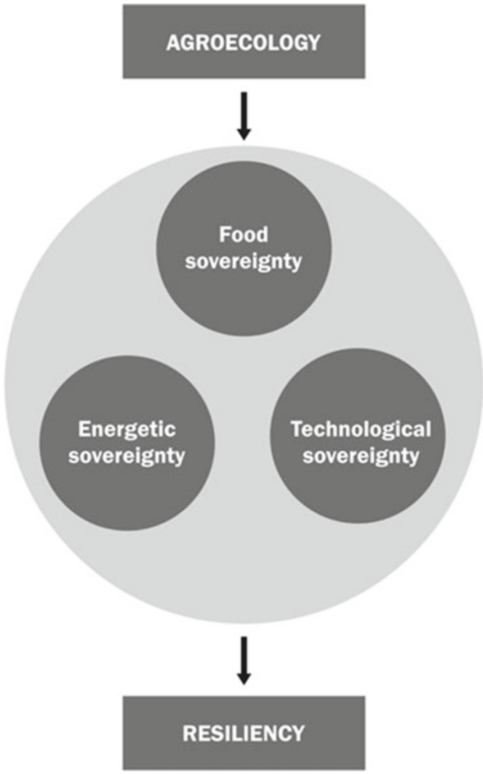

Fig. 4 The three types of sovereignty to be reached by an agricultural community or region by following agroecological principles and in the context of a resiliency strategy (Altieri et al 2011a, b)

input intensive ‘solutions’ and proprietary technologies such as transgenic crops and chemical fertilizers that not only pose serious environmental risks but have proven to be inaccessible and inappropriate to poor and small farmers that play a key role in global food security.

In addition to climate change, repeated food price spikes, shortages of good-quality land and water, and rising energy costs will prove major challenges to secure food security for all. This is why the agroecological strategy also aims at enhancing energy and technological sovereignty (Fig. 4). Energy sovereignty is the right for all rural people to have access to or generate sufficient energy within ecological limits from sustainable sources. Technological sovereignty refers to the capacity to achieve the two other forms of sovereignty by optimizing agrobiodiversity designs that efficiently use local resources and encourage synergies that sponsor the functioning of agroecosystems. This new paradigm of the “three sovereignties” gives agroecology a greater scope as a tool to determine the minimum acceptable values for food production, biodiversity conservation, energy efficiency, etc., allowing rural communities to assess whether or not they are advancing towards a basic state of food, energy and technological sovereignty in a context of resiliency.

Governments have a major role to play such as providing incentives for farmers to adopt resource- conserving technologies and revive public agroecological research and extension programs suited to the needs and circumstances of smallholder farmers, their associations and networks. National governments need to increase poor people's access to land, seeds, water and other resources vital pre-requisites for rural food security. All this must be accompanied by initiatives that enable the creation of, and access to, markets that return fair prices for small-scale producers, and protect peasants from global trade policies and dumping that do not safeguard the strategic position of domestic producers in national food systems.

It is time for the international community to recognize that there is no other more viable path to food production in the twenty-first Century than agroecology. Developing a resilient agriculture will require technologies and practices that build on agro-ecological knowledge and enable smallholder farmers to counter environmental degradation and climate change in ways that maintain sustainable agricultural livelihoods. The need to scale up the agroecological approach is long overdue and in fact is the most robust food provisioning pathway for humanity to take under current and predicted and difficult climate, energy, financial and social scenarios. Whether the potential and spread of local agroecological innovations described above, is scaled up to reach all the small farmers of a region cannot be left only to the political will of governments. It will largely depend on the ability of the various actors (including consumers) and organizations involved in the agroecological revolution to make the necessary alliances to exert pressure so that farmers can gain increasing access to agroecological knowledge as well as to land, seeds, government services, solidarious markets, and so on. Rural social movements understand that dismantling the industrial agrifood complex and restoring local food systems must be accompanied by the construction of agroecological alternatives that suit the needs of small-scale producers and the low-income non-farming population while opposing corporate control over production and consumption (Vanderploeg 2009). Of key importance will be the formulation of an agroecological research agenda with the active participation of farmers in the process of technological innovation and dissemination through Campesino-Campesino models where researchers, extension workers and NGO technicians can play a major facilitating role (Altieri and Toledo 2011).

References

Action Aid (2010) Farmer –led sustainable agriculture. http://www.actionaid.org/publications/smallholder-led-sustainable-agriculture-actionaid-international-briefing. Accessed 16 Apr 2012

Altieri MA (1995) Agroecology: the science of sustainable agriculture. Westview Press, Boulder

Altieri MA (1999) Applying agroecology to enhance productivity of peasant farming systems in Latin America. Environ Dev Sustain 1:197–217

Altieri MA (2002) Agroecology: the science of natural resource management for poor farmers in marginal environments. Agric Ecosyst Environ 93:1

Altieri MA (2004) Linking ecologists and traditional farmers in the search for sustainable agriculture. Front Ecol Environ 2:35–42

Altieri MA (2009) Agroecology, small farms and food sovereignity. Mon Rev 61:102–111

Altieri MA, Koohafkan P (2008) Enduring farms: climate change, smallholders and traditional farming communities, vol 6, Environment and development series. Third World Network, Malaysia

Altieri MA, Toledo VM (2011) The agroecological revolution in Latin America. J Peasant Stud 38:587–612

Altieri MA, Rosset P, Thrupp LA (1998) The potential of agroecology to combat hunger in the developing world. 2020 Brief. IFPRI, Washington, DC

Altieri MA, Lana MA, Bittencourt H, Kieling AS, Comin JJ, Lovato PE (2011a) Enhancing crop productivity via weed suppression in organic no--till cropping systems in Santa Catarina, Brasil. J Sustain Agric 35:1–15

Altieri MA, Funes F, Petersen P (2011b) Agroecologically efficient agricultural systems for smallholder farmers: contributions to food sovereignty. Agron Sustain Dev. doi:10.1007/s13593-011-0065-6

Bachmann L, Cruzada E, Wright S (2009) Food security and farmer empowerment: a study of the impacts of farmer-led sustainable agriculture in the Philippines. Masipag-Misereor, Los Banos

Campesina V (2010) Sustainable peasant and small family farm agriculture can feed the world. Via Campesina Views, Jakarta

CGIAR (2012) Achieving food security in the face of climate change. Final report from the commission on sustainable agriculture and climate change. http://ccafs.cgiar.org/commission/reports. Accessed 2 May 2012

Christian Aid 2011 Healthy harvests: the benefits of sustainable agriculture in Africa and Asia. A Christian Aid Report, UK.

de Schutter, O. 2010. Report submitted by the Special Rapporteur on the right to food. UN General Assembly. Human Rights Council Sixteenth Session, Agenda item 3 A/HRC/ 16/49.

ETC Group (2009) *Who will feed us? Questions for the food and climate crisis*. ETC. Group Comunique #102

Funes F, García L, Bourque M, Pérez N, Rosset P (eds) (2002) Sustainable agriculture and resistance: transforming food production in Cuba. Food First Books, Oakland

Funes-Monzote FR (2009) Agricultura con futuro: la alternativa agroecologica para Cuba. Estación Experimental Indio Hatuey, Matanzas

Garrity D (2010) Evergreen agriculture: a robust approach to sustainable food security in Africa. Food Secur 2:3–20

Gliessman SR (1998) Agroecology: ecological process in sustainable agriculture. Ann Arbor Press, Michigan

Godfray C, Beddington JR, Crute IR, Haddad L, Lawrence D, Muir JF, Pretty J, Robinson L, Toulmin SM (2010) Food security: the challenge of feeding 9 billion people. Science 327:812–818

Holt-Gimenez E (2000) Measuring farmers' agroecological resistance after Hurricane Mitch in Nicaragua: a case study in participatory, sustainable land management impact monitoring. Agric Ecosyst Environ 93:87–105

Holt-Gimenez E (2006) Campesino a Campesino: voices from Latin America's farmer to farmer movement for sustainable agriculture. Food First Books, Oakland, Oakland

Holt-Gimenez E, Patel R (2009) Food rebellions: the real story of the world food crisis and what we can do about it. Fahumu Books and Grassroots International, Oxford

IAASTD (International Assessment of Agricultural Knowledge, Science and Technology for Development) (2009) Agriculture at a crossroads. In: International assessment of agricultural knowledge, science and technology for development. Global report. Island Press, Washington, DC

IFAD (2004) The adoption of organic agriculture among small farmers in Latin America and the Caribbean. http://www.ifad.org/evaluation/public_html/eksyst/doc/thematic/pl/organic.htm. Accessed 28 Apr 2012

Koohafkan P, Altieri MA (2010) Globally important agricultural heritage systems: a legacy for the future. UN-FAO, Rome

Koohafkan P, Altieri MA, Gimenez EH (2011) Green agriculture: foundations for biodiverse, resilient and productive agricultural systems. Int J Agric Sustain. http://dx.doi.org/10.1080/14735903.2011.610206

Lappe FM, J. Collins and P. Rosset (1998) World Hunger; twelve myths . Frances Moore Lappé, A Grove Press Book, New York

Lin BB (2007) Agroforestry management as an adaptive strategy against potential microclimate extremes in coffee agriculture. Agric For Meteorol 144:85–94

Machin-Sosa, B., A.M. Roque-Jaime, D.R. Avila-Lozano, and P. Rosset. 2010. Revolucio´n Agroecolo´gica: el Movimiento de Campesino a Campesino de la ANAP en Cuba. Habana: ANAP.

Murgueitio E, Calle Z, Uribea F, Calle A, Solorio B (2011) Native trees and shrubs for the productive rehabilitation of tropical cattle ranching lands. For Ecol Manage 261:1654–1663

Natarajan, M, and R.W. Willey, 1996. The effects of water stress on yields advantages of intercropping systems. Field Crops Research 13: 117-131

Owenya MZ, Mariki ML, Kienzle J, Friedrich T, Kassam A (2011) Conservation agriculture (CA) in Tanzania: the case of Mwangaza B CA farmer field school (FFS), Rothia Village, Karatu District. Int J Agric Sustain 9:145–152

Parrot N, Mardsen T (2002) The real green revolution: organic and agroecological farming in the south. Green Peace Environmental Trust, London. www.greenpeace.org.uk/MultimediaFiles/Live/FullReport/4526.pdf

Petersen P, Tardin JM,Marochi F (1999) Participatory development of notillage systems without herbicides for family farming; the experience of the Center-South region of Paraná. In: Environment, development and sustainability. Dordrecht, Kluwer Academic Publishers (special issue on Sustainable Agriculture)

Philpott SM, Lin BB, Jha S, Brines SJ (2008) A multi- scale assessment of hurricane impacts on agricultural landscapes based on land use and topographic features. Agric Ecosyst Environ 128:12–20

Pretty J, Hine R (2009) The promising spread of sustainable agriculture in Asia. Nat Res Forum 24:107–121

Pretty J, Morrison JIL, Hine RE (2003) Reducing food poverty by increasing agricultural sustainability in the development countries. Agric Ecosyst Environ 95:217–234

Pretty J, Toulmin C, Williams S (2011) Sustainable intensification in African agriculture. Int J Sustain Agric 9:5–24

Reij CP, Smaling EMA (2008) Analyzing successes in agriculture and land management in Sub-Saharan Africa: is macro-level gloom obscuring positive micro-level change? Land Use Policy 25:410–420

Rosset, P.M., and M.A. Altieri. 1997. Agroecology versus input substitution: a fundamental contradiction of sustainable agriculture. Society and Natural Resources, 10(3), 283–95.

Rosset PM, Machín-Sosa B, Roque-Jaime AM, Avila-Lozano DR (2011) The Campesino-to-Campesino agroecology movement of ANAP in Cuba. J Peasant Stud 38:161–191

Stoop WA, Uphoff N, Kassam A (2002) A review of agricultural research issues raised by the system of rice intensification (SRI) from Madagascar: opportunities for improving farming systems. Agric Syst 71:249–274

Toledo VM, Barrera-Bassols N (2009) La memoria biocultural: la importancia ecológica de las sabidurías tradicionales. ICARIA Editorial, Barcelona

UK Food Group (2010) Securing future food: towards ecological food provision. http://www.ukfg.org.uk/pdfs/Securing_future_food.pdf. Accessed 4 May 2012

UNCTAD/UNEP (2008) Organic agriculture and food security in Afric*a*, New York: United Nations. http://www.unctad.org/en/docs/ditcted200715_en.pdf. Accessed 27 Apr 2012

Uphoff N (2002) Agroecological innovations: increasing food production with participatory development. Earthscan, London

Van der Ploeg JD (2009) The new peasantries: new struggles for autonomy and sustainability in an era of empire and globalization. Earthscan, London, 356 p

Transforming Agriculture for Sustainability: The Art and Science

Harold Schroeder

Abstract There is an urgent need for organizations in the agricultural sector and the food industry to adopt sustainable working practices to reduce the environmental impacts of their activities while meeting the challenge of an expected 50% increase in global demand for food. As in other sectors, however, organizations in agriculture and the food industry face economic pressures and must balance sustainability considerations with the need to operate a viable and competitive business. The adoption of sustainable working practices can make sound business sense, resulting in increased profits and other benefits; however, sustainability is still not a management priority in many organizations and relatively few have achieved the kind of holistic sustainability initiatives associated with business benefits.

Building on the findings from recent research on sustainability, this article argues that sustainability must be viewed in organizational transformation terms and that, like other forms of large-scale business change, it can only succeed if addressed using an "art and science" approach. Like other organizational transformations, sustainability initiatives are subject to a high rate of failure, often due to a lack of attention to the "art" or the people-related aspects of change. However, there is also a need to apply "science" to organizational change, in the form of project management and other specialist tools and techniques.

Drawing on recent sustainability literature, the article highlights aspects of art and science that are crucial for maximizing the business benefits of sustainability. It highlights the importance of achieving the right balance of art and science and of adopting a holistic approach when implementing sustainability initiatives. This is important if business benefits are to be achieved as well as positive environmental and social impacts. Since the agricultural sector and related food industries have such a big environmental footprint, it will be particularly important to adopt an art

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and science approach to transforming working practices in these sectors if real progress in sustainable agriculture is to be made.

Keywords Sustainable working • Sustainable business practices • Sustainability • Organizational transformation • Art and science • Business benefits • Organizational culture • Strategic • Sustainability • Soft skills

1 Introduction

There is an urgent need for organizations in the agricultural sector and the food industry to adopt sustainable working practices to reduce the environmental impacts of their activities while meeting the challenge of an expected 50% increase in global demand for food over the next 50 years (Tilman et al. 2002). According to the United Nations' Food and Agriculture Organization, agriculture and related industries are responsible for around one third of global warming and climate change (FAO 2001); other ecological impacts of agriculture include "land degradation, limits to water availability, loss of biodiversity *(and)* declining agricultural genetic diversity". In the social sphere, sustainable agriculture means providing fair and safe conditions of employment and the payment of a living wage to farmers and agricultural workers, to ensure that negative impacts of the sector such as "labor abuses; and the decline of the family farm" are avoided in future, and that agriculture supports economic development in the developing countries from which many materials and products are sourced (National Geographic n.d.) (Photo 1).

As in other sectors, however, organizations in agriculture and the food industry face economic pressures and must balance sustainability considerations with the need to operate a viable and competitive business. The good news is that the adoption of sustainable working practices can also make sound business sense: across all sectors of the economy, there is growing evidence that organizations that successfully incorporate sustainability in a holistic way report increased profits and other business benefits. According to the recently published Third Annual Sustainability Global Executive Survey, more organizations than ever before are now putting sustainability on their business agendas and recognize the need to do so in order to remain competitive. Even more significantly, among survey respondents with sustainability initiatives, almost a third reported increased profits as a result. Despite this, the research also revealed that many organizations are struggling to reconcile sustainability with their core business objectives, and that sustainability ranks only eighth among agenda items perceived to be important to top management. The researchers comment that "we may be reaching critical mass with companies taking sustainability seriously, but we have not yet achieved a similar critical mass with companies profiting from sustainability" (Kruschwitz and Haanaes 2011, p. 71).

Building on the findings from this and other recent research on sustainability, this article argues that sustainability must be viewed in organizational transformation

Photo 1 Sustainable working practices will be necessary if the environmental footprint of agriculture can be reduced while global demand for food soars

terms and that, like other forms of large-scale business change, it can only succeed if addressed using an "art and science" approach. While the importance of the people-related aspects of organizational change are increasingly being recognized in the literature, in practice there is continuing evidence that many mission-critical projects fail due to a neglect of the "art" of transformation. The article discusses several key aspects of art and science which are crucial in order to ensure that organizations maximize the business benefits of sustainability and highlights the importance of achieving the right balance of art and science when implementing sustainability initiatives. Since the agricultural sector and related food industries have such a big environmental footprint, it will be particularly important to adopt an art and science approach to transforming working practices in these industries if real progress in sustainability is to be made.

2 The Importance of Sustainability

In the business context, the concept of sustainability has been closely associated with the "triple bottom line", a phrase first coined by Elkington (1998) to highlight the importance of considering social and environmental as well as economic factors when calculating the costs of business activity and value generated. These social and environmental factors include, for example, health and safety, human rights, product safety, pollution and energy use, among others. The need for organizations to pay greater attention to the social and environmental impacts of their activities has increased as public awareness and media attention to these factors has grown over time. There is now a general expectation that companies will exhibit responsible social and environmental behaviour and demand the same from their suppliers (Dao et al. 2011); the point has now been reached, it has been argued, at which those who do not live up to this are likely to be pushed out of the market (Piasecki 2009) (Photo 2).

The focus on sustainability in the business world also been reinforced by the development of stock market indices such as the Dow Jones Sustainability Index, which tracks the performance of global leaders in sustainability against economic, environmental and social criteria, and by global environmental league tables such as Newsweek's annual Green Rankings, which highlight the environmental performance of the world's largest publicly traded companies (Yarett 2011). There has also been an increase over time in social and environmental legislation or standards, bringing the requirement or at least the public expectation for organisations to monitor and report on their performance in these areas. Many large organizations and an increasing number of smaller firms publish sustainability reports or include social and environmental performance reporting in their annual reports. Maintaining a positive brand image and attracting investment are becoming largely dependent on the sustainability record of an organization and its suppliers, which is often very transparent and subject to closely monitoring by environmental groups, investors and the general public alike. Pressures on firms to adopt sustainable working practices are not just external: there is increasing evidence that a well implemented sustainable approach to working is associated with business benefits such as reduced operating costs, increase revenue, better brand image and more effective employee engagement (Haanaes et al. 2011).

As a result, more and more organizations, across all sectors and size bands, are adopting sustainability initiatives – with no apparent decrease in this trend even in the economic downturn (Haanaes et al. 2011). Even among small and medium enterprises (SME), a rapid increase in the adoption of sustainability strategies has been documented in recent years (Chartered Accountants of Canada, American Institute of CPAs, Chartered Institute of Management Accountants 2010). That's the positive news; but these top-line positive findings conceal equally strong evidence that many companies are struggling to successfully incorporate sustainability into their working practices or that there is a gap between rhetoric and practice (Deloitte 2010; Haanaes et al. 2011). For example, the Third Annual Sustainability Global Executive Survey revealed that "most companies" are struggling

Photo 2 Sustainability makes good business sense

to define sustainability in a way that is relevant to their business (Kruschwitz and Haanaes 2011), while other studies have revealed that sustainability is still being addressed in a peripheral way in many organisations, with a primary focus on "green" initiatives which are planned and implemented separately from their core business strategies. An extensive review of the web sites of leading global companies revealed a lack of sustainability targets and big gaps in practice (Hubbard 2011); similarly a review of leading companies in Australia companies concluded that there was "little tangible evidence that sustainable business practices are being implemented" and that "business does not even claim to be environmentally responsible except as rhetoric" (Brennan et al. 2011, p. 56). Although more and more organisations are implementing sustainability strategies, the evidence suggests that sustainability is still given a low priority compared with other management agenda items and that relatively few are adopting sustainable working in a way that will help drive profits and business growth (Haanaes et al. 2011; Kruschwitz and Haanaes 2011).

In summary, organizations in all sectors of the economy, including agriculture and related industries, are coming under increasing pressure to adopt sustainability initiatives, yet there remains a big gap between those organizations that have done so successfully and are reaping business benefits as a result, and those who are struggling with their approaches to sustainability or have only incorporated sustainability on the margins of their business. The following sections highlight best practice in sustainability and show how an art and science approach to organizational transformation is crucial in the achievement of this.

3 Sustainability as Organizational Transformation

What is becoming evident is that those organizations which are leading the way in sustainability and also experiencing strong business performance are those in which sustainability has been implemented in a holistic way into all areas of the organisation (Aberdeen Group 2010; MIT Sloan Management Review and the Boston Consulting Group 2011). When this is achieved, their sustainability program often becomes an important aspect of corporate branding and a powerful marketing tool.

For example, Walmart's "Sustainability 360" program is underpinned by the three broad goals of (1) being supplied 100% by renewable energy; (2) creating zero waste and (3) selling products that sustain our resources and the environment. The company's 2011 Global Responsibility Report highlights its holistic approach to sustainability, stressing that "Sustainability 360 lives in every corner of our business – from associate job descriptions to our interactions with suppliers – and guides our decisions based on improving the environment, supply chain and communities where we operate and source" (Walmart 2011). Similarly, the Starbucks website includes extensive reporting on the corporation's sustainability and corporate social responsibility initiatives. Starbucks emphasises in its corporate literature its Environmental Stewardship initiatives, focused on the long-term sustainability of coffee-growing communities, as well as the company's green building, energy and water conservation and recycling program (Starbucks Corporation website).

Successfully integrating sustainability into all areas of a business is easier said than done, however, since this will often require extensive changes not only in the way the business is organized and its strategic goals, but in corporate culture and the attitudes and mind sets of organizational leaders, employees and other stakeholders. This type of holistic approach to sustainability involves what Svenssen and Wagner (2011) refer to as a "transformative" approach involving the "achievement of genuine and continuous business sustainability and *awareness* at strategic, tactical and operative levels of business" (italics added). Becoming a sustainable organization is likely to involve extensive and inter-related changes across virtually all organizational functions and areas of activity, including but not confined to strategic planning, finance, product and service design, procurement and supply chain management, management and leadership, operational work, customer relations and human resource management. The ability to successfully execute these changes will depend heavily on the commitment and engagement of people involved with or affected by them. Like other major organisational transformations, therefore, the implementation of sustainability programs requires an "art and science"-based approach.

To recap, sustainability has been proven to contribute to strong business performance when it is adopted in a holistic way throughout all areas of organizational practice and culture. The changes needed to achieve this require a combination of art and science, as discussed in the following section.

4 The Art and Science of Transformation for Sustainability

The case for art and science in transformation for sustainability is based on evidence from the organisational change literature that business transformations in general are subject to a high rate of failure. This indicates that, on average, less than half of organizational change projects succeed in meeting their objectives, while staying on schedule and within budget. Increasingly, the empirical evidence from employer surveys is that it is the people-related aspects of change, such as weak leadership or difficulties in changing employee attitudes, which are most difficult to achieve and often lead to project failure (Economist Intelligence Unit 2009; IBM Corporation 2008; McKinsey and Company 2010).

The art involved in an organisational change initiative comprises the "softer" skills relating to human behaviour and interactions, as well as the personal attributes so important in business that are often defined in terms such as "acumen" or "intuition". These art skills include, for example, leadership, business acumen, communications and adaptability. In contrast, the science comprises the skills and knowledge required to use formal processes, techniques and tools in planning, implementing and managing organisational change. These include, for example, requirements analysis, risk identification, financial planning and performance measurement. The distinction between art and science can also be conceptualized in terms of "right brain" and "left brain" thinking respectively, as illustrated in Fig. 1. In any transformation initiative, it is essential to achieve the right balance of art and science skills (Fig. 2).

Like other organizational transformations, therefore, sustainability initiatives are subject to a high rate of failure, often due to a lack of attention to the "art" or the people-related aspects of change. However, there is also a need to apply "science" to organizational change, in the form of project management and other specialist tools and techniques. Achieving the right combination of art and science is crucial if sustainability is to be successfully adopted in the holistic way that is needed to contribute to business performance as well as the achievement of positive environmental and social impacts. The importance of combining art and science to achieve transformation for sustainability is discussed further in the following section.

Left Brain - *the "science"*	Right Brain - *the "art"*
Logical	Random
Sequential	Intuitive
Rational	Holistic
Analytical	Synthesizing
Objective	Subjective
Looks at parts	Looks at wholes

Fig. 1 *Left* and *Right* Brain Thinking. The "science" of transformation can be conceptualized in terms of what is often referred to as "left brain" thinking, or a logical, analytical and objective approach. In contrast, the "art" of transformation can be conceptualized as "right brain" thinking or a more intuitive, subjective and holistic approach

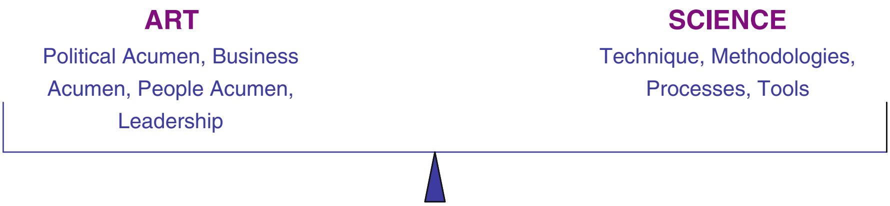

Fig. 2 The *Art* and *Science* of Transformation Scale. In any transformation initiative, such as a sustainability program, it is essential to apply the right combination of "art" and "science" skills. The appropriate balance of art and science will depend on the factors such as the scale of the project and numbers of stakeholders involved, but there is generally a need to combine various methodologies, techniques and tools (science) with people-related skills and the more intuitive abilities often defined in terms of "acumen"

5 Combining Art and Science for Sustainability

As noted above, a holistic approach involving the integration of sustainability into all areas of the organization is important if companies are to gain business benefits from their sustainability programs. First, this requires the application of science skills in systematically reviewing all functional areas and their inter-relationships, identifying sustainability goals and performance criteria, developing implementation plans and identifying roles and responsibilities. While this lays the groundwork for improvements in sustainability, achieving them also involves an extensive range of art skills. For example, these are needed to effectively communicate the objectives of the initiative and the reasons for it to all stakeholders, and to secure and maintain their commitment and contributions to achievement of the desired outcomes. The art of transformation for sustainability will ideally also include strategies for involving the input of employees and other stakeholders in the design of sustainability programs and the identification of specific areas in which sustainability gains can be achieved in order to help meet top-line goals.

The need for art skills in sustainability goes further than this, however: becoming a sustainability-driven organization requires a shift in balance from left-brain to right-brain thinking, particularly in the setting of business objectives and performance measures. This is necessary in order to expand the organisational focus from traditional financial indicators of business success to wider definitions of costs and value incorporating social and environmental dimensions. Traditional business performance measurement has primarily been based on tangible, quantifiable indicators, such as numbers of widgets produced per hour, or turnover per month. However, many of the business benefits of sustainability are more intangible or qualitative in nature, including for example improved public attitudes to the brand or increased employee engagement. On the other hand, the implementation of a sustainability program may even involve increased short-term costs in order to achieve reduced long-term costs and greater profits (Butler et al. 2011). These characteristics of sustainability programs create challenges for traditional science-based accounting and performance management systems, and call for more creative approaches to assessing performance and the increased use of qualitative performance measures. At least until recently, relatively few organisations were successfully achieving this: a 2008 employer survey reported that major barriers to the successful integration of sustainability into financial strategy included the difficulties of measuring its impact on financial performance and shareholder value (cited in Butler et al. 2011).

Overcoming such barriers requires a change in organisational culture and a relative shift in focus from science to art but also an investment in organisational learning and knowledge accumulation about social and environmental issues. Having roles dedicated to the promotion of sustainability can be important way of achieving this; individuals with relevant knowledge and awareness can be appointed to these roles, or provided with suitable training and development; these then act as sustainability "champions", educating other employees and stakeholders through organisational communications and ensuring that sustainability stays firmly on the business agenda. The more senior the champion the better, perhaps: the Aberdeen Group (2008) found evidence that many companies rated "best in class" in sustainability had appointed executive leaders to head their green supply chain initiatives, and these played a key role in communicating progress with internal and external stakeholders, building commitment and improving collaboration in pursuit of their sustainability goals. The "soft" skills of managers and team leaders at all levels of the organisation are also important in contributing to the success of sustainability initiatives, according to a 2010 survey conducted by the International Society of Sustainability Professionals (ISSP), the most important of these include communication skills, the ability to influence, inspire and motivate others, team-building skills and problem-solving (Johnson 2011).

Communications and people-management skills are indeed among the most important factors in ensuring the success of a sustainability program, but it is fundamentally the combination of art and science that is crucial, especially in relation to stakeholder management. Monitoring and securing progress towards sustainability goals in the supply chain can be especially challenging for many major corporations with extensive supply networks. In the case of Walmart, for example, more than

100,000 suppliers globally participate in the Sustainability 360 program, requiring an efficient system for collecting sustainability data and tracking performance against specified sustainability goals. Such systems must be effective not only for monitoring and performance measurement purposes but also to engage and motivate participants to improve sustainability in their business. One approach to this is provided by the example of Proctor and Gamble's Supplier Environmental Sustainability scorecard system, which generates supplier ratings on the basis of performance and can presumably influence the participants' prospects for future business with the corporation (GreenBiz Staff 2010).

External stakeholders play a particularly important role in the success or otherwise of sustainability initiatives. A major corporation's record and reputation on sustainability can be severely damaged if their overseas suppliers are found to be using unethical or illegal working practices, such as child labor, or if the hazardous components in its products are not being safely disposed of after use. Similarly, a company's efforts to introduce recyclable packaging materials may backfire if sales drop because customers dislike the new packet designs, and sustainability plans and policies will be no more than the paper they are printed on unless they are adopted by employees into everyday working practices. Analysis techniques such as stakeholder mapping and SWOT* analysis provide the structures and methods for documenting program-related risks and opportunities, but to identify and understand how to manage those arising from stakeholder interests and concerns requires people-related skills, and the ability to design communications and other measures that will be effective in securing the commitment to and engagement in the program of the respective stakeholder groups (*Strengths, Weaknesses, Opportunities, Threats, see wikipedia). This point applies as much to internal as to external stakeholders: for example, securing the commitment of finance officers to the program is likely to require an emphasis on the expected long-term reduction in cost and increase in profit or efficiencies; where external suppliers are concerned, it will generally be important to stress collaboration and the benefits to them of being associated with the sustainability program, rather than placing too much emphasis on monitoring and scrutinizing their business practices.

The above represent just a few examples of ways in which a combination of art and science are crucial in transformation for sustainability. Evidence of the effectiveness of this approach can be found in the finding of sustainability surveys. For example, those companies that were found to be profiting most from their sustainability programs in the Second Annual Sustainability and Innovation Survey and the Third Annual Sustainability Global Executive Survey were reported to have an analytical approach to sustainability, including the development of a formal business case for their program, and the use of scientific methods such as scenario planning and strategic analysis. At the same time they displayed strong evidence of a change in organisational thinking and culture, with sustainability being regarded as core and being discussed by respondents to the survey in terms of both tangible and intangible benefits such as process improvements, innovation opportunities and business growth (Haanaes et al. 2011; Kruschwitz and Haanaes 2011).

As discussed in this section, it is the combination of art and science that is necessary for the successful implementation of a sustainability initiative. The application of art skills are especially important in communicating the importance of the program to all stakeholders and securing their engagement and commitment to the achievement of sustainability goals, and in the development of new approaches to measuring performance which do not rely exclusively on financial or other quantitative metrics. At the same time, knowledge of and expertise in a range of tools and techniques are important for the effective development, management and monitoring of a program designed for the achievement of business benefits as well as positive environmental and social impacts.

6 Conclusion

In conclusion, organisations in all sectors are facing increasing internal and external pressure to adopt sustainability initiatives, and there is a pressing need for greater awareness and understanding of how to incorporate these in a strategic way into all areas of the organisation. The issue is particularly pressing in agriculture and related industries, which have an especially large environmental footprint and are reported to be responsible for around one third of man-made emissions that are contributing to climate change.

However, research indicates that sustainability as an add-on or peripheral "green" program is a risky approach and one which is likely to represent a business cost; in contrast, a holistic program in which sustainability is integrated into all organizational functions and stakeholder groups can be an effective driver of business growth as well as environmental and social benefits. This requires a transformational approach based on the application of art and science, to ensure that sustainability becomes embedded into the structure and culture of the organisation. Since the benefits of sustainability are often long-term and largely intangible, this is essential to ensure continued commitment to a program and minimize the risk of slipping back into "old" ways of doing things.

References

Aberdeen Group (2010) The sustainable supply chain. Downloaded from http://www.aberdeen.com/Aberdeen-Library/6676/RA-supply-chain-sustainability.aspx. Accessed 15 Sep 2012

Brennan L, Binney W, McCrohan J, Lancaster N (2011) Implementation of environmental sustainability in business: suggestions for improvement. Australas Mark J 19:52–57. doi:10.1016/j.ausmj.2010.11.007

Butler JB, Henderson SC, Raiborn C (2011) Sustainability and the balanced scorecard: integrating green measures into business reporting. Manag Account Q 12(2):1–10

Chartered Accountants of Canada, American Institute of CPAs, Chartered Institute of Management Accountants (2010) Evolution of corporate sustainability practices: perspectives from the UK, US

and Canada. Downloaded from http://www.cimaglobal.com/Documents/Thought_leadership_docs/CIMA_AICPA_CICA%20sustainability_report.pdf. Accessed 15 Sep 2012
Dao V, Langella I, Carbo J (2011) From green to sustainability: information technology and an integrated sustainability framework. J Strateg Inf Syst 20:63–79. doi:10.1016/j.jsis.2011.01.002
Deloitte (2010) Sustainability in business today: a cross-industry view. Downloaded from http://www.deloitte.com/assets/Dcom-UnitedStates/Local%20Assets/Documents/IMOs/Corporate%20Responsibility%20and%20Sustainability/us_es_sustainability_exec_survey_060110.pdf. Accessed 15 Sep 2012
Economist Intelligence Unit (2009) The burning platform: how companies are managing change in a recession. Downloaded from http://www.celerantconsulting.com/Downloads/ResearchReviews/Celerant%20-%20EIU_Burning%20platform.pdf. Accessed 15 Sep 2012
Elkington J (1998) Cannibals with forks: the triple bottom line of 21st century business. New Society Publishers, Gabriola Island, BC; Stony Creek, CT
Food and Agriculture Organization of the United Nations. Agriculture and Consumer Protection Department (2001) Agriculture's role in climate change. http://www.fao.org/ag/magazine/0103sp2.htm. Accessed 15 Sep 2012
GreenBiz Staff (2010) P&G launches the latest supplier sustainability scorecard. http://www.greenbiz.com/news/2010/05/12/procter-gamble-launches-the-latest-suplier-sustainability-scorecard. Accessed 15 Sep 2012
IBM Corporation (2008) The enterprise of the future: IBM Global CEO Study 2008. Downloaded from http://www.ibm.com/ibm/ideasfromibm/us/ceo/20080505/. Accessed 15 Sep 2012
Haanaes K, Balagopal B, Arthur D et al (2011) First look: the second annual sustainability & innovation survey. MIT Sloan Manage Rev 52(2):77–83
Hubbard G (2011) The quality of the sustainability reports of large international companies: an analysis. Int J Manag 28(3, Part 2):824–848
Johnson D (2011) Sustainability: spin or substance? ISHN.com 45(5):28–30
Kruschwitz N, Haanaes K (2011) First look: highlights from the Third Annual Sustainability Global Executive Survey. MIT Sloan Manage Rev 53(1):87–89
McKinsey & Company (2010) Global forces: how strategic trends affect your business. Downloaded from http://tophqbooks.com/books/280312. Accessed 15 Sep 2012
MIT Sloan Management Review and the Boston Consulting Group (2011) Sustainability: the "embracers" seize advantage. MIT Sloan Management Review Research Report, Winter 2011
National Geographic (n.d.) Sustainable agriculture. http://environment.nationalgeographic.com/environment/habitats/sustainable-agriculture/. Accessed 15 Sep 2012
Piasecki B (2009) The surprising solution: creating possibility in a swift and severe world. Sourcebooks, Naperville, Ill
Starbucks Corporation website. http://www.starbucks.com/responsibility. Accessed 15 Sep 2012
Svensson G, Wagner B (2011) Transformative business sustainability. Eur Bus Rev 23(4):334–352. doi:10.1108/09555341111145735
Tilman D, Cassman KG, Matson PA, Naylor R, Polasky S (2002) Agricultural sustainability and intensive production practices. Nature 418:671–677. doi:10.1038/nature01014
Walmart (2011) Global responsibility report. Downloaded from http://walmartstores.com/sites/responsibilityreport/2011/. Accessed 15 Sep 2012
Yarett I (2011) The world's green giants, Newsweek 2011 green rankings. Newsweek 158(17):50

Organic Bread Wheat Production and Market in Europe

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Abstract This chapter is a first attempt to analyse bottlenecks and challenges of European organic bread wheat sector involving technical, political and market issues. From 2000, the organic grain market has largely increased in Western Europe. To balance higher consumer demand there is a need to increase organic production by a new transition and technical improvement. Bread wheat is grown in

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a variety of crop rotations and farming systems where four basic organic crop production systems have been defined. Weeds and nitrogen deficiency are considered to be the most serious threat inducing lowest grain yield under organic production. The choice of cultivar, green manure, fertilization and intercropping legumes – grain or forage – are efficient ways to obtain high grain quality and quantity.

The economic viability of wheat production in Europe is also affected by subsidies from European Union agri-environmental programs. Support has been granted to organic farming since the beginning of the 1990s. Direct payments from European regulation combined with premium prices paid by consumers had compensated the lowest crop production. In the European Union, the current cycle of the Common Agricultural Policy (CAP) is due to end in 2013. Discussions are now under way to support the policy for the period 2014–2020. With the increasing consumer awareness of their food, the growth of the organic sector may continue in the near future. However, financial crisis in some countries may lead to stagnation or even decline of consumer demand.

The development of the organic grain sector is actually confronted to others challenges as quality and safety value. Technological ways of milling and baking may also improve baking quality and nutritional value of organic flour, as well as it prevents mycotoxin contamination. For instance, milling process strongly influences flour characteristics. Stone milling improves nutritive value when characteristics remain very stable independently of the milling yield while flour characteristics from roller milling appear very susceptible to the milling yield. The economic efficiency of the bread wheat sector is also influences by the existence of an adequate marketing structure answering consumers' requirements. This paper is a contribution of a multidisciplinary group of researchers involved in AGTEC-Org project using peer review, statistical data and interviews of key actors: farmers, advisers or experts (coreorganic.org/research/projects/agtec-org/index.html).

Keywords Organic agriculture • Food-chain • Bread wheat • Baking quality • Yield • Farming systems

1 Introduction

Since the beginning of the 1990s, organic farming has rapidly progressed in almost all European countries. In 2010, 5.1% of the total utilised agricultural area in the EU member states (EU-27) was cropped organically with nine million hectares (Willer and Kilcher 2012). Compared with 2000 (4.5 million hectares), the organic land has more than doubled in Europe. The organic supply is currently one of the most expanding sectors of the food industry in many European countries. Across Europe, the market for organic food grew substantially from 2000 by more than 8% per year with approximately 19.6 billion € in 2010. However, the importance of organic farming varies across the EU-27 and considerable differences exist in organic food consumption. In the Western European countries around 5% of the total food market is organic whereas the new EU members have a very low consumption of less than 1% (Source Eurostat, Willer and Kilcher 2012). While the organic grain market has developed rapidly, the growth in production has not matched the growth in demand. Consequently, Western European countries have been net importers of cereals, oilseeds, legumes, potatoes and vegetables from Eastern Europe, Australia, Canada and the United States and widespread scarcity of organic cereals led to discernible price increases at both the producer and consumer level. Organic wheat is considered an essential part of the small grains production because of their importance use for human nutrition in Western Europe (Michelsen et al. 1999).

The purpose of this chapter is to analyse bottlenecks and challenges of the organic bread (soft) wheat production and market in Europe. The objectives of this paper are (i) to provide data on existing organic wheat production and market, (ii) to characterise the main organic cropping systems comprising wheat production, (iii) to analyse the profitability of the organic wheat production and (iv) to define the main characteristic of the wheat-flour market in Europe. This paper also highlights the diversification of the organic wheat sector in Europe by analysing wheat production and market in Denmark, Italy, Austria, Switzerland and France, key actors in the organic sector in EU-27.

2 Methodology

The result of this paper are based on published, statistical database and on expertise and knowledge obtained through interviews of farmers, advisers or researchers coordinated by the scientific partners of the AGTEC-Org project (http://www.coreorganic.org/research/projects/agtec-org/index.html). The paper presents results from a survey realised in Denmark, Italy, Austria, Switzerland and France together with statistical data and literature reviews.

Statistical data were collected both from European database (EuroStat, IFOAM) and the national statistics of the selected countries. In these dataset, the area, share and growth of organic production, arable land and wheat production have been analysed. The main characteristics of the farming systems such as crop rotation, type and size of animal husbandry, machinery used, crop management and performance of organic wheat has been described both by interviews of farmers and/or key informants and national literature review. The main characteristics of bread wheat-flour sector have been described by interviews of millers, bakers and/or key actors. Twenty-six interviews were done with key informants.

3 Results and Discussion

3.1 Principles of Organic Crop Production

Organic agriculture attempts to sustain ecological balance through the design of farming systems, preservation or establishment of habitats and maintenance of genetic and agricultural diversity (IFOAM 2009). A fundamental strategy of organic farming is the prevention of problems through the careful design and management of the farming systems, rather than the reliance of off-farm interventions to solve problems. The farm design should work with natural ecological processes, taking advantage of soil nutrient and water cycles, energy flows, beneficial organism and natural pest controls. Long-term soil fertility is maintained by promoting soil biological activity and diversity, recycling of farmyard manure and diversifying crop rotation with grains and forage crops. The soil is conserved by ground covers and green manures, and if possible by reduced tillage. A key element is the reliance on farm resources within a relatively closed farming system, rather than depending on inputs that are imported from outside the farm.

In general, organic grain systems are characterized by higher crop diversity and wider crop rotations compared to comparable conventional systems (Stockdale et al. 2002). Within a crop group, diversity is often higher, e.g. cereal patterns are less dominated by winter wheat and winter barley, than in conventional farming. Crop diversification can deliver many simultaneous agronomic benefits (e.g. reduced weed pressure, Bond and Grundy 2001) and ecological benefits as e.g. reduced pest and pathogens incidence (Finckh and Wolfe 2006).

3.2 Organic Production in the European Union (EU)

The total organic area in the EU-27, fully converted and under conversion, increased from 2.3 million hectares in 1998 to 9 million hectares in 2010. This corresponded to an annual average growth rate of nearly 15%. In the surveyed countries, Austria (19%) and Switzerland (11%) have the largest share of total organic area in the total Utilised Agricultural Area (UAA) with more than 10% in 2010 (EuroStat Data 2010). Italy followed with a 8% share. Denmark had 6% share whereas France had one of the lowest share in the EU member states with 3%. France deviated from the other countries having a high contribution in the EU-27 UAA but a low share in the organic area. In EU-27, arable land accounts for 41% of the agricultural area converted to organic farming with more than four million hectares. The main use of the organic arable land is production of cereals (1.7 million hectares representing less than 3% of the total cereal area in Europe), followed by forage cultivation (1.5 million hectares). Pulses, oilseeds and root crops play an important role in organic farming to diversify the crop rotation. Wheat is the most important organic cereal in EU-27 with ca 432.000 ha in production. Durum wheat (109.031 ha) is particularly grown in Italy while large areas for soft wheat production are found in Germany (76.000 ha), France (50.169 ha) and Austria (36.719 ha). From 2000 to 2010, France and Austria have recorded positive growth of more than 25%. Denmark and Germany follows with a share of 12%. The development of organic soft wheat in Italy and Switzerland is low or null while the organic share has largely increased for others products. Concerning the supply of organic products in Europe, Italy accounted for nearly 18% of all organic crops in the EU-27 when the others countries account far less like 9% for France, 6% for Austria and around 2% for Denmark. The largest markets of organic products were Germany, France, UK and Italy. Denmark, Austria and Switzerland had the highest market shares.

3.3 Crop Production Systems in Organic Farming

Until recently, most organic farms included both crops and livestock production. From the beginning of the 1990s, organic agriculture has become much more specialized, and crop rotations have been simplified in arable farming systems. Current organic cropping systems occupy a continuum in a spectrum of intensification and diversification, characterised by different levels of inputs, crop diversity and crop management practices. Four basic types of cropping systems comprising soft wheat production were specified from the survey. These four cropping systems were: mixed cropping systems, organic arable systems in temperate regions, irrigated grain systems and extensive grain systems. The description of each farming system will focus on crop rotation (duration, place of organic wheat), proportion of legumes in the rotation, crop management on organic wheat and economic efficiency

3.3.1 Mixed Organic Farming Systems

Traditionally, the organic farming systems combined livestock (in general dairy production) and crop production within a relatively closed farming system. These systems are common in temperate or Atlantic climates with medium to high precipitation, over 700 mm year^{-1}. The cropping systems are based on a large share of N-fixing crops (more than 40% of the total area) such as grass/clover leys or lucerne, associated with cereals, silage maize or root crops in a long term rotation (8–11 years). Representative crop rotations in mixed organic farms are illustrated in Table 1. The proportion of winter wheat varied from 20 to 30% in the crop rotation.

In order to make maximum use of the large quantities of nitrogen released following forage legumes incorporation, crops with high N-demand as spring or winter cereals are usually grown after these crops. Silage maize or root crops (potatoes or sugar beet) grown at a less favourable position in the crop rotations receive animal manure in large quantities. Diseases are usually prevented by the crop diversity within the crop rotation and the use of robust varieties. Weeds are prevented by crop rotation, preventive measures in the cultivation, e.g. ploughing, and curative measures during growth, e.g. comb weeder. Nonetheless, in the survey weeds were indicated as the main problem experienced in these rotations although the high share of perennial leys (clover/grass) to some extent should eliminate annual species.

The economic efficiency is partly ensured thanks to weak expenditure (no off-farm inputs) with the valorisation of the main crops by livestock. Therefore, the higher labour cost, due to weed and soil tillage operations, is largely compensated by higher product prices.

Table 1 Representative crop rotations including winter wheat in mixed farming systems

Year	Austria[a]	France(1)[b]	Denmark[c]	France(2)[b]
1.	Clover grass	Lucerne	Clover grass	Lucerne
2.	Clover grass	Lucerne	Clover grass	Lucerne
3.	**Winter wheat**	**Winter wheat**	**Winter wheat**	Lucerne
4.	Maize	Spring wheat	Spring oat	**Winter wheat**
5.	Field bean	Pea	Spring barley with undersown clover grass	Spring barley
6.	Triticale	**Winter wheat**		Sunflower
7.	Winter barley	Winter barley with undersown Lucerne		Pea or Lentil
8.				**Winter wheat**
9.				Winter cereal
10.				Pea
11.				**Winter wheat**

Source
[a] Rinnofner et al. (2008)
[b] Gerber and Fontaine (2009)
[c] Source Interviews with Senior scientist Ib S. Kristensen, University of Aarhus & Senior advisor Jens Erik Ørum, Institute of Food and Resource Economics, University of Copenhagen

3.3.2 Organic Arable Farming Systems in Temperate Regions

The farming system representative for particularly Western and Central Europe have progressively increased their parts of grain crops in the organic cropping systems. The livestock production is null in stockless farming systems or very limited compared to the grain production. The poor profitability of leys has diminished their importance while high premium for cereals and root crops have encouraged their cultivation. Current arable organic rotations consist of cereals, grain legumes, root crops or field vegetables and annual leys. As organic markets for new crops develop, crops as sugar beet and oilseeds may be grown more widely. Some farmers also invest in root crops or field vegetables requiring heavy investments but providing presently high net margins. Examples of different crop rotations in arable organic systems are illustrated in Table 2. The proportion of winter wheat varied from 20 to 40% in the crop rotation.

Composted off-farm manures and other organic sources (i.e. green compost, organic fertilizers) may be used to meet crop N demand. In some cases, the introduction of 1 or 2 year fallow with clover grass or lucerne tends to improve N soil fertility-building. These farms are also characterised by frequent cultural practices including soil tillage, more intensive weed and pest control and increased organic fertilizer input. Sowing date, row width and seed rate will influence the incidence of diseases and weeds through manipulation of the canopy structure. The methods of reduced tillage are generally not employed on more intensive

Table 2 Representative crop rotations in arable systems

Year	France(1)[b]	France(2)[b]	Switzerland[c]	Austria[a]
1.	Pea	Pea or Field bean	Ley grass	Field bean
2.	**Winter wheat**	**Winter wheat**	**Winter wheat**	**Winter wheat**
3.	Barley	Winter barley or Triticale	Maize	Maize
4.	Potato or Field vegetable	Sunflower	**Winter wheat** or Spelt	Spelt
5.	(Field vegetable)	**Winter wheat**	Spring barley	Triticale
6.			Ley grass	
7.			Ley grass	

Source
[a] Rinnofner et al. (2008)
[b] Gerber and Fontaine (2009)
[c] Source: interviews from Klaus Steiner, Coordinator Cereal-Pool of Bio Suisse & Bertrand Bollag, category manager of arable crops, Bio-Suisse

managed organic farms because of the benefits of ploughing in burying plant residues and weed seeds.

The economic efficiency is ensured thanks to high performance in yield and quality and premium price for cereals, root crops or field vegetables. These farming systems induced higher labour and operating costs (i.e. fertilization and equipment).

3.3.3 Irrigated Grain Systems

Irrigated organic farms are typically found in Mediterranean or Continental climates with regular water deficit (precipitation <700 mm year^{-1}). These cropping systems are based on a balanced proportion of spring crops, mostly irrigated, as maize, sunflower, soybean or pea associated in the crop rotation (4–6 years) with winter cereals (i.e. wheat, barley, triticale). The crop rotations contain large proportion (more than 30%) of N-fixing legumes as monoculture or intercrop for grain legumes or as cash crop or undersown as grass leys as clover grass. Examples of different crop rotations of irrigated organic farms are illustrated in Table 3. The proportion of winter wheat varies from 20 to 33% in the crop rotation.

The N management is generally based on high rate of N-fixing grain legumes and off-farm organic sources (manures, organic fertilizers or others sources as green waste) for partially meeting the N demand of winter cereals. On some farms, there is currently a risk of unbalanced crop rotations in favour of few profitable crops, e.g. premium quality and high yielding grain, inducing potential development of specialized weed flora. Weed management is ensured combining preventive methods (crop sequence choice, soil tillage management, use of false seedbed technique, use of cover crops) and curative methods, e.g. hoeing and harrowing, on the whole crop rotation (Bàrberi 2002).

Table 3 Representative crop rotations in irrigated grain systems

Year	Italy[a]	France(1)[b]	France(2)[b]
1.	Maize	Maize	Soya or Pea
2.	**Winter wheat**	Soya Bean	**Winter wheat**
3.	Vegetables	**Winter wheat**	Maize
4.	Potato		Fever Bean
5.	Vegetables		**Winter wheat**
6.			Maize

Source
[a]AIAB (Italian Association for Organic Agriculture) and CRA (Council of Researches in Agriculture)
[b]Gerber and Fontaine (2009)

Table 4 Type of crop rotations in extensive organic farming systems

Year	France(1)[b]	France(2)[b]	Italy[a]
1	Luzerne	**Winter wheat**	Durum wheat
2	Luzerne	Sunflower	Sunflower
3	**Winter wheat**	Faba Bean	Durum wheat
4	Winter barley		Pea or Faba bean or Lentil or Lupin
5	Rye + Lentil		
6	Spring barley		

Source
[a]AIAB (Italian Association for Organic Agriculture) and CRA (Council of Researches in Agriculture)
[b]Gerber and Fontaine (2009)

The economic efficiency is mostly ensured thanks to premium price and high yield and quality performance of irrigated crops. The level of external inputs is strongly linked to the farmer's financial capacity.

3.3.4 Extensive Organic Farming Systems

These farmers generally grow a diversity of crops including small grains, oilseed crops and legumes under dry conditions (precipitation <700 mm year^{-1}) without irrigation. Moreover, niche market crops such as aromatic or medical plants and alternative small grains (e.g. kamut, millet) can be found as a means of increasing profit even though it requires management flexibility and presents marketing challenges. Examples of different crop rotations of extensive organic farms are illustrated in Table 4. The proportion of winter wheat varies from 0 to 33% in the crop rotation.

The extensive grain systems are generally based on reduced land management (no tillage, limited weed control) and limited use of external inputs (composted manure or organic fertilizers). The farm management is based on low input regarding mechanization, limited usage of external inputs and/or available family labour. These systems could in general be developed under two contrasting scales; on small-scale family farms - under 10 ha in Southern Europe - but in most cases on large-scale organic farms: over 200 ha in Central and Eastern Europe. The economic efficiency is mostly ensured by limited costs, large scale economies or by added value due to direct marketing, even the average yield is lower than theses ones observed in the organic arable farming systems.

3.4 *Profitability of Organic Wheat in Europe*

The economic viability of wheat production in Europe is clearly affected by the support payments, the technical performance but also by the existence of an adequate marketing structure.

3.4.1 Political Support

Growing consumer demand for organic food and the consequent increase in organic production led to the introduction of Council Regulation (EEC) 2092/91 in 1991. This regulation has been amended on several occasions, in particular in 1999 to cover organic livestock production (N° 1804/99) and in 2007 with the new EU Council regulation, (N° 834/2007) on organic production and labelling emphasizing environmental protection, biodiversity and animal welfare. In the European Union, the current cycle of the Common Agricultural Policy (CAP) is due to end in 2013. Discussions are now under way to reform the policy for the period 2014–2020. Under European Union's agri-environmental programs (Pillar II of the CAP, rural development), support has been granted to organic farming since the beginning of the 1990s. Therefore, the organic sector is also supported by premium prices paid by the consumers. The surveyed countries show large differences in the support payments, both in terms of the levels of payments and eligibility, and policy towards organic farming. For instance, Michelsen et al. (1999) showed that EC 2078/92 payments amounted to approximately 20% of profits in Austria and Denmark, while in France they amounted to only 4% of profits, due to both the relatively low level of the payments and the fact that only converting productions were eligible for payments. Over the last decade, support programmes for organic production, e.g. agri-environmental programmes, subsidies for new equipment, support for certification etc., have been consolidated by national or regional programmes. A further important support measure for organic farming are organic action plans. In 2011, 26 countries and regions in Europe had an action plan, many of them with quantitative targets. Austria, for instance, aimed to have 20% organically managed agricultural land by the end of 2010 an aim that was almost achieved by mid-2010 when 19.7% of the agricultural land was organic. On the opposite, the organic action plan launch in

Table 5 Organic wheat yields (t ha^{-1}) in the surveyed countries

	France	Denmark	Austria	Italy	Switzerland
Grain yield Min value	2.5	1.0	3.0	3.5	3.0
Grain yield Max value	5.0	4.0	5.5	6.0	6.5

France in 2007 aimed to have 10% organically managed agricultural land by the end of 2012 when only 3% of the agricultural land was organic by the end-2010.

3.4.2 Yield, Price and Costs

In Europe, crop yield in organic farming are on average 60–70% of those under conventional management (Padel and Lampkin 1994; David et al. 2005). However, yield differences could be higher for intensive cereal crops such as organic wheat where grain yields varied from 30 to 70% compared to the conventional yields. Table 5 illustrates yield variability in the surveyed countries where conventional wheat yields varied from 6 to 8.2 t ha^{-1} (source Eurostat).

Although some information is available, credible and systematic price information is very scarce and collected mainly by non-government organizations or research institutes. For instance, Hamm et al. (2002) collected data of several markets and reported high price premiums for organic wheat. In the European Union, farmers are receiving premiums for organic wheat from 30% up to 200% depending on countries and sales channels. Prices for organic cereal used for human nutrition can reach 100% above conventional prices, whereas the price increase for animal feed is maximum 30%. Over the last 10 years, these premiums have fluctuated considerably over time in coherence with the large variation observed on the world cereal market. The premia of organic wheat reported from Austria and France are above 150%, about 50% in Switzerland, while the price level for conventional wheat is high, and 30–40% in Denmark. The premium is around 80% in Italy where the domestic market for organic food is not consolidated.

For the consumer, the premium of organic cereals is approximately 60% ranging from 40% for whole wheat to 75% for flour. The farm gate prices also varied between the different marketing channels, with prices realised via direct marketing (weekly market or Community-Supported Agriculture initiatives) often being twice as those received from supermarkets. On the other hand, costs strongly varied for the different farming systems and marketing channels. Reduction of variable costs could be achieved by restricted use and replacement of off-farm inputs, e.g. fertilizers and pesticides, with on-farm sources, e.g. organic manures or N fixed crops. In general, labour costs are 10–20% higher on organic arable farms than on comparable conventional farms (Offermann and Nieberg 1999). Nonetheless, the labour per ha UAA has been reduced in the latest years in organic arable systems because of the increase in farm size and the development of labour-saving technology, e.g. combined harrow and sowing techniques or mechanical weed control. On the

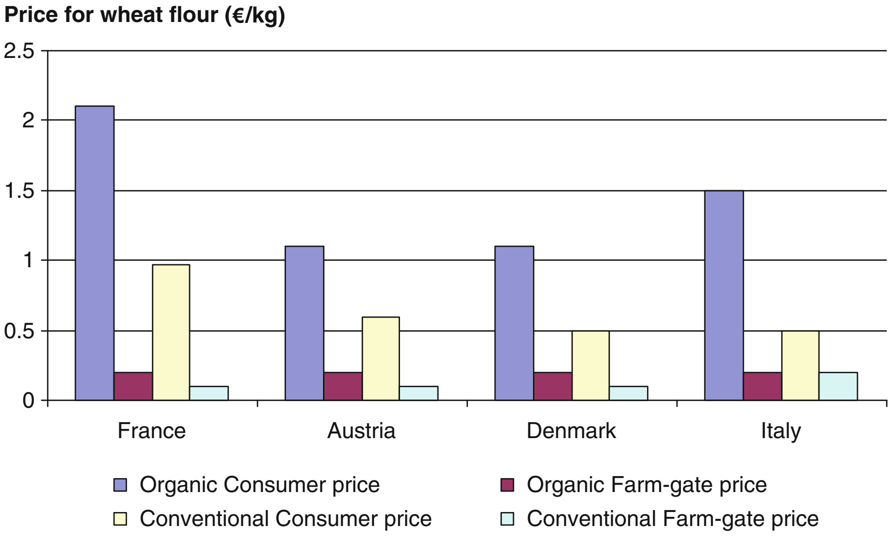

Fig. 1 Farm gate and consumer price for wheat flour in France, Austria, Denmark and Italy (From European Commission 2005)

contrary, family farmers (e.g. extensive grains systems or some mixed farming systems) have maintained their structure according to the family labour force. The greater importance of marketing and processing (e.g. direct sale of wheat flour) on organic farms may imply higher investments. Offermann and Nieberg (2000) concluded that high profits observed in organic arable farms relative to comparable conventional farms were essentially due to high prices offered to organic products. Nonetheless, the large difference between farm-gate and consumer price for organic products strongly limit the development and expansion of the organic cereal sector. Figure 1 shows that in France and Italy, large price increases of organic products are realised within the market chain, but not at farm level.

The key question is whether the relative profitability of organic wheat will still be maintained if the premium paid by consumers is reduced. First, it depends on the combined growth in consumer demand and production level, which should result in upward price pressure on organic foods in comparison to the likely decline in production costs. Secondly, it is also determined by direct policy support compensating yield gap. Finally, the profitability of organic grains should be also guaranteed by the improvement of yield performance compensating the decline of the price premium (IFOAM 2009).

On-farm wheat processing is also a valuable option to improve profitability. Processing can range from cleaning and packaging to milling flour or to producing baked goods from the grains. Adding value also adds additional costs. Depending on the type of activity, the producer may need to make considerable investments in equipment and time. Recently, organic farmers have innovated in new ways of direct selling as Community-Supported Agriculture (CSA) initiatives or farmers shops.

3.5 *The Organic Wheat and Flour Market in Europe*

In the last two decades, the market for organic foods has developed fast throughout Europe. Organic products have been frequently associated with attributes such as local origin and supply, small-scale units of production and direct selling from producer to consumer. Nevertheless, at present organic markets are mainly based on highly industrialised and concentrated units of production, distributed through mainstream retail channels (Mette Wier and Carmen Calverley 2002). The organic wheat and flour market has been diversified over time. In its early days, the organic food market developed outside the conventional sales channels. Traditionally, direct sales from producer to consumer and specialised shops (e.g. La Vie Claire in France, NaturaSi in Italy) provided organic products for target consumers interested in non-conventional products (whole food or healthy food). The quantities of organic wheat-based products sold through these channels were often small, with only small segments of consumers being reached. In the beginning of the 2000s, the development was strongly supported by the wholesalers entering the organic market. Supermarkets were important actors in developing sales through new (occasional or regular) consumers. Therefore, the growing demand of organic cereals (Michelsen et al. 1999) induced the arrival of new operators (e.g. bakers and industries) from the conventional sector. Even though a mix of different sale channels can be found in all European countries, these are not considered of equal importance. For instance, the organic wheat-based market in Switzerland is mostly centralised by two wholesalers (Migros and Coop), specialised shops are dominant in Austria while a combination of the different sales is found in Italy, Denmark and France. The present situation for sale channels in Switzerland and France is shown in Fig. 2. Organic wheat production was traditionally traded mainly inside countries or with neighbouring EU countries. In the recent years, the development of the domestic market in Western European countries has limited exports, mainly from Germany

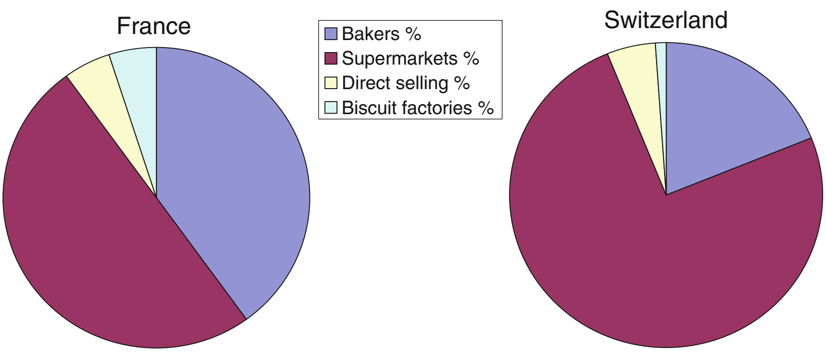

Fig. 2 Rate of the different sale channels of organic wheat-based products in Switzerland and France (Source: Data 2007 of the Federal Statistical Office, Switzerland; Data 2007 for France in David and Joud 2008)

and Italy, and increased imports from other countries. The grain trade has recently increased between Western European countries and Eastern European countries, including important exporters like Romania, Hungary and Czech Republic.

3.6 Technical Issues

Organic farming systems face a number of technical challenges, particularly related to crop nutrition, weed (Bàrberi 2002), pest and disease management (van Bruggen 1995). Organic wheat is affected by regular agronomic and climatic limiting factors (Casagrande et al. 2009; David et al. 2005). Currently, weeds are considered to be the most serious threat in organic wheat production while diseases and pests are of relatively minor concern. In a survey of 32 growers of organic winter wheat, Taylor et al. (2001) reported that the major problem was perceived to be weeds (75%), with diseases and pests to be of relatively minor concern (19 and 9%, respectively). Fear of ineffective weed control is also perceived by the farmers as one of the major obstacles for conversion from conventional to organic production (Albrecht 2005). The productivity of organic wheat is also restricted by the limited supply of N (David et al. 2005; Olesen et al. 2002). By choosing cultivars with good disease resistance, the organic farmer should be able to keep disease problems within acceptable limits. However, the choice of suitable cultivars for organic wheat is still limited, as plant breeding has been largely directed at providing cultivars adapted to conventional farming (Phillips and Wolfe 2005). Moreover, for the organic grower, disease resistance is only one aspect to be considered and choice of cultivars may be strongly influenced by other factors such as grain quality, ability to compete with weeds, N use efficiency and availability of organically produced seed. On-going research currently evaluate performance of composite cross populations compared with pure lines to buffer the population against a wide range of environmental variation recently observed with global climate change (Wolfe et al. 2008).

Organic cereal production has a greater reliance on organic sources and internal cycling of N (Watson et al. 2002; Dawson et al. 2008) than conventional cropping. The absence or limited proportion of forage or cash crop legumes in some farming systems as well as the decline of animals on farm increase the risk of N deficiency on crops with high N-demand as winter wheat. Changes in the regulation of organic standards have limited the use of fresh manure or slurry from conventional farms. As an example, current regulations on organic farming in Denmark allow arable farmers to add up to 70 kg total N total ha^{-1} in form of conventional manure. Swiss farmers are not longer to apply conventional manure causing regular N deficiency. The lack of synchronization of crop nitrogen requirements and availability of soil nitrogen (N) from organic matter and materials, such as crop residues, green manures and composts, is known to affect wheat yield and quality. There is a crucial challenge to improve N use efficiency through genetic improvement (Le Gouis et al. 2000; Dawson et al. 2008), to adapt fertilizer management for organic fertilizers and, finally, to develop decision-support systems for N management (David et al. 2005).

Photo 1 Undersowing clover in winter wheat (Source F Celette)

Innovative cropping systems have to be designed to reduce the need of external inputs and limit energy consumption. For instance, cereal-legume mixed crops are often used in organic farming as an efficient way to reduce the need of external N inputs (Jensen 1996). Legumes and non-legumes may complement each other in the use of N sources since although both use soil inorganic N sources (Corre-Hellou et al. 2006; Gooding et al. 2008). In cereal-grain legume intercrops, the satisfaction of grain N demand is improved by increasing soil N availability per plant while legumes rely mainly on N_2 fixation. Cereal-legume mixed crops are also better competitive against weeds than legume sole crops (Hauggaard-Nielsen et al. 2001) (Photo 1).

Spatial diversification by intercropping systems can provide benefits, summarized by Vandermeer (1989) and Malezieux et al. (2008), as increased productivity and stability, better use of resources, reduction of losses due to pests, diseases and weeds, and higher economic gain.

In organic farming systems, frequent mechanical treatments (seedbed preparation, weed control, N application) could increase risk of soil compaction and loss of soil microbial activity whereas soil quality is essential to preserve long term fertility. The adoption of conservation tillage may on some soils decrease mechanical treatments and improve soil fertility (Peigné et al. 2007).

3.7 Mycotoxin Contamination

The presence of mycotoxins is a major concern for wheat growers. In conventional agriculture, fungicides applied at specific times during grain development may reduce the occurrence of the mycotoxin producing pathogens *Fusarium* spp. In organic systems, specific control strategies include diverse crop rotation with limited cereal proportion, frequent ploughing, reduced nitrogen fertilisation levels, selection of resistant genotypes and the use of variety mixtures with variable flowering dates to limit risk of ear disease. Organic wheat might be expected to be more susceptible to mycotoxins because it is not treated with fungicides. However, several authors (Birzele et al. 1998; Schollenberger et al. 2002; Vanova et al. 2008) found that the concentration of deoxynivalenol (DON) in organically produced wheat was not greater than the DON concentration in wheat produced by integrated or conventional management. The degree of toxin contamination of wheat flour increases with increasing ash content (Trigo-Stockli et al. 1996). Since the fungal growth is concentrated in the hull, the processing methods that remove different outer layers of the grain may reduce the degree of mycotoxin contamination.

3.8 Bread Making Quality of Organic Wheat

When comparing organic and conventional wheat, various authors have shown lower protein content (Gooding et al. 1993; Poutala et al. 1994; Woese et al. 1997; Carcea et al. 2006; Mäder et al. 2007; Zörb et al. 2009), gluten, dough mixing tolerance, and loaf volume (Feil and Stamp 1993; Annett et al. 2007). Haglund et al. (1998) emphasized that, regardless of the cultivation system used, wholemeal flour with protein content lower than 12% required less mixing for optimum dough development. As a consequence, small-scale bakers adapt their baking processes to organically grown flours with low protein content. Additionally, organic flours are more frequently used for wholemeal bread than conventional flours. Requirements regarding proteins contents are less for whole-meal products than for refined white flour. Although organic agricultural practices vary widely, genotype selection and optimum management of N fertilization can yield wheat protein content similar to that obtained with synthetic fertilizers (Gooding et al. 1993; Salomonsson et al. 1995; Kleijer and Schwärzel 2006). For organically grown wheat, several authors (Salomonsson and Larsson-Raznikiewicz 1985; Lu et al. 2005; Zörb et al. 2009) attributed the decrease of acetic acid insoluble glutenins to N shortages in the heading period before flowering. During this period, the mineralization of compost or residues from N fixing preceding crop was too low and resulted in lower-than-expected elastic properties and loaf volume. Late, organic fertilization with a low level of N (under 100 kg N ha^{-1}) may give more gluten and higher loaf volume if the N input is efficiently used (Wirries and Biining-Pfaue 1995; David et al. 2004). Besides protein quantity, protein quality and baking performance could be affected by N nutrition. Technological ways of milling and baking process may also influence baking quality of organic bread. For instance, milling yield strongly influences flour characteristics and baking value of roller milled flour (unpublished data from Agtec-Org).

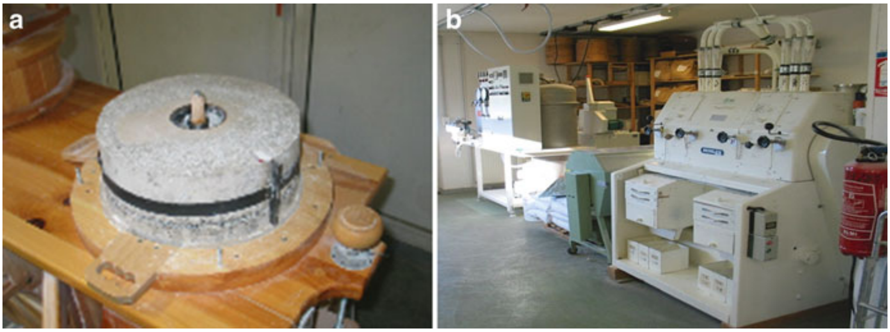

Photo 2 (**a**) Stone miller (**b**) Roller miller (Source INRA)

3.9 Nutritive Value

In several countries, consumption of bread made from organic wheat is linked to the consumption of whole-meal bread (100% flour yield from grain). The nutritive value of whole meal is generally considered superior to that of refined flour. AGTEC-Org project demonstrated that stone milling improved nutritive value when characteristics remain very stable independently of the milling yield (Photo 2).

Whole wheat grains are an excellent source of dietary fibre and various antioxidants (flavonoids, phenolic acids, phytic acid, selenium, and vitamin E) because these compounds are concentrated in bran and germ (Decker et al. 2002; Stracke et al. 2009). However, more research is needed to evaluate the effect of health beneficial secondary metabolites in organic cereals.

4 Conclusion

The production of organic bread wheat develops much slower than the demand from consumers in Europe. From the late 1990s, demand for organic bread wheat coupled with inadequate supply has resulted in record-level prices and significantly increased imports. The organic bread wheat production should be secured from domestic sources when imports from outside EU may introduce additional levels of economic risk associated with transportation and quality.

Wheat yield in organic farming varied from 30 to 70% of yield of conventional cropping when higher premia for organic wheat may to some extent compensate for this. To maintain and expand organic wheat production, the crop management needs to be optimized and the grain yield should be improved to become economically more attractive to the farmers. First, there is a crucial need to maintain diversity within cropping system, to support ecological health, control weeds and improve N management by N fixing crops, while organic farmers are increasingly being pressured by forces of the market to re-adopt the factory principles of specialization and control (Kirchenmann 2000).

Bread wheat is grown in a variety of crop rotations and farming systems. Wheat offers greatest comparative advantage relative to other crops, because of its ability to control weed seed germination and improve soil quality through its deep rooting growth habit and high root biomass. Nonetheless, it is essential to manage nutrient nutrition and control annual and perennial harmful weeds. Premium price for special quality can be obtained by optimized choice of genotype, crop rotations and adjusted organic fertilizer inputs. In order to participate in the premium price of organic wheat products, farmers may get involved in further processing and marketing.

There are no indications of increased risk of mycotoxin contamination in organic wheat but different baking characteristics of organic wheat may require adaptation of other baking processes than with conventional wheat. Organic wheat producers will have to fulfil the technological needs of bakers; even the requirements are diverse, in Europe, from small artisan bakers to large enterprises. Then, there is a need to investigate the effect of different growing regimes and technological processes on baking quality and performance.

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References

Albrecht H (2005) Development of arable weed seedbanks during the 6 years after the change from conventional to organic farming. Weed Res 45:339–350

Annett LE, Spaner D, Wismer WV (2007) Sensory profiles of bread made from paired samples of organic and conventionally grown wheat grain. J Food Sci 72:S254–S260

Bàrberi P (2002) Weed management in organic agriculture: are we addressing the right issues? Weed Res 42:177–193

Birzele B, Backes F, Berleth M, Kramer J (1998) Mycoflora, DON and OTA contents of winter wheat in the years 1995 until 1997. Rev Med Vet (Toulouse) 149:527–535

Bond W, Grundy AC (2001) Non-chemical weed management in organic farming systems. Weed Res 41:383–405

Carcea M, Salvatorelli S, Turfani V, Mellara F (2006) Influence of growing conditions on the technological performance of bread wheat (*Triticum aestivum* L.). Int J Food Sci Technol 41(Suppl 2):102–107

Casagrande M, David C, Valantin-Morison M, Makowski D, Jeuffroy M-H (2009) Factors limiting the grain protein content of organic winter wheat in south-eastern France: a mixing-model approach. Agron Sustain Dev 29:565–574

Corre-Hellou G, Fustec J, Crozat Y (2006) Interspecific competition for Soil N and its interaction with N_2 fixation, leaf expansion and crop growth in pea-barley intercrops. Plant Soil 282: 195–208

David C, Joud S (2008) Etat des lieux de la collecte du blé biologique panifiable en France. Ind des céréales 159:21–26
David C, Jeuffroy MH, Laurent F, Mangin M, Meynard JM (2004) The assessment of a decision making tool for managing the nitrogen fertilization of organic winter wheat. Eur J Agron 23:225–242
David C, Jeuffroy M-H, Henning J, Meynard J-M (2005) Yield variation in organic winter wheat: a diagnostic study in the southeast of France. Agron Sustain Dev 25:213–223
Dawson JC, Huggins DR, Jones SJ (2008) Characterizing nitrogen use efficiency in natural and agricultural ecosystems to improve the performance of cereal crops in low-input and organic agricultural systems. Field Crops Res 107(2):89–101
Decker E, Beecher G, Slavin J, Miller HE, Marquart L (2002) Whole grains as a source of antioxidants. Cereal Foods World 47:370–373
European Commission (2005) Organic farming in the European Union. Facts and figures, 30 p. http://ec.europa.eu/agriculture/organic/files/eu-policy/data-statistics/facts_en.pdf
Feil B, Stamp P (1993) Sustainable agriculture and product quality: a case study for selected crops. Food Rev Int 9:361–388
Finckh MR, Wolfe MS (2006) Diversification strategies. In: Cooke BM et al (eds) The epidemiology of plant disease. Springer, Dordrecht, pp 269–308
Gerber M, Fontaine L (2009) Les grandes cultures biologiques en France: Etat des lieux des rotations pratiquées par region. ITAB Doc, 52 p
Gooding MJ, Davies WP, Thompson AJ, Smith SP (1993) The challenge of achieving breadmaking quality in organic and low input wheat in the UK – a review. Asp Appl Biol 36: 189–198
Gooding M-J, Kasynova E, Ruske R, Hauggaard-Nielsen H, Jensen E-S, Dahlmann C, von Fragstein P, Dibet A, Corre-Hellou G, Crozat Y, Pristeri A, Romeo M, Monti M, Launay M (2008) Intercropping with pulses to concentrate nitrogen and sulphur in wheat. J Agric Sci 145(5):469–475
Haglund A, Johansson L, Dahlsted L (1998) Sensory evaluation of wholemeal bread from ecologically and conventionally grown wheat. J Cereal Sci 27:199–207
Hamm U, Gronefeld F, Halpin D (2002) Analysis of the European market for organic food. Organic market initiatives and rural development, vol 1. School of Management and Business, University of Wales, 129 p
Hauggaard-Nielsen H, Ambus P, Jensen ES (2001) Interspecific competition, N use and interference with weeds in pea-barley intercropping. Field Crop Res 70:101–109
IFOAM (2009) In: Niggli U, Slabe A, Schmidt O, Halberg N, Schlüter M (eds) Vision for an organic food and farming research agenda for 2025. Organic knowledge for the future. FIBL, Frick, p 46
Jensen ES (1996) Grain yield, symbiotic N_2 fixation and interspecific competition for inorganic N in pea-barley intercrops. Plant Soil 182:25–38
Kirchenmann F (2000) In: Allard G, David C, Henning J (eds) Organic agriculture faces its development: the future issues. INRA Editions, Paris, pp 63–74
Kleijer G, Schwärzel R (2006) G. Qualité boulangère du blé d'automne dans les essais d'homologation extenso et bio. Rev Suisse Agric 38:31–34
Le Gouis J, Beghin D, Humez E, Pluchard P (2000) Genetic differences for N uptake and N utilisation efficiencies in winter wheat12. Eur J Agron 12(3–4):163–173
Lu C, Hawkesford MJ, Barraclough PB, Poulton PR, Wilson ID, Barker GL, Edwards KJ (2005) Markedly different gene expression in wheat grown with organic or inorganic fertilizer. Proc R Soc B 272:1901–1908
Mäder P, Hahn D, Dubois D, Gunst L, Alföldi T, Bergmann H, Oehme M, Amado R, Schneider H, Graf U, Velimirov A, Fließbach A, Niggli U (2007) Wheat quality in organic and conventional farming: results of a 21 year field experiment. J Sci Food Agric 87:1826–1835
Malezieux E, Crozat Y, Dupraz C, Laurans M, Makowski D, Ozier-Lafontaine H, Rapidel B, de Tourdonnet S, Valantin-Morison M (2008) Mixing plant species in cropping systems: concepts, tools and models. Agron Sustain Dev 28:1–20

Michelsen J, Hamm U, Wynen E, Roth E (1999) The European market for organic products: growth and development. In: Dabbert et al (eds) Organic farming in Europe: economics and policy, vol 7. Universität Hohenheim, Hohenheim
Offermann F, Nieberg H (1999) Economic performance of organic farms. In: Dabbert et al (eds) Organic farming in Europe: economics and policy, vol 5. Universität Hohenheim, Hohenheim
Olesen JE, Petersen BM, Berntsen J, Hansen S, Jamieson PD, Thomsen AG (2002) Comparison of methods for simulating effects of nitrogen on green area index and dry matter growth in winter wheat. Field Crops Res 74:131–149
Padel S, Lampkin N (1994) Farm-level performance of organic farming systems: an overview. In: Lampkin N, Padel S (eds) The economics of organic farming. CAB International, Wallingford, pp 201–221
Peigné J, Ball B, Roger-Estrade J, David C (2007) Is conservation tillage suitable for organic farming? A review. Soil Use Manage 23(2):129–144
Phillips SL, Wolfe MS (2005) Evolutionary plant breeding for low input systems. J Agric Sci 143:245–254
Poutala RT, Kuoppamaki O, Korva J, Varis E (1994) The performance of ecological, integrated and conventional nutrient management systems in cereal cropping in Finland. Field Crops Res 37:3–10
Rinnofner T, Freyer B, Friedel JK (2008) Nachhaltige Fruchtfolgesysteme für den Energiepflanzenanbau. Division of Organic Farming, University of Natural Resources and Applied Life Sciences, Vienna
Salomonsson L, Larsson-Raznikiewicz M (1985) Quantitative and qualitative studies of proteins of wheat from different growing systems. Swed J Agric Res 15:133–138
Salomonsson L, Salomonsson AC, Olofsson S, Jonsson A (1995) Effects of organic fertilizers and urea when applied to winter wheat. Acta Agric Scand Sect B Soil Plant Sci 45:171–180
Schollenberger M, Suchy S, Terry Jara H, Drochner W, Miiller H-M (2002) Fusarium toxins in wheat flour collected in an area in southwest Germany. Int J Food Microbiol 72:85–89
Stockdale EA, Shepherd MA, Fortune S, Cuttle SP (2002) Soil fertility in organic farming systems – fundamentally different? Soil Use Manage 18:301–308
Stracke BA, Eitel J, Watzl B, Mäder P, Rüfer CE (2009) Influence of the production method on phytochemical concentrations in whole wheat (*Triticum aestivum* L.): a comparative study. J Agric Food Chem 57:10116–10121
Taylor BR, Watson CA, Stockdale EA, McKinlay RG, Younie D, Cranstoun DAS (2001) Current practices and future prospects for organic cereal production: survey and literature review. HGCA Research review no. 45. Home-Grown Cereals Authority, London, UK
Trigo-Stockli DM, Deyoe CW, Satumbaga RF, Pedersen JR (1996) Distribution of deoxynivalenol and zearalenone in milled fractions of wheat. Cereal Chem 73:388–391
Van Bruggen AHC (1995) Plant disease severity in high-input compared to reduced-input and organic farming systems. Plant Dis 79(10):976–984
Vandermeer JH (1989) The ecology of intercropping. Cambridge University Press, Cambridge/Melbourne
Vanova M, Klem K, Misa P, Matusinsky J, Hajslova J, Lancova K (2008) The content of Fusarium mycotoxins, grain yield and quality of winter wheat cultivars under organic and conventional cropping systems. Plant Soil Environ 54(9):395–402
Watson CA, Myrbeck A, Løes A-K, Salomon E, Schroder J, Bengtsson H, Stockdale EA (2002) A review of farm-scale nutrient budgets for organic farms in temperate regions. Soil Use Manage 18:264–273
Wier M, Calverley C (2002) Market potential for organic foods in Europe. British Food Journal 104(1):45–62
Willer H, Kilcher L (eds) (2012) The world of organic agriculture – statistics and emerging trends 2012. FIBL/IFOAM, Bonn, p 338
Wirries FM, Biining-Pfaue H (1995) Wheat from organic agriculture. An improved analytical description of gluten quality. In: Sontag G, Pfannhauser W (eds) Current status and future trends in analytical food chemistry. Proceeding of the 8th European conference on food

chemistry (Euro Food Chem VIII) (Euro Food Chem VIII), vol 2. Austrian Chemical Society, Vienna, pp 527–530

Woese K, Lange D, Boess C, Bogl KW (1997) A comparison of organically and conventionally grown foods – results of a review of the relevant literature. J Sci Food Agric 74:281–293

Wolfe M, Boyd HE, Clarke S, Haigh ZEL, Jones H (2008) Wheat populations: population performance and stability in organic and non-organic environments. In: Proceeding of the 2d ISOFAR conference, Modena, June 2008, pp 676–679

Zörb C, Betsche T, Langenkämper G (2009) Search for diagnostic proteins to prove authenticity of organic wheat grains (*Triticum aestivum* L.). J Agric Food Chem 57:2932–2937

Organic Farming of Vegetables

Margit Olle and Ingrid H. Williams

Abstract Agriculture began organically. For many centuries, humans farmed without synthetic biocides or inorganic fertilizers, relying on organic fertilizers derived from plants and animals, and protecting crops from pests and diseases using naturally-occurring materials. From the second half of the nineteenth century growers around the world successfully developed and refined farming systems that relied on synthetic biocides or inorganic fertilizers. However, during the past two or three decades there has been a change once more towards organic cultivation. Here we review the effects of organic cultivation on the production quantity and quality characteristics of vegetables. Analyses of studies reported in the literature showed the following: (1) Organic cultivation affected the growth of vegetables positively in 43% of studies and negatively in 57% of studies. (2) Organic cultivation affected the yield of vegetables 59% positively, 29% negatively and 12% did not have any significant influence. (3) Organically grown vegetables have, in most studies (65%), better nutritional value than conventionally grown ones; 20% were not significantly different and only in 15% was there a reduction in nutritional value. Nitrate levels were lower in 86% of studies with organic cultivation and greater in only 14% of studies. (4) Organic cultivation of vegetables uses a variety of methods for disease and insect control: hot water, hot air and electron treatment, biological seed treatment groups like microorganisms, plant extracts and inducers of resistance, solarization for nematode control, biopesticides, insect net. (5) Weed control is the most difficult part of vegetable production in organic cultivation. (6) Efficient methods against weeds are tillage, mulching, flaming, hot water treatment. If the

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proper technology is used, the organic cultivation of vegetables is not so time- and money-consuming and produces vegetables of better quality and nutritional value with no pesticide residues.

Keywords Growth • Nutritional quality • Organic cultivation • Plant protection • Vegetables • Weeds control • Yield

1 Introduction

Agriculture began organically. For many centuries, humans farmed without synthetic biocides or inorganic fertilizers, relying on organic fertilizers derived from plants and animals, and protecting crops and animals from pests and diseases using naturally-occurring or minimally-processed materials (Kristiansen 2003). In 1842, Lawes started the first factory for the manufacture of artificial fertilisers in the UK, and, in 1843, he together with the chemist Gilbert, initiated the first of a series of long-term field experiments to measure the effect on crop yields of inorganic and organic fertilisers; some of these continue to this day at Rothamsted Research. Together Lawes and Gilbert laid the foundations of modern scientific agriculture and established the principles of crop nutrition. Throughout the following century, growers around the world successfully developed and refined farming systems that relied increasingly on synthetic biocides or inorganic fertilizers. During the past two or three decades the situation has begun to change. Increasing concerns about food quality, farm worker health, rural development, and the environmental impacts of farming systems, for example, have focused the attention of policy makers, consumers, researchers and farmers on alternative productions systems, including organics (Kristiansen 2003).

Organic farming is estimated to be growing at 30% a year worldwide in response to market forces (Ashley et al. 2007). The demand for certified organic produce, especially vegetables, currently exceeds supply and, in many cases, produce attracts premium prices (Ashley et al. 2007). Organic agricultural production is undergoing a rapid transformation as the demand for healthier food and more environmentally-sound production increases globally. Large producers are adopting organic practices to meet the growing demand (Smukler et al. 2008).

Organic fertilizers offer many benefits for horticulture. Firstly, they release nutrients slowly, which means that plants are fed over a long period. Secondly, this slow release avoids over-feeding thus avoiding providing too much nitrogen, phosphorous or potassium, all of which can be harmful in too high doses. Thirdly, they create a good environment for beneficial soil organisms, such as earthworms, which improve the soil structure by incorporating organic matter well down into the topsoil creating drainage and air tunnels while so doing (Stein 2009).

In organic farming, a number of methods are used to maintain soil fertility. These include: (1) crop rotation, which ensures that one crop does not deplete the soil of the nutrients that it uses most; (2) cover crops to protect against soil erosion; (3) the planting of special crops known as “green manures” that are ploughed back into the soil to enrich it; and (4) the addition of aged animal manures and plant wastes, also known as compost, to the soil. The distinguishing feature of these fertility management practices is the addition of organic matter to the soil, in the form of plant and animal wastes, to preserve soil structure and provide food for soil microorganisms. With these methods, plant nutrients are released slowly to the soil over time (Worthington 2001).

Well-managed organic vegetable production systems can provide food security and a healthy diet for humans, while being less harmful to the environment and more efficient in natural resource use. According to the online literature survey by Juroszek and Tsai (2008), tomato is the vegetable most commonly researched in organic farming, followed by lettuce, carrot and cucumber.

The objective of this review is to give an overview of the effects of organic cultivation on the production quantity and quality characteristics of vegetables.

2 Germination and Growth

A germination survey of the first crop of radish showed that the germination ratio was lower in an organic plot than in a plot with reduced agricultural chemicals (Akira et al. 2003). The second germination survey compared mulch; organic mulch was not practical because it resulted in a low germination ratio (Akira et al. 2003).

Lettuce seedlings grown with organic fertiliser showed an increased growth rate compared to those grown with traditional fertiliser. By contrast, cabbage and cauliflower seedlings grown with organic fertiliser showed a reduced growth rate (Botrini et al. 2004). The optimum pH of growing media for lettuce in organic cultivation was close to 6 and the optimum electrical conductivity (EC) was lower than 2 dSm^{-1}. Increasing pH and especially EC reduced growth, and conductivity about 3 dSm^{-1} or higher had an inhibitory effect on growth when pH was about 7 or higher (Loncaric et al. 2009). The impact of row covers on cucumber plant growth was significant. Use of row covers increased vine length, flower count, leaf area and leaf count (Nair and Ngouajio 2010). Soil amendment treatments consisted of combinations of the following: poultry compost, poultry litter, dairy compost, dairy manure, blood meal, feather meal, and Fertrell™ 5-5-3. Poultry compost resulted in the greatest plant growth in all trials (Rauton 2007). Overall, organic cultivation positively affected vegetable growth in 43% of studies from the literature and negatively affected growth in 57% of studies.

3 Yield

Application of commercial organic fertilizer at the current recommended level of 151 kg/ha resulted in higher vegetable yields than application of commercial inorganic fertilizer at the current recommended level of 660 kg/ha. The Ca and Mg

Table 1 Effect of compost and mineral fertilizer treatments on the average yield and nitrates in cucumbers harvested in 2007 and 2008

Treatment	Fruit yield kg per plot (20 m^2)	Nitrates in fruits (mg/kg)
100% mineral N	112.5	92.7
100% mixed compost	10.8	0.0
100% plant compost	37.5	0.0
100% animal compost	10.5	0.0
F test L.S.D 0.05	**1.71	**1.85

**Significant between treatments at $p<0.05$ (Mahmoud et al. 2009)

Table 2 Annual yields (average value and standard deviation) for integrated and organically-grown vegetables on sandy loam soil (*L*) and sand (*A*) during 1994–1998

Crop	Yield (integrated) (t/ha)	Yield (organic) (t/ha)	(Organic/integrated) yield ratio (%)
Cabbage (loam soil)	51.3 ± 10.5	36.1 ± 7.4	70
Carrot (sand)	47.2 ± 13.7	38.8 ± 12.8	82
Pea (loam soil)	4.5 ± 1.2	3.6 ± 1.2	80
Pea (sand)	2.8 ± 0.6	2.4 ± 0.4	85
Potato (sand)	31.4 ± 5.0	27.6 ± 7.1	88

Fjelkner-Modig et al. (2000)

in the organic fertilizer, which are absent from the inorganic fertilizer, could have contributed to the higher yield (Alimi et al. 2007). Organic treatments resulted in higher carrot root production compared to conventional treatments (Bruno et al. 2007). Lettuce yield was significantly higher when organic fertilization was used, compared to a mineral nutrient supply (Porto et al. 2008). Cabbage and tomato yields produced in the organic system were higher than those in the conventional system (Ma et al. 2009). Applications of compost led to higher marketable yields of cucumber (Nair and Ngouajio 2010). All the associations of the lettuce and rocket as well as their sole crop had better productive performance under the organic fertilization and the regrowth of rocket increased the agronomic efficiency of the intercropping system (Oliveira et al. 2010). Soil amendment treatments consisted of combinations of the following: poultry compost, poultry litter, dairy compost, dairy manure, blood meal, feather meal, and Fertrell™ 5-5-3; poultry compost resulted in the greatest yield in all trials (Rauton 2007). Yield could be higher in organic cultivation of vegetables because organic management of soil fertility affects soil dynamics and plant metabolism, resulting in differences in plant composition and nutritional quality. Soil that has been managed organically has more microorganisms. These produce many compounds that help plants, including substances such as citrate and lactate that combine with soil minerals and make them more available to plant roots (Worthington 2001).

Mahmoud et al. (2009) obtained lower yields of cucumber from organic treatments than from conventional treatments (Table 1). Similarly organic onions gave lower yields than those grown conventionally (Ashley et al. 2007). Examples of yields of vegetables grown in integrated versus organic production systems are

Table 3 Yield, dry matter, and content of nitrates and nitrites of Monanta carrots from organic and intensive conventional farms in 1997 × ± SD (average value and standard deviation)

Analyzed parameter in relation to fresh mass	Organic 1, n 3 = 10	Conventional 2 , n = 10
Yield in autumn 1997 in t/ha	37.5 ± 14.1 a 4	50.0 ± 23.1 a 4
Dry matter (g/100 g)	11.55 ± 0.84 a	11.10 ± 0.80 a
Nitrates (mg/kg)	52.2 ± 36.8 a	209.7 ± 175.0 b 5
Nitrites (mg/kg)	0.57 ± 0.42 a	1.06 ± 0.42 b

Rembialkowska (2003)
(1) Torun and Plock provinces
(2) Warszawa province
(3) n number of samples; 1 sample = result for 1 farm
(4) Values in the same rows marked with the same letters do not differ significantly
(5) Values in the same rows marked with different letters are statistically significantly different

presented in Table 2 (Fjelkner-Modig et al. 2000). Boteva and Rankov (2007) found that plant mass was greatest in head lettuce varieties, when cultivated on alluvial-meadow soil after fertilizing with 10 t/da farmyard manure, and for celery-lettuce varieties after fertilization with 15 t/da (treatments were: 0, 1, 5, 10, 15 and 20 t/da farmyard manure). Cabbage and cauliflower seedlings grown with organic fertiliser had lower fresh weight compared with traditional fertilization, particularly at the end of growth period (Botrini et al. 2004). The marketable yield of onions grown with chemical fertilizer was 64.5 t ha^{-1}, while that of onions grown with organic fertilizer (applied six times in February and March) was 46.4 t ha^{-1}, i.e. a 28% reduction (Lee et al. 2009). Conventional treatment also gave higher yields than organic in tomato and French bean (Thakur et al. 2010); this may be due to chemical fertilizers containing a few mineral substances, principally nitrogen, potassium and phosphorus and the addition sometimes of trace minerals. These fertilizers dissolve easily in the water present in soil (Worthington 2001).

The foliar application of seaweeds, a mixture of compost tea and argan by-product, and a mixture of seaweeds and Argan by-products had no effect on the quantity of bean, eggplant, tomato and melon crops produced (Bourgol 2005). Spent mushroom (*Pleurotus ostreatus*) cultivation substrate and mature compost amendments showed no significant effect on cucumber production (Ehaliotis et al. 2005). Rembialkowska (2003) found no significant differences in yield of carrots grown with organic vs. conventional cultivation (Table 3).

High application rates of mature compost, however, in a more fertile soil, resulted in yields comparable to those obtained with sheep manure (Ehaliotis et al. 2005); application of sheep manure almost doubled cucumber production compared to the control (non-amended soil), whereas application of plain seaweed reduced it by 40% (Ehaliotis et al. 2005). Four organic transition strategies including tilled fallow, mixed-species hay, low intensity open-field vegetable production and intensive vegetable production under high tunnels, each with and without annual compost amendment, were analyzed for nematode communities and soil properties. In this study, tomato yield under high tunnels plots exceeded yield in other treatments

Table 4 Key items of nutritional and sanitary value of organic compared with conventional vegetables

Increased contents	Reduced contents	Comparable contents
Dry matter	Pesticide residues (mostly absent)	Most minerals
Some minerals (iron, magnesium)	Nitrates	Beta-carotene
Anti-oxidants: Vitamin C (potatoes), Polyphenols in vegetables, Salicylic acid in vegetables		

Lairon (2010)

Table 5 Numbers of studies of organic crops shown to have higher, lower or equal nutrient content compared with conventionally grown crops

Nutrient	Higher		Equal		Lower	
Reviewer	Woese	Heaton	Woese	Heaton	Woese	Heaton
Protein quality	3	–	0	–	0	–
Nitrate	5	0	10	2	25	14
Vitamin C	21	7	12	6	3	0
B-carotene	5	–	5	–	3	–
B-vitamins	2	–	12	–	2	–
Calcium	21	–	20	–	6	–
Magnesium	17	–	24	–	4	–
Iron	15	–	14	–	6	–
Zinc	4	–	9	–	3	–
Minerals	–	7	–	6	–	1
Dry matter	–	10	–	8	–	1

Bordeleau (2002)

potentially due to the season extension and higher N availability (Briar et al. 2011). The high tunnel growing strategy increased tomato yield. The results showed that the type of fertilizer applied significantly affected yield of lettuce (Masarirambi et al. 2010). Organic cultivation affected the yield of vegetables 59% positively, 29% negatively and 12% did not have any significant influence.

4 Nutritional Quality

The nutritional and sanitary value of organic compared with conventional vegetables can be found in Table 4 (Lairon 2010). Table 5 gives results from studies of organic crops shown to have higher, lower or equal nutrient content compared with conventionally grown crops (Bordeleau et al. 2002). For leafy vegetables as well as for root vegetables and tubers, a trend for higher dry matter content in organic foodstuffs has been found while no significant difference has been obtained for fruit vegetables (Lairon 2010). Similarly, organically grown crops had higher dry matter content than integrated grown crops (Fjelkner-Modig et al. 2000). Organically grown cucumber contained higher dry matter (Ma et al. 2009). On the other hand, no statistical difference was observed in the percentage of dry matter among the following different treatments: control (no fertilization), chemical fertilization, chicken manure, cattle manure, worm manure, and organic compost, with the exception of the value observed at the organic compost treatment, which was the lowest (3.7%) (Abreu et al. 2010). Contrary to the findings of Lairon (2010), cabbage and cauliflower seedlings grown with organic fertilizer showed a reduction of dry weight compared with traditional fertilization, particularly at the end of growth period (Botrini et al. 2004).

Regarding vegetables (carrot, beetroot, lettuce, kale, leek, turnip, onion, celeriac and tomato) a trend has been observed for higher levels of iron and magnesium expressed on a nutritional quality and safety of organic food (Lairon 2010). Lettuce seedlings grown with organic fertiliser had a higher N and K uptake than those grown with traditional fertilisation. By contrast, cabbage and cauliflower seedlings grown with organic fertiliser showed a reduction of nutrient content, particularly at the end of growth period (Botrini et al. 2004).

Cabbage and cauliflower seedlings grown with organic fertiliser showed a reduction of chlorophyll content compared with traditional fertilization, particularly at the end of the growth period (Botrini et al. 2004). Regarding water-soluble vitamins, the most studied has been Vitamin C (ascorbic acid), a key vitamin for which higher daily intakes are recommended. Studies on tomato, celeriac and kale showed higher vitamin C levels in organically-grown products. In contrast, no difference was found during studies in leek, carrot or beetroot (Lairon 2010). The vitamin C content of an organic fruit or vegetable is 27% more, on average, than a comparable conventionally grown fruit or vegetable (Worthington 2001). Similarly, in leafy vegetables, leaf concentrations of vitamin C were significantly higher in organic-fertilized than in chemically-fertilized vegetables (Xu et al. 2003). By contrast, the vitamin C content was not influenced by the growing system in the study of Fjelkner-Modig et al. (2000). These results may be due to the vitamin content of a plant depending on a number of factors such as climate, genetic properties, fertilizer and soil. Organically grown cucumber contained higher vitamin C (Ma et al. 2009). Nitrogen from any kind of fertilizer affects the amounts of vitamin C and nitrates as well as the quantity and quality of protein produced by plants. When a plant is presented with a lot of nitrogen, it increases protein production and reduces carbohydrate production. Because vitamin C is made from carbohydrates, the synthesis of vitamin C is reduced also. Because organically managed soils generally present plants with lower amounts of nitrogen than chemically fertilized soils, it would be expected that organic crops would have more vitamin C, less nitrates and less protein but of a higher quality than comparable conventional crops (Worthington 2001). Conventionally grown carrots showed higher content of nitrates than organic carrots (Rembialkowska 2003; Table 3). Mahmoud et al. (2009) found that nitrates were present in conventionally grown cucumber fruits but absent from those grown organically (Table 1).

Table 6 Differences in nutrient content between organic and conventional vegetables: mean percent difference for four nutrients in five frequently studies vegetables

	Nutrient[a]			
Vegetable	Vitamin C	Iron	Magnesium	Phosphorus
Lettuce	+17	+17	+29	+14
Spinach	+52	+25	−13	+14
Carrot	−6	+12	+69	+13
Potato	+22	+21	+5	0
Cabbage	+43	+41	+40	+22

Worthington (2001)

[a] Plus and minus signs refer to conventional crops as the baseline for comparison. For example, vitamin C is 17.0% more abundant in organic lettuce (conventional 100%, organic 117%)

Fat-soluble vitamin and carotenoid contents have been the subjects of some studies. A review of 27 related studies reported β-carotene levels in vegetables with no noticeable differences found overall between organic and conventional foodstuffs. By contrast, a positive relationship between N-fertilization and β-carotene levels has been reported in carrots, while a recent study on organic vs. conventional tomatoes showed higher contents of β-carotene in the former (Lairon 2010). Organically grown cucumber contained higher sugar (Ma et al. 2009). In leafy vegetables, leaf concentrations of sugars were significantly higher in organic-fertilized than in chemical-fertilized vegetables (Xu et al. 2003).

Depending on season, organic vegetables overall may contain at least 30–50% less nitrates than conventional ones (Worthington 2001; Porto et al. 2008; Lairon 2010). This was confirmed by a lower nitrate content of plants when the percentage of organic N increased (Mahmoud et al. 2009). The analysis of the scientific literature showed that, in most of the experiments, nitrate content clearly reduced by using organic procedures (Pimpini et al. 2005). In leafy vegetables, leaf concentrations of nitrate were lower in organic-fertilized than in chemical-fertilized vegetables (Xu et al. 2003).

Muramoto (1999) concluded the following from his research:

1. Nitrate levels exceed the maximum levels specified by European Commission Regulation much more often in conventional than in organic spinach.
2. Nitrate levels tend to be higher in organic spinach grown using guano and Chilean nitrate than in spinach grown using compost.
3. Nitrate levels in spinach are affected by the rate and type of nitrogen fertilizer applied, and also by soil nitrification activity, soil texture, and harvest time.
4. Organic growers may reduce nitrate concentration in spinach using methods such as pre-plant soil nitrate testing, compost based fertility management, afternoon to evening harvest, and petiole removal.
5. Nitrate levels in California-sampled Iceberg and Romaine lettuce are safe regardless of season and farming practice.

Organic crops contained significantly more iron, magnesium, and phosphorus than conventional crops (Worthington 2001). Differences in the nutritional content between organic and conventional vegetables can be seen in Table 6 (Worthington 2001). Plants

Fig. 1 Photos of organic carrot and organic red beet experiments in Estonia in Kurenurme in August 2011. Experiments were carried through on the fields of self-employed entrepreneur Galina Rehkli (Photos are taken by Margit Olle)

produced by bounce back compost were higher in Ca, Fe and Zn contents on a fresh mass basis than plants produced by cattle manure, followed by those produced using inorganic fertilizers and lastly chicken manure (Masarirambi et al. 2010).

Potassium fertilizer can reduce the magnesium content and indirectly the phosphorus content of at least some plants. When potassium is added to soil, less magnesium is absorbed by plants and because phosphorus absorption depends on magnesium, less phosphorus is absorbed as well. Potassium is presented to plants differently by organic and conventional systems. Conventional potassium fertilizers dissolve readily in soil water presenting plants with large quantities of potassium while organically managed soils hold moderate quantities of both potassium and magnesium in the root zone of the plant. Given these plant responses, it would be expected that organic crops would contain larger amounts of magnesium and phosphorus than comparable conventional crops (Worthington 2001).

This study shows that alternative, organic fertilizers have similar or even better positive effects than farmyard manure and that they can contribute to the improvement of the nutritional value of the vegetables produced (Pavla and Pokluda 2008).

Organically grown vegetables have, in most cases (65%), better nutritional value than conventional ones. Fifteen percent of cases showed a reduction in nutritional value and 20% results were not significantly influenced. 86% of results showed a reduction and 14% of results showed an increase in nitrate levels in organic cultivation.

5 Plant Protection

Organic carrot and red beet experiments in Kurenurme, Estonia in 2011 produced very good quality crops with almost no disease and insect attack on plants (Fig. 1). Tomato spotted wilt was the most important disease in the organic tomato cropping system, resulting in smaller plant development, fewer flower clusters and lower yield. In the conventional tomato cropping system, the disease was kept under control, and the population of thrips, the virus vector, occurred at lower levels than in the organic tomato cropping system (Bettiol et al. 2004). Downy mildew incidence was similar between treatments with fertilizer and no fertilizer. Mineral fertilization did not increase downy mildew incidence compared to the organic treatments (Goncalves et al. 2004). Overall, the physical seed treatments (hot water, hot air and electron treatment) resulted in a moderate to good control of the respective diseases. Also, from all biological seed treatment groups (microorganisms, plant extracts and inducers of resistance) candidates with promising control properties could be identified. The influence of the biological methods on emergence was often more prominent under greenhouse than under field conditions (Schmitt et al. 2006).

The nematophagous fungus, *Pochonia chlamydosporia* (Goddard) Zare & Gams, has been investigated as a potential biological control agent for use in integrated pest management strategies for *Meloidogyne incognita* (Kof & White) Chitwood in vegetable crops. The fungus significantly reduced nematode infestations in soil following a tomato crop, in a strategy that combined the use of the fungus with crop rotation (Atkins et al. 2003). Solarization associated with organic fertilization has potential to be used in nematode control and to reduce the need for application of pesticides (Silva et al. 2006).

The incidence of thrips on onions was similar between fertilized and not fertilized treatments. Treatments with mineral fertilization presented the same incidence of thrips on onions when compared to organic fertilization (Goncalves and Silva 2003). Results from one study suggest that a combination of using reflective mulch and host plant resistance can additively suppress whitefly infestations, which have particular importance in the fast-growing organic vegetable production industry (Simmons et al. 2010).

There are control methods for disease and insect control in organic cultivation of vegetables (hot water, hot air and electron treatment, biological seed treatment groups like microorganisms, plant extracts and inducers of resistance, solarization for nematode control, biopesticides, insect net).

6 Weed Control

Weed management is a major constraint in organic production. It can be expensive and time-consuming and severe crop yield losses may be incurred when weeds are not adequately controlled. Research on organic weed management in herb and vegetable production is increasing internationally (Kristiansen 2003).

There are several ways to control weeds in organic vegetable production (Table 7). Nowadays, corn gluten meal is used in organic cultivation systems for weed control (Anonymous 2006). Corn gluten meal is a non-selective pre-emergence or preplant-incorporated herbicide that inhibits root development, decreases shoot length, and reduces plant survival. The development of a mechanized application system for the banded placement of corn gluten meal between crop rows (seed row not treated) has increased its potential use in organic vegetable production, especially

Table 7 Summary of direct (or physical) and indirect (or cultural) weed control practices used in organic herb and vegetable production

Direct/physical methods	Indirect/cultural methods
Tillage: mechanical cultivation of soil before and during the cropping phase	**Rotation**: varying crops, cover crops, fallows and grazing over time
Hand weeding: manually hoeing or pulling weeds	**Cover crops**: green manure or other crop grown in fallow period to suppress weed growth by competition and allelopathy
Mulching: organic materials normally used, use of woven plastic 'weed mat' is restricted	**Prevention**: reducing weed seedling numbers prior to cropping phase, and avoiding weed seed production at all times
Slashing: slashing or mowing using hand-operated or tractor-mounted implements	**Timing**: strategic timing of planting/sowing, tillage, fertilizing and irrigating, plant-back after cover crop
Grazing: a wide range animals used, usually in rotation, rarely within cropping phase (e.g. poultry)	**Planting density**: increased usually, but some crops (e.g. cotton) use wider spacing to allow access for tillage implements
Biological control: classical; inundative, mycoherbicides methods available	**Intercropping**: growing two or more crops in close proximity to improve resource capture
Solarisation: requirements to be effective, limitations (e.g. selective control only)	**Crop and cultivar selection**: sow vs. transplant, growth rate, canopy density & closure
Thermal methods: various flame, steam, hot water, infra red implements used, use of burning is restricted	**Precision placement**: irrigation and fertilizer applied close to crop (e.g. drippers)
	Soil management: modify pH, fertility and specific nutrients

Kristiansen (2003)

in direct-seeded vegetables. This research determined that a corn gluten meal free planting strip (corn gluten meal applied between crop rows) provided increase crop safety for direct-seeded squash compared with broadcast applications. Furthermore, these results have implications for all direct-seeded organic vegetable crops once the optimum corn gluten meal (CGM) application rates and corn gluten meal free strip width can be determined for specific vegetables to maximize crop safety, yields, and weed control efficacy (Webber et al. 2010). Webber and Schrefler (2006) added that the granulated formulation worked well at all application rates and application configurations. The powdered CGM did not flow easily, and its delivery was inconsistent and unreliable when used in the solid application configuration.

In addition more effective methods against weeds can be found: (1) Six methods of stale seedbed preparation were compared on certified and transitional organic land (Boyd et al. 2006). The flamer and the clove oil herbicide had the lowest number of weeds emerging with the crop following stale seedbed formation. (2) Field experiments were conducted from 1998 to 2000 to study the effect of summer cover crop and in-season management system on weed infestations in lettuce. The results indicate that prior summer cover crops can improve both conventional and organic vegetable production systems (Ngouajio et al. 2003). (3) Ammonium nonanoate provided consistent control of weeds across a large range of application volumes.

The results indicate that ammonium nonanoate has excellent potential as an organic herbicide (Webber et al. 2011).

Weed control is the most difficult part of vegetable production in organic cultivation. Good methods against weeds can be: tillage, mulching, flaming and hot water treatment.

7 Conclusion

Findings from the literature suggest the following: (1) The growth of vegetables was affected positively in 43% and negatively in 57% of all studied cases from the literature on organic cultivation. (2) Organic cultivation affected the yield of vegetables 59% positively, 29% negatively and 12% did not have any significant influence. (3) Organically grown vegetables have, in most cases (65%), better nutritional value than conventional ones. Fifteen percent cases showed a reduction in nutritional value and 20% of results were not significantly influenced. 86% of results showed reduction and 14% of results showed an increase in nitrate levels in organic cultivation. (4) Methods are available for disease and insect control in organic cultivation of vegetables (hot water, hot air and electron treatment, biological seed treatment groups like microorganisms, plant extracts and inducers of resistance, solarization for nematode control, biopesticides, insect net). (5) Weed control is the most difficult part of vegetable production in organic cultivation. (6) Good methods against weeds include tillage, mulching, flaming and hot water treatment. If the proper technology is used the organic cultivation of vegetables is not so time- and money-consuming and the trend is that, with organic cultivation, vegetables with better quality and better nutritional value with no pesticides residues can be produced.

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References

Abreu IMO, Junqueira AMR, Peixoto JR, Oliveira SA (2010) Microbiological quality and productivity of lettuce under chemical and organic fertilization. Ciênc Tecnol Aliment, Campinas 30(1):108–118

Akira M, Yasuhiro N, Masahiro T (2003) Development of standard techniques for organic cultivation of vegetables. (3). Fertilization management methods for organic cultivation. B. Effects of organic fertilization on growth and biological properties (radish). Annual report of the Gifu Prefectural Research Institute for Agricultural Sciences in Hilly and Mountainous Areas, pp 261–264

Alimi T, Ajewole OC, Olubode-Awosola OO, Idowu EO (2007) Organic and inorganic fertilizer for vegetable production under tropical conditions. J Agric Rural Dev 1:120–136

Anonymous (2006) Organic and conventional vegetable production explored. Agric Res (April):20–21

Ashley R, Bishop A, Dennis J (2007) Intensive organic vegetable production integrated development. A report for the Rural Industries Research and Development Corporation. RIRDC Publication No 04/121 RIRDC Project No: DAT-37A, 46 pp

Atkins SD, Hidalgo-Diaz L, Kalisz H, Mauchline TH, Hirsch PR, Kerry BR (2003) Development of a new management strategy for the control of root-knot nematodes (*Meloidogyne* spp) in organic vegetable production. Pest Manag Sci 59:183–189

Bettiol W, Ghini R, Galvão JAH, Siloto RC (2004) Organic and conventional tomato cropping systems. Sci Agric (Piracicaba, Braz) 61(3):253–259

Bordeleau G, Myers-Smith I, Midak M, Szeremeta A (2002) Food quality: a comparison of organic and conventional fruits and vegetables, Ecological agriculture. Den Kongelige Veterinœr- og Landbohøjskole, Denmark

Boteva K, Rankov V (2007) Effect of organic and organic-mineral fertilization on yield in some lettuce varieties. Plant Sci 44(6):539–541

Botrini L, Magnani G, Graifenberg A (2004) Effects of organic fertilization on growth and quality of lettuce (*Lactuca sativa* L.) and cabbage (*Brassica oleracea* L.) seedlings. Colt Prot 33(11):71–77

Bourgol A (2005) Effect of foliar fertilization on growth and yield of organic vegetables grown under plastic house in south of Morocco. Thèses et Masters, Centre International de Hautes Etudes Agronomiques Méditerranéennes Bari, 69 pp

Boyd NS, Brennan EB, Fennimore SA (2006) Stale seedbed techniques for organic vegetable production. Weed Technol 20:1052–1057

Briar SS, Miller SA, Stinner D, Kleinhenz MD, Grewal PS (2011) Effects of organic transition strategies for peri-urban vegetable production on soil properties, nematode community, and tomato yield. Appl Soil Ecol 47(2):84–91

Bruno RLA, Viana JS, SilvaVF BGB, Moura MF (2007) Production and quality of seeds and roots of carrot cultivated under organic and mineral fertilization. Hortic Bras 25:170–174

Ehaliotis C, Zervakis GI, Karavitis P (2005) Residues and by-products of olive-oil mills for root-zone heating and plant nutrition in organic vegetable production. Sci Hortic 106(3):293–308

Fjelkner-Modig S, Bengtsson H, Stegmark R, Nystrom S (2000) The influence of organic and integrated production on nutritional, sensory and agricultural aspects of vegetable raw materials for food production. Acta Agric Scand Sect B Soil Plant Sci 50:102–113

Goncalves PAS, Silva CRS (2003) Impact of the organic fertilization on onion thrips incidence. Hortic Bras 21(3):459–463

Gonçalves PAS, Silva CRS, Boff P (2004) Incidence of downy mildew in onion growing under mineral and organic fertilization. Hortic Bras 22(3):538–542

Juroszek P, Tsai HH (2008) Research needs in organic vegetable production systems in tropical countries with a focus on Asia. In: 16th IFOAM organic world congress, Modena, Italy, 16–20 June 2008, 4 pp

Kristiansen PE (2003) Sustainable weed management in organic herb and vegetable production. A thesis submitted for the degree of Doctor of Philosophy of the University of New England, June 2003, 225 pp

Lairon D (2010) Nutritional quality and safety of organic food. A review. Agron Sustain Dev 30:33–41

Lee JT, Ha IJ, Kim HD, Moon JS, Lee SD (2009) Times and frequencies of additional fertilization to improve nutrient efficiency of organic liquid fertilizer for onion organic cultivation. Korean J Hortic Sci Technol 27(1):30–36

Lončarić Z, Vukobratović M, Ragalyi P, Filep T, Popović B, Karalić K, Vukobratović Ž (2009) Computer model for organic fertilizer evaluation. Poljoprivreda (Osijek) 15(2):38–46

Ma CH, Chen JH, Yang RY, Palada MC, Ou SC, Lin YH, Chen LH (2009) Monitoring soil and vegetable quality under six fertilization strategies in organic and conventional farming systems. In: The 9th international conference of the East and Southeast Asia federation of soil science societies, pp 373–374

Mahmoud E, El-Kader NA, Robin P, Akkal-Corfini N, El-Rahman LA (2009) Effects of different organic and inorganic fertilizers on cucumber yield and some soil properties. World J Agric Sci 5(4):408–414

Masarirambi MT, Hlawe MM, Oseni OT, Sibiya TE (2010) Effects of organic fertilizers on growth, yield, quality and sensory evaluation of red lettuce (*Lactuca sativa* L.) 'Veneza Roxa'. Agric Biol J North Am 1(6):1319–1324

Muramoto J (1999) Comparison of nitrate content in leafy vegetables from organic and conventional farms in California. Center for Agroecology and Sustainable Food Systems University of California, Santa Cruz Santa Cruz, CA 95064 831/459-2506, 64 pp

Nair A, Ngouajio M (2010) Integrating rowcovers and soil amendments for organic cucumber production: implications on crop growth, yield, and microclimate. HortScience 45(4):566–574

Ngouajio M, McGiffen MEJ, Hutchinson CM (2003) Effect of cover crop and management system on weed populations in lettuce. Crop Prot 22(1):57–64

Oliveira EQ, Souza RJ, Cruz MCM, Marques VB, Franca AC (2010) Productivity of lettuce and rocket in intercropping system under organic and mineral fertilization. Hortic Bras 28:36–40

Pavla B, Pokluda R (2008) Influence of alternative organic fertilizers on the antioxidant capacity in head cabbage and cucumber. Not Bot Hort Agrobot Cluj 36(1):63–67

Pimpini F, Gianquinto G, Sambo P (2005) Organic vegetable production: evolution, base principles and quality of products. Italus Hortus 12(4):31–44

Porto ML, Alves JC, Souza AP, Araujo RC, Arruda JA (2008) Nitrate production and accumulation in lettuce as affected by mineral nitrogen supply and organic fertilization. Hortic Bras 26:227–230

Rauton RSW (2007) Soil fertility management in organic vegetable production. Partial fulfillment of the requirements for the degree Master of Science Plant and Environmental Sciences of the Graduate School of Clemson University, 67 pp

Rembialkowska E (2003) Organic farming as a system to provide better vegetable quality. Acta Hortic 604:473–479

Schmitt A, Jahn M, Kromphardt C, Krauthausen HJ, Roberts SJ, Wright SAI, Amein T, Forsberg G, Tinivella F, Gullino ML, Wikström M, Wolf J, Groot SPC, Werner S, Koch E (2006) STOVE: seed treatments for organic vegetable production. In: Presented at the European joint organic congress, Odense, Denmark, 2 pp

Silva MG, Sharma RD, Junqueira AMR, Oliveira CM (2006) Effect of solarization, chemical and organic fertilization on the control of nematode on greenhouse lettuce. Hortic Bras 24:489–494

Simmons AM, Kousik CS, Levi A (2010) Combining reflective mulch and host plant resistance for sweetpotato whitefly (Hemiptera: Aleyrodidae) management in watermelon. Crop Prot 29:898–902

Smukler SM, Jackson LE, Murphree L, Yokota R, Koike ST, Smith RF (2008) Transition to large-scale organic vegetable production in the Salinas Valley, California. Agric Ecosyst Environ 126:168–188

Stein S (2009) A review of organic fertilizers. http://www.helium.com/items/1431343-a-review-of-organic-fertilizers. Accessed 17 May 2011

Thakur KS, Dharminder K, Amit V, Thakur AK, Mehta DK (2010) Effect of organic manures and biofertilizers on growth and yield of tomato and French bean under mid hills of Himachal Pradesh. J Hill Agric 1(2):176–178

Webber CL, Shrefler JW (2006) Corn gluten meal applicator for weed control in organic vegetable production. J Veg Sci 12(4):19–26

Webber CL, Shrefler JW, Taylor MJ (2010) Influence of corn gluten meal on squash plant survival and yields. HortTechnology 20(4):696–699

Webber CL, Shrefler JW, Brandenberger LP, Taylor MJ, Carrier LK, Shannon DK (2011) Weed control efficacy with ammonium nonanoate for organic vegetable production. Int J Veg Sci 17(1):37–44

Worthington V (2001) Nutritional quality of organic versus conventional fruits, vegetables, and grains. J Altern Complement Med 7(2):161–173

Xu HL, Wang R, Xu RY, Mridha MAU, Goyal S (2003) Yield and quality of leafy vegetables grown with organic fertilizations. Acta Hortic 627:25–33

Biomass Gasification Crops for the Climatic Range of New Zealand

Richard Renquist and Huub Kerckhoffs

Abstract Plant biomass can be used for multiple forms of bioenergy and there is a very large potential supply, depending on which global assessment is most accurate with regard to land area that could be available for biomass production. The most suitable plant species must be identified before the potential biomass production in a particular region can be quantified. This in turn depends on the degree of climatic adaptation by those plant species. In the range of climates present in New Zealand biomass crop growth has less restriction due to water deficit or low winter temperature than in most world regions. Biomass production for energy use in New Zealand would be best utilised as transport fuel since 70% of the country's electricity generation is already renewable, but nearly all of its transport fossil fuel is imported. There is a good economic development case for transport biofuel production using waste streams and biomass crops. One promising conversion technology is thermochemical gasification.

This review identified the most suitable crop species and assessed their production potential for use as the feedstock to supply a gasification plant making biofuel, within the climatic range present in New Zealand. Information from published work was used as a basis for selecting appropriate crops in a 2-year selection and evaluation process. Where there were knowledge gaps, the location-specific selections were further evaluated by field measurements, by distinguishing three categories of growth habit (perennials, summer and winter annuals), by identifying a high-yielding benchmark species for each category and by the use of crop models to simulate yields in 'marginal' site conditions. This review demonstrates how these elements constitute a methodological tool to quantify the rapid screening and ranking of species. The data presented have superseded much of the speculative information on suitability of species for the potential development of a biofuel industry.

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1 Introduction

Plant biomass can be used for multiple forms of bioenergy and there is a very large potential supply, e.g., the Billion Ton Study in the USA (U.S. Dept of Energy 2011; Boundy et al. 2010) and in the EU a study by the European Environmental Agency (EEA 2006) that expressed the primary biomass potential in energy units (Joules) and also million tons of oil equivalent per year. Global scale assessments of how much land will be available for biomass production were reviewed in 2005 (Lemus and Lal) and updated in recent years (Beringer et al. 2011). This review is focused on identifying the most suitable crop species and assessing their production potential for use as bioenergy feedstocks within the climatic range present in New Zealand.

The context for bioenergy development in New Zealand is that roughly 70% of the country's electricity generation is already renewable, but nearly all of its transport fuel is imported (NZ Energy Data File 2011). The country faces rising costs and less certain supply of fossil transport fuels. The most compelling use for purpose-grown biomass is therefore its conversion to transport biofuels, as opposed to heat and electrical energy (Hall and Gifford 2007). Furthermore, New Zealand uses very little coal, so replacing transport fossil fuel is also the best way to reduce greenhouse gas emissions, apart from agricultural ruminant methane. The current government is interested in new energy sources that offer economic development opportunities. The government's New Zealand Energy Strategy has, therefore, incorporated components of the New Zealand Bioenergy Strategy (BANZ 2011), a document by the Bioenergy Association of New Zealand that makes the economic development case for the use of bioenergy using waste streams and biomass crops.

Among the 'biomass to biofuel' conversion technologies, thermochemical gasification is one that is developing well (van der Drift et al. 2000; Franco et al. 2009; Pang 2011; Rauch 2011). It differs from the biological process of biogas production by anaerobic digestion and has been used in the past with either coal or wood as its bioresource/feedstock. This review will assess the use of herbaceous plant species for biomass production within the New Zealand climatic range for the specific end use of thermochemical gasification to produce biofuels.

The products of gasification in Fig. 1 (Rauch 2011) are hydrogen and carbon monoxide, created at specific high temperatures in the absence of oxygen and with careful control of feedstock transit time. This gaseous mixture, once known as 'producer gas', is now called syngas or product gas. It can be used as engine fuel in gaseous form or converted to synthetic liquid fuel, either diesel or petrol. The process has been referred to as 'biomass to syngas to liquid fuel' or BTSL (Pang 2011). The syngas is converted to synthetic diesel via the Fischer-Tropsch thermochemical process, which is also being advanced by current research.

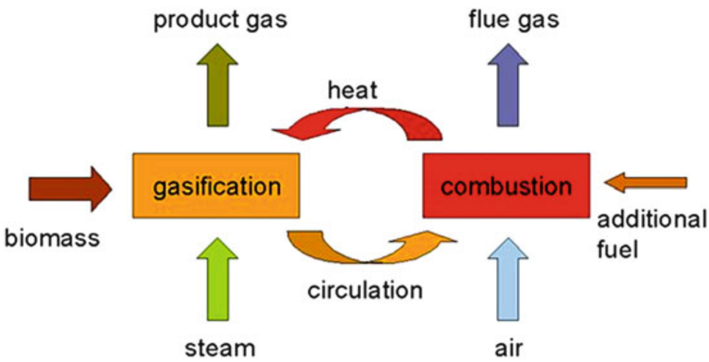

Fig. 1 Working principle of the fast internal circulating fluidized bed (*FICFB*) gasifier with steam as gasification agent, such as used at the Gussing, Austria plant (Figure courtesy of the Vienna University of Technology (www.ficfb.at))

This chapter is primarily a literature review with supporting local assessments to select the best herbaceous crop or weed species, within the context of New Zealand soil types and climatic range that would provided suitable biomass resource for a gasification plant.

The initial literature review was conducted from 2008 to 2010 during which time other activities were also completed to fill the knowledge gaps for species and cultivars not previously tested in New Zealand. We developed estimates of species ranking if grown in 'marginal' sites. This type of land, while lower yielding, is considered more suitable for achieving sustainability objectives.

This paper includes a section that describes the procedure we used to select the best species from the long list of candidates in a time-efficient manner, since highly definitive field trials in the range of climates would require a decade or longer; the section therefore has more aspects of a 'Methods' paper than would usually be found in a review.

This review and screening process has identified a 'short list' of the most promising non-woody species for biomass production and generation of biofuel in New Zealand. The details of their final selection and subsequent field trials will be the subject of a following research paper.

2 Benefits of Biomass for Energy

2.1 Security of Energy Supply

It is a given that an energy supply based on use of non-renewable fossil fuels is not sustainable in the long term. Since this review has a geographical focus, it is relevant that New Zealand imports 97.5% of the oil and petroleum-based liquid fuels it consumes (New Zealand Energy Data File 2011) and therefore also has a security issue related to such delivery. This could arise even before the world petroleum supply is depleted, such that alternative domestic fuel production would be required. Oil is also produced from New Zealand wells, but 95% is bound by export contracts.

2.2 Greenhouse Gas Reduction

Given the strong evidence for anthropogenic contributions to climate change, the displacement of fossil fuels is a technology change that will be beneficial and probably critical to future-proof current and following generations. This is the basis for active bioenergy research programmes internationally. One study considered the aspect of carbon sequestration from growing perennial energy crops in degraded land (Lemus and Lal 2005). The beneficial impact on net greenhouse gas emissions would be from both carbon sequestration and use of the biomass to replace fossil fuels. The latter aspect was also assessed in a 2004 study (Clifton-Brown et al.).

2.3 Energy Crop Research

Energy crops were a topic of considerable interest after the global 1970s oil supply/price crises. Some research continued and it has greatly increased with oil price rises/spikes in recent years. Large research programmes are in progress by the International Energy Agency (Sims et al. 2008) and its Bioenergy division (Bauen et al. 2009; Fritsche et al. 2009; IEA Bioenergy Executive Committee 2009); in Europe (Amon et al. 2007; Ceotto and Di Candilo 2010) and in the USA Biomass Program and biofuel programmes (Perlack et al. 2005; U.S. Dept of Energy 2011; Propheter et al. 2010; Propheter and Staggenborg 2010). Bioenergy programmes are also being set up in the larger developing countries like Brazil (Brito Cruz 2009) and China (Li 2010). Increased research emphasis in the USA is also being placed on breeding of species to enhance their traits as biomass crops (Simmons et al. 2008).

Archontoulis (2011) has noted that most published experimental data from energy crops is quite recent. While species already grown for agricultural uses are well understood in terms of their physiological and agronomic aspects, newer biomass crops especially those that could be classes as ‘weed’ species are less well described.

2.3.1 Agronomic Aspects

Much of the research emphasis on new biomass species has been on agronomic aspects of their production. Several reports, with a focus on dry mass yield, suggest there is a good potential to produce fuels and other types of energy from biomass crops. The range of species being researched in Europe include hemp, kenaf, maize, sorghum (Amaducci et al. 2000; Zegada-Lizarazu et al. 2010) and cardoon (Angelini et al. 2009). Cropping systems research includes energy crops in rotations, some of them dual-purpose species (Zegada-Lizarazu and Monti 2011) and mixed food/energy crop systems that also use food crop residues for energy (Amon et al. 2007; Karpenstein-Machan 2001). Improved tillage practices can have a positive environmental benefit, as will be considered in Sect. 3. Changing from conventional tillage to no-till is shown to enhance C sequestration and decrease CO_2 emissions (West and Marland 2002).

2.3.2 Physiological Aspects

A deeper understanding of new biomass species through physiological research will enhance agronomic practices with these crops. Examples are research characterising the mechanisms of crop response to water and nutrients. Such papers are covered is previous reviews (Bessou et al. 2010) and physiological aspects are reported in several recent research papers on the newer biomass species, for example cynara, kenaf and sunflower (Archontoulis et al. 2011). There are other examples for sunflower (Steer et al. 1993) and sorghum (van Oosterom et al. 2010). Physiological issues such as a crop's impact on the nitrogen cycle are relevant enough to be considered below (Sect. 3.4). Otherwise, since the focus of this review is on species selection for biomass production in New Zealand, physiologically-oriented papers on crop species that have biomass potential will not be reviewed here.

2.3.3 Socio-Economic Aspects

The potential for extensive use of land to produce energy crops raises socio-economic issues to consider. Since a new industry would be established this would require associated infrastructure development and could involve population migration back to rural areas. However, a change of land use from food crops to energy crops is under scrutiny in terms of the socio-economic impacts. A large increase in food prices in 2008 was attributed to use of maize grain and soybeans for fuel in North America. However, a closer analysis showed there were also price impacts from commodity market speculation involved (Mueller et al. 2011). Another study examined socio-economic effects of different facets along biofuel industry development pathways (Duer and Christensen 2010). As with crop physiological aspects, these will not be the reviewed in this paper.

3 Sustainability Issues Using Biomass for Energy

3.1 Land Use Change

Environmental issues with food production (e.g., overuse of fertiliser contributing to nitrate leaching, pesticide use and pesticide residues) have been recognised for many years and are expected to be more challenging as food demand escalates in the coming decades. So, it is not surprising that proposals to use land for the purpose of replacing fossil fuels have raised controversy. The sources of biomass for both food and biofuels need to be produced in a sustainable way, with the net carbon and nitrogen footprints in equilibrium. There is also the moral issue of placing transport biofuel - in part a discretionary consumer product - in competition with food - an essential human need - for use of crop land. For an overview on land use change see Howarth and Bringezu (2009). Direct use of a food species as biomass and use of the best arable land for biofuel in a world that will need to grow more food for a predicted ten billion people by 2050 can be challenged as non-sustainable (Blanco-Canqui and Lal 2009; Davis et al. 2009; Katola and Salmi 2010).

A follow-on issue that has been identified for some cropping situations is **indirect land use change**, since the previous use, eg, tropical rain forest with very high carbon storage, may mean that decades of biofuel production are required before the benefits of replacing fossil fuels will compensate for the carbon debt created by land use change (Ceotto and Di Candilo 2010; Dale et al. 2010; IEA Bioenergy Executive Committee 2009). In Brazil, where biofuel production from sugarcane is often assessed as sustainable, the effects of indirect land use changes were determined by one analysis to exceed the benefits of biofuel substitution (Lapola et al. 2010).

The above efforts to quantify this indirect effect have been useful, but doing so is complex. It has been noted by others that its inclusion in the sustainability standard being applied to biofuels differs from the standard applied to land use change for food production (Kim et al. 2009).

3.2 Land Area Requirements for Biomass Crops

It will be important to predict during the next few decades how much surplus agricultural land could be sustainably diverted to feedstocks for biofuels. Earlier studies of how much land will be available for biomass production were reviewed in 2005 (Lemus and Lal). One later assessment looked in particular at the global amount of abandoned agricultural land available for biomass production (Campbell et al. 2008). Beringer et al. (2011) looked at potential bioenergy production given the environmental constraints and agricultural needs in the context of a global analysis. An assessment of the biofuel production potential using the arable and pastoral lands in Europe was made by Fischer et al. (2010a, b). Another analysis considered the impacts of regional (European Union) policies for biofuel supply on global land use and food production (Banse et al. 2011). A model for southern Australia of the effect of a shift to large-scale biofuel production (Bryan et al. 2010) showed that using food crops like wheat and canola for biofuel was more profitable than their use for food, but the beneficial effects on greenhouse gases and replacing fossil fuels were outweighed by the reduction in food production. There were specific regions within southern Australia where land use for biofuels could be beneficial overall.

The assumptions used in different models result in widely differing calculations of how much land is potentially available for biomass cropping. Bessou et al. (2010) compared the predictions of three global-scale models when the assumed level of agricultural intensification by 2050 was low (organic-type systems), medium and very high. At the low input/intensification level the land required for food would be double the current area, leaving no land for energy crops. For the other two models reviewed the surplus land area available for energy crops at the highest scenario of each is calculated to be 1.3 and 3.6 Gha, respectively (Bessou et al. 2010). These require what may be overly optimistic gains in food crop yields, up to 4.6 times 1998 yields, in order to create 'surplus' land.

3.3 Water Use by Biomass Crops

Water use by biomass crop species needs to be considered at the paddock, the landscape and global scale. At the farm or paddock scale the usual assumption is that biomass crops should be unirrigated. The two bases for this are: (1) the capital cost of irrigation systems is too high for what will need to be a low- to moderate-value crop in order to result in economic energy production, and (2) there are ethical/environmental issues of diverting the water resource from food production or of sourcing it from either surface waters that provide environmental services or non-renewable groundwater resources (De Fraiture and Berndes 2009).

Even for unirrigated biomass production the amount of water transpired is a significant consideration at the global scale. Such an analysis was first done a decade ago (Berndes 2002) which demonstrated the importance of taking the water use into consideration in both the production of energy crops and the industrial processes for conversion to biofuels. With respect to the choice of biomass crops that analysis also presented the wide range in water use efficiency differences between species. Projections of water requirements in 2050 if bioenergy provided 50% of total energy, or biofuel provided 30% of transport, are that the transpiration would be nearly half of that for total food production (De Fraiture and Berndes 2009).

3.4 Nitrogen Cycle and Use by Crops

Nitrogen fertilization is an effective tool for improving the efficiency with which cropland is used. The gain in crop productivity will offset the emission used to produce mineral fertilizers (Ceotto 2005). Unfortunately, nitrogen applied to crops as fertilizers and manure is inefficiently used in most cropping systems. Unused fractions contaminate surface and ground water resources (Pierce and Rice 1988). Losses occur via denitrification, volatilization and leaching (Ceotto and Di Candilo 2010). Galloway et al. (2002) defined reactive nitrogen as all biologically active, photochemically reactive and radiatively-active nitrogen compounds present in the biosphere and atmosphere of earth, and includes inorganic reduced and oxidized forms of nitrogen and organic compounds as urea, amines and amino acids. When it enters agro-ecosystems, reactive nitrogen derived from either synthetic fertilisers or legumes has equally negative environmental impacts.

The reduction of reactive nitrogen in agricultural systems is therefore an important sustainability issue. Growing biomass crops has the potential to reduce the problem. One means to do this is the same as for food crops, i.e. to improve the yield of dedicated energy crops so that production can be achieved on a limited land area. Another strategy is to exploit the potential of dual purpose crops on arable land (Ceotto and Di Candillo 2010). When the crop residues or whole dedicated energy crop in a rotation is converted to bioenergy via e.g., combustion and gasification, the reactive nitrogen is neutralised.

In terms of relative production of damaging reactive nitrogen, crops with a high yield at low nitrogen supply are the lowest producers. Some of the better biomass species have high nitrogen use efficiency, which is a significant environmental advantage resulting in less ground water and runoff pollution derived from nitrogen fertilisers. It also makes them more cost effective.

When legumes are used in a crop rotation, the fixed nitrogen can be taken up and eventually released back in to the atmosphere as benign N_2 if the following crop is used as a bioenergy feedstock for the appropriate conversion technology.

3.5 Life Cycle Assessment

A rigorous assessment of sustainability usually involves a Life Cycle Assessment (LCA) analysis of biofuel production (Börjesson et al. 2010; Katola and Salmi 2010; Davis et al. 2009; Wortmann et al. 2010; Patterson et al. 2008; Blanco-Canqui and Lal 2009). An important aspect of sustainability usually assessed is the relative greenhouse gas production of different fuels. LCA has proven very useful to assess the relative merits of potential future biomass species (Rettenmaier et al. 2010). Some studies have successfully identified biofuels that are relatively poor choices in terms of energy balance and/or environmental impacts (Davis et al. 2009).

The appropriate scope for an LCA is often from ‘cradle to farm gate.’ In one such analysis of perennial biomass crops in Italy (Monti et al. 2009) four biomass species were compared to a food crop rotation in terms of ecological impact on a per hectare basis and on energy impacts. The per-hectare impacts of all four were about half those of the wheat/maize rotation. Three of the four also had much lower impacts than the fourth biomass crop on an energy basis as well, which is clearly essential for an effective energy crop.

3.6 Use of ‘Marginal’ Land for Bioenergy Crops

A species having low input requirements is also likely to be better adapted to utilise ‘marginal’ land. This is not only in the interest of the grower/landowner, creating a new land use for such areas, but is a key aspect of making the biofuel production from biomass sustainable. In order to use performance in 'marginal' land as a species selection criterion, as intended in this review, then 'marginal' itself needs to be reconsidered and better defined. This need has been noted in other analyses of biofuel production (Ceotto and Di Candilo 2010; Robertson et al. 2010; Davis et al. 2009; Dale et al. 2010).

There are several complexities to consider in defining 'marginal' (Dale et al. 2010), but allowing for such considerations, marginal sites can be defined as those which provide on average suboptimal growing conditions for major food or feed crops in the relevant climatic zone. Marginal sites are also defined according to properties of the soil, the topography and the reliability of key weather factors like favourable rainfall and temperature. This is why the term 'marginal site' may be preferable to 'marginal land.'

4 Species Screening, Energy Crop Criteria

Identifying the desirable characteristics of a biofuel crop has been reviewed before (e.g. Ceotto and Di Candilo 2010). We conclude that an ideal New Zealand biofuel crop should possess the following key attributes:

- a species already in New Zealand or having qualities such as sterile seed that enable speedy regulatory approval for importation
- easy to establish, even on 'marginal' land
- can be established by minimum/no-tillage techniques
- early spring growth to compete strongly with weeds
- deep rooting to access subsoil water and preferably a perennial growth habit
- good solar radiation capture and high daily growth rate over a long period
- very high or high dry mass yield
- nutrient and water requirements are low relative to yield
- resilient to the site limitations (e.g., frost or water deficit)
- easy to manage (minimal pest control needs)
- biomass production is above ground
- easy to harvest
- the delivered biomass has a moisture content no higher than that of wood
- has a low nitrogen concentration and low or moderate ash content, and
- can be stored dry or ensiled.

These attributes of an ideal bioenergy crop reveal how to go about improving energy crops in terms of yield and net energy gain (Ceotto and Di Candilo 2010) and feedstock traits such as ash content (Monti et al. 2008), as well as environmental sustainability. Low nitrogen content is both a reflection of lower industrial fertiliser use and lower release of N_2O. Perennial plants usually have better nutrient recycling due to underground storage organs.

This section describes the biomass species we identified as candidates for evaluation. The international literature search results in 2008 came from biomass studies largely aimed at liquid fuels and pyrolysis studies using waste stream biomass, but more recent searches also identified more papers on bioenergy from dedicated crops. Commercial biofuel literature was also a useful source as to which species are attracting interest as biofuel feedstock.

The literature review identified a wide range of potential biomass species. These included crops known to have high dry mass yield in New Zealand arable soils, resident weed species with observed prolific growth, advanced cultivars of arable crop species that could be introduced to New Zealand and overseas biomass crop or weed species with traits such as sterility that would enable introduction to New Zealand.

A compilation of recent New Zealand field data on high biomass arable crops and some weed species, and new dry mass field measurements in commercial crops or small plots were designed to add preliminary New Zealand information on less well-studied species.

This review used additional criteria particular to the research project it was part of, a biomass gasification research project. The net requirement by the operator of a gasifier unit is for feedstocks that collectively can be grown, stored and supplied year-round at a relatively low cost per tonne dry mass.

High dry mass was the best criterion for initial ranking of prospective biomass species. This process was structured by distinguishing three categories of growth habit: summer annual species, perennials and winter annual species, to facilitate direct comparisons of species for which there is only limited information with those that are well-characterised crop species of the same type.

The following subsections provide lists of species (categorised by crop growth habit) and literature review findings for each that provide (1) a brief description of their potential as biomass crops based on yield, (2) relevant aspects of each species' agronomy and (3) whether there are issues making it less favourable to use as a crop in New Zealand.

Some of the species information from New Zealand is specific to geographic regions of the country. Figure 2 can be referred to, noting that low latitudes are in the north end of the country.

4.1 Perennial Species

4.1.1 Lucerne (*Medicago sativa*)

Criteria match for dry mass yield: Lucerne is a widely-grown species in New Zealand, with proven high dry mass yields. Douglas (1986) summarised yield results from 57 different crops/treatments from various authors investigating lucerne growth as far back as 1965, covering all of the major climates and growing environments in which lucerne is grown. Under rain-fed conditions in the South Island highest yields were obtained from lowland soils on alluvium, ca. 15–20 tonnes dry mass per hectare (t DM/ha). In other climates/soil types (e.g. lowland soils on loess and/or fine gravels, hill and upland soils on loess and where rainfall was

Fig. 2 New Zealand map showing regions where the yield of species was assessed. The numbering key for regions discussed in the review is: *1* Northland, *2* Waikato, *3* Hawke's Bay, *4* Canterbury, *5* Southland

350–550 mm/year) lucerne yielded much lower (ca. 8–9 t DM/ha). Crops grown in the North Island under rain fed conditions and on soils derived from recent alluvium were also the highest yielding whilst those grown on soils with volcanic parent material were generally lower yielding (Douglas 1986).

The recent New Zealand research record confirms that lucerne has very high biomass yields, >20 t DM/ha in deep soils in warm parts of the North Island with adequate rainfall (Shaw et al. 2005b). Yields can be equally good in the best South Island soils (Brown et al. 2003). Lucerne is widely adapted to marginal sites with lower water holding capacity as the crop has a strong tap root and is capable of utilising water from deep in the soil profile.

Agronomy: Douglas (1986) also presented data indicating that available water capacity (AWC) has a large, linear effect on lucerne yield with an extra 63 kg DM/ha per mm of AWC. This was particularly true on light stony soils, but the effect is diminished on soils with higher water holding capacity, such as lowland soils on alluvium (Douglas 1986). The recent lucerne research programme by Brown et al. (2000, 2003, 2005a, b, 2006) and Teixeira et al. (2007a, b, c, 2008) were on a deep, high water holding soil. One study by Brown (2003) reported yields of 21.3 in year 1, declining after year 3–17.5 t DM/ha in year 5. Shaw et al. (2005b) reported on non-irrigated North Island lucerne trials in the Hawke's Bay and Waikato regions. On deep high water-holding soil in Hawke's Bay the yield was 9.4 t DM/ha in year

Fig. 3 Lucerne (*Medicago sativa*). When comparing dry mass yield to other biomass species that are only harvested once per year it should be noted that more harvesting effort is required for lucerne, with three or four harvests per year

1 and 22.0 t/ha the next 2 years. The Waikato crop was grown on a hill soil with only moderate water holding capacity (marginal in that respect). This crop yielded 5.4 t/ha in the year it was sown, 17.4 t/ha in year 2 and 14.6 t/ha in year 3.

Lucerne can be considered sufficiently well researched to use in the engineering model for supplying biomass to a gasification biofuel plant and also to use as a species with documented New Zealand biomass production for comparing to yields of less familiar species.

Issues: Lucerne usually has high value as livestock forage, so it may be more expensive for the biofuel plant to purchase than other biomass species. Multiple harvests are also a cost factor (Fig. 3).

4.1.2 Giant Miscanthus (*Miscanthus x giganteus*)(Mxg)

Criteria match for dry mass yield: reported dry mass yields have been high to very high in Europe. The most promising genotype is *Miscanthus x giganteus* (or Mxg). Peak yields are achieved as early as the third year (Lewandowski et al. 2000; Clifton-Brown et al. 2004) or not until the sixth year (Christian et al. 2008) and are higher in warmer climates. Mediterranean research has compared several energy crop candidate species and found Mxg to be a consistent high performer with irrigation or summer rainfall: 27 t DM/ha in Italy (Cosentino et al. 2007) and 28–38 t DM/ha in Greece (Danalatos et al. 2007). Since Mxg was only recently introduced to New Zealand (Brown 2009) the best guide to its yield potential is from an analysis using a UK crop model, which simulated a 13 year mean yield for a site in New Zealand (2008 report by A. Hastings, commissioned by Peter Brown). The peak DM in early winter averaged 27 t DM/ha, while late winter mean DM (the time of harvest) was

18.7 tDM/ha. The mean yields included 6 years with some yield reduction predicted due to water deficit. Details are provided in Sect. 7.2.3.

Agronomy: European research has compared several genotypes (Clifton-Brown et al. 2001). Findings from several UK trials led to release of a Production Guide (DEFRA 2001). Mediterranean research has compared several energy crop candidate species and found Mxg to be a consistent high performer, but Mxg does require irrigation or summer rainfall in Italy (Cosentino et al. 2007) and Greece (Danalatos et al. 2007). Research on harvest timing has indicated that while peak dry mass is in early winter the better time to harvest is after several tonnes of dry mass has been translocated to the rhizome system, along with nutrients to supply early spring growth. The yield at that time is usually 5–10 tDM/ha below the peak (Clifton-Brown et al. 2004).

Physiology: Agronomic and environmental research with Miscanthus led to publication of a growth model, MISCANFOR, in the UK (Hastings et al. 2009). Other studies have quantified response to irrigation and nitrogen (Cosentino et al. 2007). Miscanthus has a low nitrogen content, which is environmentally advantageous because it requires less nitrogen fertiliser to grow and because combustion of the biomass produces less reactive nitrogen than burning fossil fuels or other crop species that are higher in nitrogen content (Ceotto and Di Candilo 2010), and environmental benefits of Miscanthus were greater than other biomass crops (Lewandowski and Schmidt 2006). There is also a positive impact on greenhouse gas emissions by replacing fossil fuels (Clifton-Brown et al. 2004).

Issues:The high cost of establishment is due to vegetative propagation of the sterile triploid Mxg and the need for modified planting equipment. For high dry mass yield Miscanthus requires rain or soil water into the summer, which is often lacking in the Mediterranean climate. While this would not be an issue in most regions of New Zealand with more than 700 mm rainfall, the marginal sites preferred for biomass crops will sometimes be defined by a combination of shallow soil and low summer rainfall. Since New Zealand has a milder winter climate than the European locations, where it has had the most testing as a biomass crop, there may be challenges with winter weed control and early re-growth from the top of the plant before harvest is complete. None of these issues appear to negate the potential of this species in many parts of New Zealand, but they will need to be researched (Fig. 4).

4.1.3 Jerusalem Artichoke (*Helianthus tuberosus*)

Criteria match for dry mass yield: While usually considered a tuber crop, the use of Jerusalem artichoke shoot biomass has been quantified and investigated for producing biogas or forage (Gunnarson et al. 1985; Wunsche 1985; Seiler 1993). The 1980s Scandanavian research documented yields from 7 to 20 tDM/ha (Gunnarson et al. 1985; Wunsche 1985). A trial with multiple shoot harvests in Minnesota (45° latitude) indicated a theoretical yield higher than 25 tDM/ha (Rawate and Hill 1985). The first New Zealand trials had shoot biomass yields in the

Fig. 4 Giant Miscanthus (*Miscanthus x giganteus*). Transplanted as small plantlets with two or more rhizome branches (**a**); height after 12 months, from mid-summer to mid-summer (**b**)

range of 13–16 tDM/ha (Kerckhoffs et al. 2011; see Table 4 in Sect. 6.2.2). Much higher shoot yields (>30 tDM/ha) have been observed in 2011–2012 trials in Hawke's Bay (unpublished).

The highest tuber yield to date (15.0 tDM/ha or 58 tFM/ha) was in Northland, from plants with both shoots and tubers harvested in the winter (Kerckhoffs et al. 2011). However, the Northland tubers had inadequate vernalisation for new spring growth (see the Physiology section).

Agronomy: As a new commercial species in New Zealand, Jerusalem artichoke is a good example of a species needing to have its growth and environmental responses characterised thoroughly. This can be guided by extensive findings in the Northern Hemisphere, although the emphasis there has been on tuber production using annual row cropping methods. If biomass is also produced in that way they the optimal seed spacing needs to be defined. In a perennial system, with some or all tubers left in the ground after the previous season, the growth habit is much different. We observed more than 100 stems/m^2 compared to 10–20 stems/m^2 in the first year. This may require different canopy management if stem population proves to be excessive for optimal use of sunlight.

Physiology: Plant development, such as biomass and nutrient allocation patterns has been investigated in North America. Shoot growth reached peak dry mass 18 weeks after planting in two trials (McLaurin et al. 1999; Swanton and Cavers 1989). However, the highest observed shoot dry mass yields (Wunsche 1985) and our unpublished 2012 results are from long-season crops. Daylength effects, particularly on early tuber-forming cultivars, appear to favour high latitudes (Wunsche 1985) over lower latitudes (Seiler 1993) for shoot dry mass production. However, cultivars vary widely in growth habit and yield, so comparing trial results with different cultivars is difficult.

New Zealand spans a wide range of latitudes, so this mass partitioning effect needs to be evaluated further for the New Zealand cultivar 'Inulinz'. Another matter to clarify is whether shoot growth peaks too soon to intercept full summer radiation. If true then one option is to harvest shoots early for dry mass, then allow the crop to regrow a full-season crop of shoots and sufficient tubers produced for a crop the following year (Rawate and Hill 1985).

Issues: The vernalisation requirement of Jerusalem artichoke tuber buds is well known (Kays and Nottingham 2008). In 2010, this was not met in northern New Zealand for the local cultivar 'Inulinz'. Further testing will be needed to define how far north the crop can be grown and still have buds vernalised to enable good perennial vegetative yield. The costs for planting and storing tubers need to be determined. Management practices need to be defined to ensure tubers do not regenerate if paddocks are used for different arable crops. No issues noted to date appear to seriously detract from this species' potential in the majority of New Zealand (Fig. 5).

4.1.4 Switchgrass (*Panicum virgatum*)

Criteria match for dry mass yield: Switchgrass has been widely tested in its native North America and its yield potential modelled throughout the USA (McLaughlin and Kszos 2005; Wright et al. 2009). Test yields ranged from 4 to 18 t DM/ha and were most often in the 10–12 t DM/ha category (Wright et al. 2009). Greater yields were sometimes observed in the southeast region of the USA with the hottest summer weather and ample rainfall. It was lower yielding than Miscanthus in direct comparisons (Heaton et al. 2008).

Agronomy: Switchgrass has a low nitrogen requirement and moderately lower water requirement, which is similar other C4 species such as Miscanthus. It persists for at least 10 years and is easy to maintain.

Issues: Switchgrass is not currently in New Zealand and would probably not qualify for introduction since it is able to spread by seed as well as rhizomes. Growth would start very late in the spring due to cool New Zealand soils and high yields would be unlikely in the temperate summer weather. Yields would also likely be low in marginal sites with low summer rainfall (Ceotto and Di Candilo 2010).

4.1.5 Reed Canary Grass (Phalaris arundinacea)

Criteria match for dry mass yield: Reed canary grass is present in New Zealand and was tested as a feedstock for biogas production in the 1980s (Stewart 1983). It is

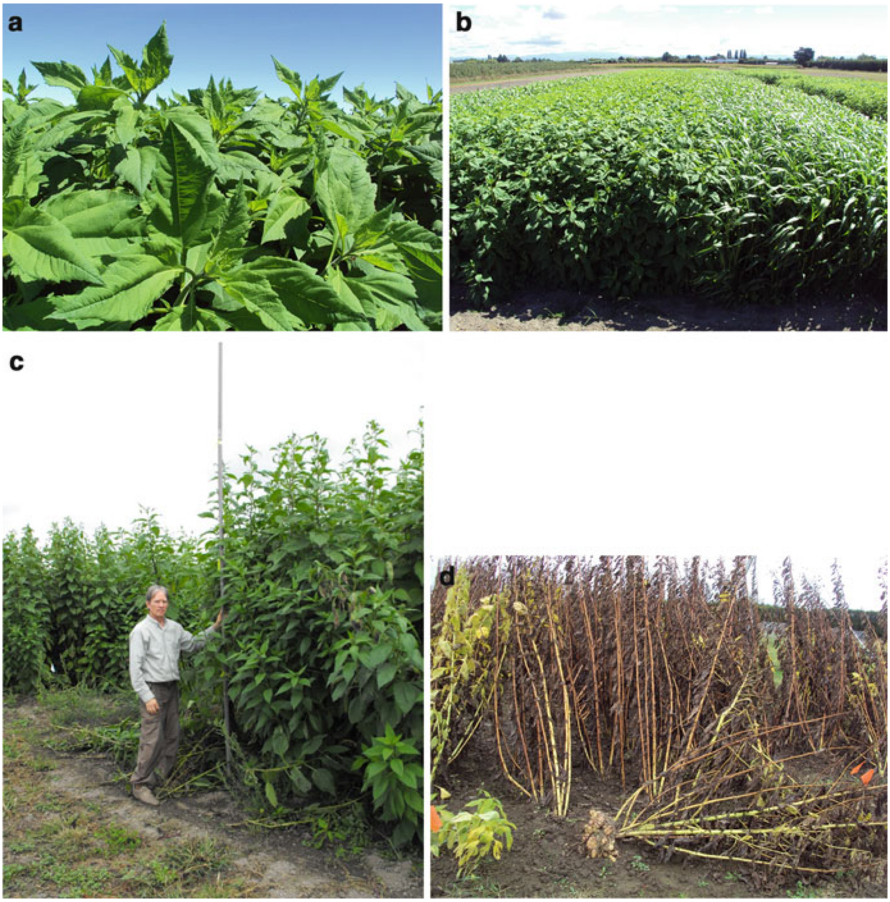

Fig. 5 Jerusalem artichoke (*Helianthus tuberosus*). Vegetative growth is rampant even in cool weather (**a**) and in Hawke's Bay region is similar to the growth and mid-summer mass of the sorghum on either side (**b**). Shoot dry mass peaks after flowering (**c**) and shoot mass is translocated to the tubers from the stage in **c** through to shoot senescence (**d**)

very hardy, grows quickly and spreads easily both by seed and by creeping rhizomes. Dry mass yield under European conditions was less than 10–12 t DM/ha in a comparison to Miscanthus and triticale (Lewandowski and Schmidt 2006).

Agronomy: The species is an inferior crop to Miscanthus in the climates of north-western Europe in terms of nitrogen use efficiency and energy use efficiency (Lewandowski and Schmidt 2006).

Issues: Reed canary grass is considered to be a weed pest in New Zealand wetlands. It is a major threat to marshes and wetlands because it can replace native species. It is difficult to eradicate once established and there could be a problem for local authorities. It is currently listed for eradication (Environment Canterbury 2011).

Fig. 6 Harding grass (*Phalaris aquatica*). Like other perennials Harding grass makes a slow start compared to the surrounding forage oat crop, planted at the same time. It grows 1.5–2 m tall, but is not higher in dry mass than shorter pasture grasses

4.1.6 Harding Grass (*Phalaris aquatica*)

Criteria match for dry mass yield: Harding grass is a tall bunchgrass of the same genus as canary reed grass. It is present in New Zealand and has been sown in pastures as a 10% component of seed mixtures. It is toxic to cows at higher levels, so the only information on its growth in pure stands was from a seed grower. Preliminary results were also obtained in a small research trial in the Hawke's Bay region (unpublished). At the end of the first season (November 2009) the Harding grass yielded much lower than plots of winter annual oats in the same trial. Harding grass produced (5.0 ± 2.2 t DM/ha) compared to oats (16.9 ± 4.3 t DM/ha). In the following season the Harding grass plots were damaged, but they would have been expected to yield between 7 and 12 t DM/ha based on the long-term experience of a New Zealand seed grower (Ian Gorton, personal communication, 2009). It was clear that annual DM yield from perennial Harding grass would be far less than the combined annual yield of winter oats and a summer biomass crop. Such a large yield deficiency outweighs the benefits of using this perennial species for biomass production.

Issues: *Phalaris aquatica,* while tall-growing, has no greater dry mass than the best regular pasture grasses and it is toxic to livestock if there is >10% in pastures (Fig. 6).

4.1.7 Napier Grass (*Pennisetum purpureum*)

Criteria match for dry mass yield: Napier grass is a large perennial that can grow more than 3 m high. The leaves are susceptible to frost but the root system can remain alive if the ground is not frozen. The grass grows easily from rhizome and stem fragments and forms thick clumps with long, flat leaves which have strongly ridged midribs. Napier grass is present in New Zealand and has been tried as a biofuel feedstock (Stewart 1983).

Issues: Napier grass is listed as a pest species in New Zealand and classified as an Unwanted Organism by the Department of Conservation (Biosecurity NZ 2011b) and is also listed as an invasive species in the Pacific Islands.

4.1.8 Cardoon or Cynara (*Cynara carduncului*s)

Criteria match for dry mass yield: Cynara (or cardoon or artichoke thistle) is a tall relative of artichoke used as an ornamental or for edible stems by those tolerate the sharp thistle features. It is known for its high biomass yield (>25 tDM/ha) under favourable conditions (Angelini et al. 2009; Gominho et al. 2011).

Physiology: Recent research into dynamics of light and nitrogen distribution in canopies (Archontoulis 2011) provided a basis for the high dry mass yield of cardoon in relation to other biomass species. The crop is very well suited to the Mediterranean climate with rainfall concentrated in the early part of its season, but in drier years may need irrigation in springtime for high yield (Archontoulis 2011). This last reference also contains photos of Cynara and kenaf.

Cardoon is costly to establish, although somewhat invasive once present. Crop handling needs to allow for its sharp spines and cardoon has higher nutrient requirements than ideal for a biomass crop. The biomass may be too high in ash content for gasification. The climatic preference is for very dry summers which are rare in New Zealand. If there is rain after the crop starts to dry it may regrow. That could make the harvested biomass too wet for storage or gasification. In one LCA analysis of four biomass species in Italy the cardoon was far worse than the other three in terms of its impacts, on an energy basis (Monti et al. 2009).

4.1.9 Giant Reed (*Arundo donax*)

Criteria match for dry mass yield: Giant reed is a clump-forming bamboo-like grass having short rhizomes and a dense root mass. It can grow up to 5 m in height. Giant reed does not spread by seed and has very high biomass yield (>25 tDM/ha) in Mediterranean climates (Ceotto and Di Candilo 2010).

Issues: Giant reed requires abundant moisture and is subject to serious damage by spring frosts. It has an ability to spread over geographic locations quickly, via natural waterways, which allows Giant Reed to overtake large areas very quickly. Giant Reed is an extremely flammable plant, even when it is green. These factors produce various results that make Giant Reed extremely undesirable in New Zealand where the winters are milder than in Europe. It is already present but the subject of control efforts (Biosecurity NZ 2012a; New Zealand Biosecurity Institute 2009).

4.1.10 Tagasaste or Tree Lucerne (*Chamaecytisus palmensis*)

Criteria match for dry mass yield: Tagasaste or tree lucerne has been studied for forage use in New Zealand (Logan and Radcliffe 1985; Lambert et al. 1989). The per-plant yields were rarely converted to yield per hectare; the only cited value was <2 t DM/ha. Tree height in experiments was less than 2 m.

Issues: Tagasaste had low yields in many years due to drought sensitivity. In warm wet conditions it was susceptible to root rots (Logan and Radcliffe 1985).

4.1.11 Pampas Grass (*Cortaderia sellowana*)

Criteria match for dry mass yield: Pampas is a giant, clump-forming grass that can grow to 4 m or more. The leaves snap readily when tugged. Dead leaf bases curl like wood shavings, unlike the related native *C. fulvida*. No annual dry mass data is available in New Zealand.

Issues: Windborne seeds allow the grass to easily spread far and wide. It readily colonises disturbed sites, quickly becomes dense and can suppress the growth of other species. It replaces ground cover, shrubs and ferns, creates a fire hazard, provides habitats for possums and rats, and impedes access (Biosecurity New Zealand 2011a). It is therefore classified as a noxious weed in two regions. It would be restricted from use as a biomass crop.

4.1.12 Toe toe (*Cortaderia fulvida*)

Criteria match for dry mass yield: The New Zealand native species of *Cortaderia* are smaller than pampas grass. No dry mass yield per hectare has been reported, but it is visually much less massive than pampas grass.

Issues: Native *Cortaderia* species are slow to establish and there are restrictions against the use of non-local ecotypes of this native species in some areas, according to New Zealand specialist W. Parker of Oratia Native Plant Nursery (personal communication, 2009).

4.1.13 "Wandering willie" (*Tradescantia fluminensis*)

Criteria match for dry mass yield: *Tradescantia,* is a rank perennial weed in shady areas but it is low growing and is not a high dry mass producer, only 7.5 t DM/ha (Standish et al. 2001). *Tradescantia* was impressive in terms of efficient use of low solar radiation, with its dry mass peaking at only 10% of full sunlight.

Issues: There could be restrictions on its cultivation and distribution due to its adverse impact on natural wooded landscapes. In the sun it would probably be overgrown by other species.

Fig. 7 Yacon (*Smallanthus sonchifolius*). Yacon is grown for its crisp root and also has massive shoot growth, but which is quite reduced by root harvest time. Note the frost burn of upper leaves

4.1.14 Yacon (*Smallanthus sonchifolius*)

Criteria match for dry mass yield: Yacon is a tall-growing perennial (2 m) with very large shoots. Their mass has not been measured in New Zealand at the peak time during the summer in a research report that focused on fresh mass of the large fleshy (edible) storage roots. At harvest time fresh mass of shoots was 15.7 t FM/ha compared to 90 t FM/ha in roots (Douglas et al. 2007). Even if the standing shoots had air dried to a moisture content of 50% before harvest the DM yield would have been <8 t DM/ha.

Agronomy: New Zealand trials found that yacon requires early spring planting and a long season to achieve high root fresh mass yields; in cooler areas the root yield was only 20–30% of the top yield in a warm site (Douglas et al. 2007). Therefore only latitudes below 38° should be considered suitable in New Zealand. Warm nights may be required for higher shoot dry mass, but these are lacking in most of New Zealand.

Issues: Yacon is quite frost tender, part of the reason most of New Zealand is considered unsuitable. The use of roots for biomass requires too much energy expenditure for harvest and there is as yet no market in New Zealand for the roots as food. This would be a prerequisite for using the shoots as a crop residue (Fig. 7).

4.1.15 Water Hyacinth (*Eichhornia crassipes*)

Criteria match for dry mass yield: Water hyacinth is a mat-forming water weed with very high productivity.

Issues: Water hyacinth entered New Zealand many years ago and became a pest species. Its current status is that it has been eradicated and has not been allowed into New Zealand since 1927, eliminating it from contention as a biomass species (Biosecurity New Zealand 2012b).

4.1.16 Cattail (Rapu in New Zealand) (*Typha orientalis*)

Criteria match for dry mass yield: The native species of this genus has the Maori name rapu. Its common name in North America is cattail and in the UK bulrush. Rapu is closely related to those northern hemisphere *Typha* species, which have been studied in relation to bioremediation of secondary sewage and for biofuel production (Shahbazi 2009). The biology of *Typha orientalis* has been detailed in northern New Zealand (Pegman and Ogden 2005), where its annual dry mass productivity was 29.1 t DM/ha, with 22.6 t DM/ha in the shoots.

Agronomy: Both due to its very high DM productivity and adaptation to sites not suited for food crops, rapu is an interesting biomass weed to consider cropping. Since many natural wetlands would be excluded from harvest for environmental reasons, commercial production of rapu would probably be on marginal, poorly drained agricultural land and this would require special landform modification to create standing water. Some current dairy pastures in the South Island West Coast, shaped into 'humps and hollows,' already have nutrient runoff problems in the hollows, so nutrient interception by rapu could make milk production more sustainable while producing biomass.

A preliminary trial in the Hawke's Bay region compared quadrat harvests in a wetland, either a two cut per season regime or a single early winter harvest. The mean DM yields were a total of 18.6 t DM/ha for the two cut regime compared to 29.7 t DM/ha for the one cut regime (unpublished data). So rapu has a very high peak shoot DM which is adversely affected by an additional summer harvest.

Issues: Like Miscanthus (Clifton-Brown et al. 2004), the ideal timing for first biomass harvest may not be at the early winter peak dry mass, since that may reduce the yield in the following season. So some loss of shoot dry mass via translocation to the rhizome system prior to harvest is probably necessary. The requirement for standing water, coupled with the legal protection of natural wetlands, very much limits the scope for commercialisation of *Typha* as a biomass crop. Harvest would be more feasible in climates colder than New Zealand, where ponds freeze hard enough for driving equipment on the ice (Fig. 8).

4.1.17 Gorse (*Ulex europaeus*)

Criteria match for dry mass yield: The average DM yield over a 6-year growth cycle reported in a lower North Island study (Egunjobi 1971) was 9.8 t DM/ha/year plus average annual litter fall of 8.9 t DM/ha/year. This was calculated from the 60 t/ha standing biomass at age six, measured for plants that grew from seed after the site

Fig. 8 Cattail or Rapu (*Typha orientalis*). This wetland weed has a very high peak dry mass, but harvest probably needs to be delayed past the peak

was burned. A goat forage trial in the Canterbury region found the DM yield to be 19.5 t/ha/year (Radcliffe 1986). Gorse as biomass crop has strong appeal due to its wide adaptation, growth on sloping marginal land, coppicing ability and need for little or no fertiliser. It is also a legume that fixes nitrogen, sometimes enough to create a nitrogen run-off problem.

Agronomy: Gorse grows well on steep slopes in New Zealand, a category of clearly marginal land that cannot be used by most biomass crops which require slopes suitable for harvesters. It would be harvested more like a short-rotation forestry crop and would regrow from cut stems.

Issues: Gorse's shortcomings as a biomass species include its lesser harvestable dry mass (since litter would be difficult to collect) and practical management difficulties such as its nasty spines. If this species' potential was deemed worthy the latter might be overcome by in vitro plant breeding to develop a spineless form.

4.2 Summer Annual Species

4.2.1 Maize (*Zea mays*)

Criteria match for dry mass yield: Very high DM yields, many in the 25–30 t DM/ha were documented in New Zealand seed company field trials (Densley et al. 2005) and also in research trials (Booker 2008; Li et al. 2006; Reid et al. 1999; Rhodes

1977; Shaw et al. 2007). A 2009–10 trial at two marginal sites produced maize yields of 29 tDM/ha in the irrigated site and 12.6 tDM/ha in the drought-affected site (Kerckhoffs et al. 2011). The high yield and strong knowledge base (as a major New Zealand crop for grain and silage) makes maize a good benchmark to compare other summer annual biomass species to.

Agronomy: Silage maize is well-studied in New Zealand (Booker 2008; Li et al. 2006; Rhodes 1977; Sadras and Calvino 2001; Shaw et al. 2005a, b, 2007). Even in a drought year in the Waikato maize region (2007–08) the mean biomass yield across 44 trials of Pioneer® seed was 22.3 tDM/ha (B. McCarter, Genetic Technologies Ltd, personal communication) Maize response to nitrogen supply has been characterised in the New Zealand crop model AmaizeN (Li et al. 2006) and response to soil water supply has been widely studied (e.g., Sadras and Calvino 2001).

Issues: Maize is high yielding and its agronomy is well defined, and therefore a good species for assessment as a gasification feedstock in the planned engineering model in the research project. However, there are issues with its large scale use as a biomass crop. The main issue is an ethical one (discussed in Sect. 3). Maize is grown on the best arable land that could be producing important food crops. Its main use is as a feed crop (either forage or grain) for livestock; the end products are milk and meat. At the scale of New Zealand alone this is not an ethical issue, since about 90% of the meat and milk is exported and any staple food can be locally supplied to meet New Zealand food demand. At the global scale the need to increase food supply does make this an issue, although the protein foods are exported to populations already well fed, not those that are hungry (Fig. 9).

4.2.2 Sunflower (*Helianthus annus*)

Criteria match for dry mass yield: There is no published research on sunflower biomass yield in New Zealand and the international literature is predominantly on seed and oil production. The reported DM yield in Perth, Australia was 14 tDM/ha (Steer et al. 1993) and the yield was similar in Oregon, USA trials (Kiniry et al. 1992). Yield was 11 tDM/ha in Victoria, Australia (Connor et al. 1985). Dry mass yields were 10.8 tDM/ha in research in Turkey (Goksoy et al. 2004) and 12.8–13.9 tDM/ha in a study in Greece (Archontoulis 2011).

A 2005–2006 trial by the authors with a forage sunflower cultivar in a fertile Hawke's Bay soil yielded up to 17 tDM/ha at the highest plant population density among several densities that were compared (the overall average yield was 14.4 tDM/ha). This crop had a very high average growth rate of 173 kg DM/ha/day (unpublished data). A 2009–2010 trial at two marginal sites produced sunflower yields of 10.4 and 8.1 tDM/ha (Kerckhoffs et al. 2011). The limiting factor was loss of the seed to birds in one location, since seeds are typically 25% of the total dry mass (Massignam et al. 2009). At the other site the low yield was due to severe water deficit (Fig. 10).

Agronomy: Sunflower has potential as a biomass species due to its moderate dry mass yield in mildly marginal conditions and a relatively short growing season.

Fig. 9 Maize (*Zea mays*). Selection '33 M54' is a long-season type and yielded 33 t DM/ha 2 months after this photo in Northland

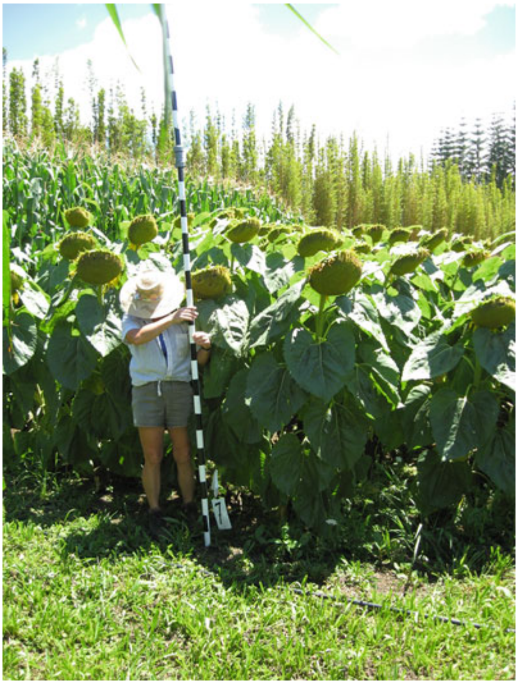

Fig. 10 Sunflower (*Helianthus annum*). Forage sunflower had lower dry mass than other species tested and has about 25% of its dry mass as seeds, which can be lost to birds

Since the aim of biomass production is to maximise sustainable yield on a year-round basis, a species with a fast growth rate that fits between other crops can satisfy a useful purpose. The irrigation response by sunflower has been studied in the Mediterranean (Goksoy et al. 2004; Sadras and Calvino 2001) and Australia (Connor et al. 1985).

Physiological aspects: In addition to soil water response (Connor et al. 1993; Steer et al. 1993) the effects of canopy architecture are very relevant to sunflower dry mass yield potential (Archontoulis 2011). Both of these high dry mass factors are less optimal in sunflower than in very high dry mass species such as cardoon and kenaf (Archontoulis 2011).

Issues: The greater drought susceptibility of sunflower than several high dry mass C4 grasses such as sorghum, maize and pearl millet makes it less adaptive to marginal soil water supply. The significant part of the total dry mass in the seeds (and the high risk of losing it to birds) and the somewhat lower dry mass yield even in good conditions are all negative factors for sunflower biomass production.

4.2.3 Sorghum (*Sorghum bicolor*)

Criteria match for dry mass yield: Dry mass yield of fibre sorghum in the north of Italy was 26.2 tDM/ha (Amaducci et al. 2000). High yields were also observed in Greece (Danalatos et al. 2009). The cooler New Zealand climate might be expected to limit yields and that has been the case based on the average yield of 15.5 tDM/ha from several NZ science reports (Cottier 1973; Taylor 1973; Taylor et al. 1974; Chu and Tillman 1976; Rhodes 1977; Piggot and Farrell 1980; Causley 1990). However, the mean would be much lower without the results in the reports by Piggot and Farrell (1980, 1984) who found that 'Sugar Drip' sweet sorghum averaged 25 tDM/ha in deep loams and well-drained fertile clays and 20 tDM/ha in dry friable soils in Northland, the warmest part of New Zealand. In the authors' 2010 trial in Northland the yield of the best subtropical sorghum cultivar was 30.3 tDM/ha (see Table 4) (Kerckhoffs et al. 2011).

Agronomy: Sorghum is not widely grown in New Zealand but its use for dairy forage is of current interest to farmers. It is generally found to yield lower than silage maize but to have greater drought tolerance and ability to recover (Singh and Singh 1995). Hybrid sorghum cultivars fall into three categories: sorghum x sorghum, sorghum x sudan and sudan x sudan crosses. New subtropical cultivars require testing of their potential to stay in vegetative mode for an extended period, increasing the biomass yield. Tests in Australia indicated high total dry mass from use of multiple cutting, for grazing as dairy feed (Johnson 2005). In the cooler New Zealand climate a higher total dry mass may be expected from a single harvest of a long-season cultivar. Effective weed control in this small-seeded crop is of agronomic importance, but provided by current herbicides.

C4 grass species usually have very high nutrient input requirements. The 'rule of thumb' of the seed company supplying the best two sorghum cultivars is that a 30 tDM/ha forage crop would remove over 500 kg/ha of nitrogen, even if a subtropical species does not produce seed. However, tissue analyses from our Northland field trial (Kerckhoffs et al. 2011) indicated that crop removal was only 240 kg N/ha.

Physiology: One feature of sorghum conducive to its use in marginal sites is better tolerance of and recovery from soil water deficit. Studies in Greece (Dercas and Liakatas 2007), India (Singh and Singh 1995) and the USA (Stone et al. 2002) have helped clarify agronomic response and physiology of water use. Nitrogen use by plant parts is another relevant aspect (van Oosterom et al. 2010) as is the effect of sowing rates on biofuel productivity (Wortmann et al. 2010).

Issues: While sorghum may grow well in conditions of low water availability, the main apparent drawback to use of sorghum for biomass production in New Zealand is that much of the country does not have warm enough temperatures for a long enough growing season. The suitable regions are below latitude 38° S. These include Northland, Waikato, Bay of Plenty, East Cape and Hawke's Bay. However, regions other than Northland could be cool enough some years to impact yields. Several of these regions have enough summer rainfall that the choice of 'marginal sites' may need to be based on yield restrictions other than soil water deficit, such as more frequent site susceptibility to cool weather. As with other agricultural crops there is also the issue that the use of sorghum as an energy crop competes with its use for livestock forage. Sorghum also has a high nitrogen fertiliser requirement. Although our trial measured nitrogen uptake by a fully matured crop to be only 240 kg N/ha, this level of nitrogen use is still an issue for a biomass crop unless the fuel conversion technology conserves nutrients. Gasification does not do so (Fig. 11).

4.2.4 Pearl Millet (*Pennesetum glaucum*)

Criteria match for dry mass yield: There has been very little use of this crop species in New Zealand, particularly for full season growth to its maximum biomass. Yield reports in Australia are on grain yield rather than biomass (Queensland Primary Industries and Fisheries 2005; Pacific Seeds 2009). Cultivars for feed seed production are short in both height and season, so forage cultivars are preferable for biomass. The potential for pearl millet to have a high yield in northern New Zealand is based on its height and growth similarities to sorghum in Australia (Pacific Seeds 2009) and on high sorghum yields in past New Zealand trials (Piggot and Farrell 1984). In the authors' 2010 trial in Northland the yield was very high, 31.2 t DM/ha (see Table 4) (Kerckhoffs et al. 2011).

Agronomy: When grown for biomass the cultural methods used are essentially the same as for subtropical cultivars of sorghum. Most information is directed at the feed quality of Pennesetum when used as forage, eg, in Queensland, Australia (Pacific Seeds 2009). The low protein content of pearl millet when grown all season rather than grazed is indicative that the nitrogen fertiliser requirement is likely to be much lower than when grown to be grazed.

Fig. 11 Sorghum (*Sorghum bicolor*). In the cooler climate of the lower North Island a sudan x sudan hybrid cultivar like 'Sprint' only yielded about 7 t DM/ha (**a**). In Northland the subtropical cultivar 'Jumbo' had a 30.3 t DM/ha yield 2 months after the photo (**b**)

Pearl millet has been found to be even more adaptive to soil water deficit than sorghum, at least in terms of grain production (Queensland Primary Industries and Fisheries 2005).

Issues: Like sorghum, pearl millet is an agricultural crop whose use as an energy crop competes with its use for livestock forage. The moderately high fertiliser inputs will require special crop management and end use of the biomass to make production sustainable (Fig. 12).

Fig. 12 Pearl millet (*Pennisetum glaucum*). The cultivar 'Nutrifeed' yielded as well as the subtropical sorghums in Northland (31 t DM/ha) 2 months after the photo

4.2.5 Hemp (*Cannabis sativa*)

Criteria match for dry mass yield: Hemp is a tall-growing short-season species grown for fibre or oil, including to a limited extent in New Zealand (McIntosh 1998). Research has focused on the production of fibre and seed oil, not biomass (McPartland et al. 2004) however the crop has reportedly yielded >20 t DM/ha in Italy, 19 t DM/ha in the Netherlands and relatively well on marginal sites (Struik et al. 2000). Models have been developed of both growth and industrial economics (Eerens 2003). The highest dry mass yields will probably come from different cultivars than used for oil and fibre. The few published reports of New Zealand dry mass yield (McIntosh 1998; Gibson 2007) indicated a wide range of yields, only the upper end of which makes hemp of interest as a principal summer crop for feedstock for gasification or other biofuel technologies. Yields of 14–20 t/ha were cited, but in several experiments they were <10 t/ha. However, industrial hemp could fill a useful niche in a biomass system since it achieved its maximum yield in a shorter time than other crops, perhaps enabling it to be grown between two high-yielding winter crops.

Recent New Zealand field measurements of dry mass, commissioned by the author in 2010, were made by Midlands Seed Ltd near Ashburton in the South Island. In plots harvested from a fibre cultivar the dry mass yield averaged 9.1 t DM/ha

Fig. 13 Hemp (*Cannabis sativa*). The height difference between an oil seed cultivar (*centre*) and the more suitable fibre cultivars (Photo courtesy of Midlands Seed Co., Ashburton, New Zealand)

(unpublished data), well below the 15 tDM/ha target deemed economically viable for summer annual crops to supply bioenergy facilities.

Agronomy: To achieve high dry mass may require sowing seed at quite a high rate (Struik et al. 2000). Nitrogen fertiliser above 100 kg N/ha had no benefit to dry mass yield (Struik et al. 2000). Hemp is also fairly adapted to periods of water deficit. A study of the economics of growing hemp fibre as a crop for land treatment of treated sewage (Eerens 2003) determined that it would be difficult even in the central North Island to produce two crops (two cuttings) as would be required for an economically viable treatment and fibre production system.

Issues: The largest hurdle to New Zealand production of hemp is the regulatory compliance costs of its growth, storage and shipment to ensure the crops do not contain illegal levels of drug THC, as found in other *Cannabis sativa* cultivars. There is also the need to document high yields in cooler South Island sites, where its use as a short crop between winter forage, grain or biomass crops would be most valuable. The best yields would be in northern New Zealand, but there are better species options there (Fig. 13).

4.2.6 Kenaf (*Hibiscus cannabinus*)

Criteria match for dry mass yield: Kenaf is a warm season annual species that grows very tall (>4 m in hot climates) with a high dry mass yield potential (Alexopoulou et al. 2000; Danalatos et al. 2006), Yields in a recent irrigation trial ranged across 19.6, 22.8 and 24.5 tDM/ha (Archontoulis 2011). Past research in New Zealand for use as paper pulp showed that in the cooler local climate the yield was <9 tDM/ha and the height was <1.7 m (Withers 1973).

Physiology: Canopy architecture findings help explain the high yield potential in the Mediterranean climate (Archontoulis 2011).

Issues: Kenaf requires warmer summers than occur in New Zealand. It is also susceptible to Botrytis infection and prone to keep growing if water is available, as is likely here. That may make it difficult to get the biomass dry enough for harvest.

4.3 Winter Annual Species

4.3.1 Tickbean (*Vicia faba*)

Criteria match for dry mass yield: Vicia faba (broad bean, fava bean) is a winter crop that has been reasonably well-researched as a forage crop in New Zealand. The dry mass yields reported in the South Island experiments were always less than 15 t DM/ha (Jones et al. 1989; Newton and Hill 1987; Rengasamy and Reid 1993). A 2011 Hawke's Bay trial with the cultivar 'Wizard' sown on 11 April and harvested 28 October yielded an impressive 24 t DM/ha (data not yet published).

Agronomy: Tickbean is of interest as a winter crop in rotation with a late-sown or short-season summer annual. This would be most feasible in regions with sufficient summer rainfall, such as Southland and several parts of the North Island. It is sown as early as possible in autumn after previous crop removal (e.g., April in New Zealand). Its cultural requirements have been described (Rengasamy and Reid 1993; Jones et al. 1989; Newton and Hill 1987). For use as forage it is harvested prior to its peak seed maturity when the feed value is not reduced by lack of water. Even for a mature harvest the soil water supply is only likely to be an issue during a rare winter drought in the eastern cropping districts of both North and South Islands. Nitrogen is fixed in the root system nodules.

Issues: Although the dry mass yield was very high in the 2011 trial, the favourable weather conditions, the timing of crop development and lack of disease may be hard to duplicate. It could be challenging to grow in marginal soil and colder South Island winters and still fit between summer crops, which also take longer in the cooler weather. In the wet winter climate there is a significant cost in keeping diseases such as chocolate spot under control. The tissue water content at harvest may also be higher than ideal for a biomass crop (Fig. 14). However, this species should be considered for the lower South Island.

4.3.2 Winter Cereals: Wheat (*Triticum aestivum*), Oats (*Avena sativa*), Barley (*Hordeum vulgare*) and Triticale (x *Triticosecale*)

Criteria match for dry mass yield: Cereal species sown in autumn or winter and harvested in early to mid summer have been shown to yield >15 t DM/ha in good arable soils in New Zealand. Dry mass yield is reported as 'whole crop yield' in cereal research, where grain yield is usually the focus. Winter wheat can have a

Fig. 14 Tickbean or broadbean (*Vicia faba*). A tall dense crop after a warmer than average Hawke's Bay winter growth season

whole crop yield >15 t DM/ha (de Ruiter 2004; Kerr and Menalda 1976; Stephen et al. 1977). Forage oats yielded 16.9 t DM/ha in the author's 2009 trial (unpublished), similar to other North Island findings (Kerr and Menalda 1976; Stephen et al. 1977; McDonald and Stephen 1979). Winter barley dry mass yields were 14.7–16.6 t DM/ha (Kerr and Menalda 1976; Scott and Hines 1991). Triticale whole crop yields can be >20 t DM/ha, both in the North Island (Scott and Hines 1991) and the southern South Island (Plant & Food Research unpublished trial results for clients). All results are yields on good arable crop land.

Agronomy: There is an active research programme that has documented soil water and nitrogen fertiliser responses in terms of grain yield (e.g., Carter and Stoker 1985). The research cited in the previous paragraph documented high biomass production of these cereal species for forage in New Zealand. The geographic focus for use of winter annual species as energy crops is the South Island, where species that require warmer conditions (such as sorghum) are not feasible. The main effort required to assess triticale (or other cereals) as energy crops is to determine their yields in marginal New Zealand sites, via research trials and/or use of crop models.

Issues: If dry mass yield is determined to be adequate (>13 t DM/ha may be sufficient if production costs are moderate) then the main issue is whether food/feed species should be used as energy crops. Another issue is the nitrogen fertiliser requirement,

Fig. 15 Triticale (*x Triticosecale*). The vegetative growth of triticale (at the rear) is much greater than the modern wheat in the foreground, and dry mass is also greater

which may be high with some cereals. If economic supply of feedstock to a gasification plant requires double cropping (having a short summer crop between winter triticale crops) then the feasibility of this, using 'marginal' sites, is also a relevant issue (Fig. 15).

5 Rapid Species Selection Approach

The review of literature presented above was the central element in meeting the first 2 year aim of a 6 year research project. However, the review was not in itself sufficient for the project aim and it was also tailored to be integrated with local New Zealand information on the performance of plant species. The aim was to obtain a reduced list of high dry mass species with suitable attributes.

A final 'short list' of the best species was reached in two steps, starting by excluding less suitable species until there was a manageable number remaining (termed pre-selection). This involved three elements, the principal one being the science literature review that is the focus of this paper (Sect. 5.1). The following two subsections describe the use of New Zealand expertise and the use of conceptual tools to structure the species comparisons using the literature information. The last element of pre-selection was the gathering of new preliminary field measurements (Sect. 5.4) to help identify the most promising 15 biomass species for the intended end use.

More detailed evaluations of the 15 pre-selected species included two additional procedures, a formal field trial in two climatic zones (Sect. 5.4) and the use of crop models (Sect. 5.5). Overall, our species selection approach may be novel in its integrated use of procedures that were all capable of delivering results within a 2-year timeframe. Sections 5 and 6 are therefore presented for the benefit of readers that may face similar time constraints in selecting among plant species for a particular use. Sections 5 and 6 therefore have more aspects of a 'Methods' paper than would usually be found in a review.

5.1 Pre-Selection of High Dry Mass Species Via Literature Review

A species selection approach with a step-wise structure was developed to achieve the project aims. The first step was a pre-selection of promising high dry mass species based on the international literature, as cited in the subsection for each species listed in Sect. 4. When a species was a widely-grown New Zealand agricultural crop then the literature review was used to specify attainable dry mass yield. Relevant information was collated for biomass productive potential for specific regions within New Zealand for potential use as an energy crop via gasification. Such species could be ranked and the best few species and cultivars identified. However, it is possible that the ranking on prime crop land will differ from the ranking on 'marginal' sites, which is the end objective of the research project. So even for major crop species there was an additional step required to estimate 'marginal site' yield before the final ranking could be made.

When a reviewed species had a reported high productivity and was likely to be adapted to part of the range of New Zealand climates, but was not present in the country, then additional procedures were required to establish its productivity ranking within 2 years. Plant introduction through New Zealand's rigorous biosecurity procedures would usually take too long. If a species had been introduced in past years, or had entered New Zealand inadvertently, then regulatory guidelines could be examined to see if the species identified in the literature review would be accepted for commercial use over extended areas of the country.

5.2 Pre-Selection of High Dry Mass Species Via Local New Zealand Expertise

Gathering local expert knowledge involved an effort to tap the institutional memories of research organisations regarding more obscure species of minor crops or weeds known to produce high dry mass. Measurements of dry mass had been recorded but not always published. This work was largely done during 1975–1985, a time of strong interest in bioenergy in New Zealand as elsewhere, due to the oil supply crises. Additional expert New Zealand advice on major arable crops came from commercial sources such as seed companies. Their records for a major crop like maize contain yield data from hundreds of field plots.

5.3 Pre-Selection Tools: Plant Growth Categories and Benchmark Species

As noted at the start of Sect. 4, the process of ranking of species in terms of high dry mass process was facilitated by distinguishing three categories of growth habit: summer annual, perennial and winter annual species, enabling more direct comparisons. Within each category it was possible to choose one species with well documented high dry mass performance in New Zealand. These are referred to as benchmark species. The research and seed company trial findings for maize and lucerne documented that these crops, when grown on fertile land with good water supply, had higher dry mass yields than other current New Zealand crop species of their types. Silage maize was designated as the benchmark for summer annuals and lucerne as the benchmark species for perennials. Details were presented in Sects. 4.1.1 and 4.2.1. Most high dry mass winter annuals are cereal grain species. The designated winter annual benchmark species was wheat (Sect. 4.3.2). All three benchmark species also shared the advantage of having crop models that are calibrated in the major production regions of New Zealand.

5.4 New Field Trial Data in New Zealand to Supplement the Literature Review

There was also a strong case, in the interest of time, to generate new field trial data to include in the species selection process. When a species was present in New Zealand, but there was inadequate dry mass data to rank it relative to other species, we planted small field trials along with familiar reference crops in order to make preliminary measurements of dry mass. This was done concurrently with the first year of the literature review. Those measurements were part of the pre-selection phase.

In the second year, more formal field trials were carried out with the more promising summer annual species/cultivars among the most promising 15 species. These generated new and climatically-relevant scientific data that is now published, therefore available as a new addition to the literature review. Results were a key part of selecting the final short list of 'best' species to assess for use in the bioenergy engineering research project.

5.5 Crop Models as a Tool to Estimate Dry Mass Yield in 'Marginal' Sites and to Compare Species

While the use of models may seem more appropriate to describe in a research paper than here, using this tool in the species selection procedure was necessary and the results are therefore reviewed. The rationale is based on the need during the final stage of species screening to rank species on how well they yield in 'marginal' sites rather than in prime crop land. Use of such sites is a more sustainable basis for bioenergy production. For previously researched benchmark species the yield data is from trials in good quality arable soils, so each of these species had to be checked as to whether it really belonged in the final list. The way to do this was via use of the crop models that could simulate yield under the environmental conditions that make a site marginal. This enabled the following step which was to compare the biomass yields of new candidate species to the 'marginal site' yields of benchmark species.

The models currently used in New Zealand are species-specific and local calibrations from the farming systems tool known as APSIM (Keating et al. 2003) developed in Australia. The three growth limiting factors that can be altered in the crop models are soil texture or depth, air temperature and rainfall. If a target yield for biomass production is set, such as 15 tonnes dry mass per hectare (t DM/ha), then the combinations of the three factors that restrict yield to 15 t DM/ha can be identified for each cropping region of New Zealand. While a benchmark species was used for this, the information that is used to assess a new species of that same growth habit type is an empirically- defined set of site conditions that represents 'marginal'. The target of 15 t DM/ha was chosen because all three benchmark species have demonstrated yields several tonnes per hectare more than that (see species details in Sect. 4).

The final step in selection of the best three to four species (in addition to the benchmark crops maize and lucerne) was to make use of any APSIM models of benchmark or other species to make more direct comparisons of available yield data. The aim for the best 'new' species is to utilise them in the subsequent 4 years of agronomic research and modelling of biomass supply to a gasification plant.

6 Overview of Species Selection

6.1 International Literature Pre-Selection

The result of the literature review was a compilation of the best 15 species to compare in more depth and the exclusion of a number of other species without further assessment.

Table 1 lists the species whose dry mass yields have previously been measured in New Zealand. See Chap. 4 for details of the basis for excluding each species from further consideration in the project on gasification feedstocks.

Table 1 Plant species in New Zealand assessed in the literature review but not included among the 15 promising species pre-selected for further evaluation during the first 2 years of the project

Common name	Scientific name	Exclusion criteria
Tagasaste or tree lucerne	*Chamaecytisus palmensis*	Low temp and disease sensitive
Toe toe	*Cortaderia fulvida*	Conservation restrictions
Wandering willie	*Tradescantia fluminensis*	Pest restrictions; low dry mass
Reed canary grass	*Phalaris arundinacea*	Pest restrictions
Napier grass	*Pennisetum purpureum*	Pest restrictions in regions
Pampas grass	*Cortaderia sellowana*	Pest restrictions in regions
Yacon	*Smallanthus sonchifolius*	Low temp sensitive; low DM

Table 2 Plant species considered due to being grown for biomass/biofuel outside New Zealand. These were assessed via literature review and by checking their status with New Zealand authorities. They were excluded from further consideration by the gasification feedstock project (full reasons are in Sect. 4)

Common name	Scientific name	Exclusion criteria
Sugarcane	*Saccharum hybrids*	Low temp sensitive
Switchgrass	*Panicum virgatum*	Requires hot summers
Jatropha	*Jatropha curcas*	For oilseed, not biomass
Water hyacinth	*Eichornia crasspies*	Prohibited pest species
Giant reed	*Arundo donax*	Restricted pest
Cardoon	*Cynara cardunculus*	Spreads by seed
Kenaf	*Hibiscus cannabinus*	Hard to get dry at harvest

Table 2 includes some species favoured for use as biofuel feedstock in other parts of the world. Sugarcane is one of the best sources of biomass and ethanol but requires tropical temperatures (Brito Cruz 2009). Jatropha has great potential as an oil seed tree crop and its cultivation is expanding in India (Jatropha World 2012), however it is used for seed production, not as a species for high total biomass. The other species are each considered in Sect. 4, where the issues that preclude them from use for gasification in New Zealand are described. Cardoon, while having several issues (see Sect. 4) may have better potential than the other species on this list. However, there is no New Zealand source of seed to test it at the necessary scale.

The outcome of the review of international literature and New Zealand expert advice was to reduce the number of candidate species to the 15 listed in Table 3. This also shows the New Zealand data sources available to compare and screen these crops. Field data is mostly from maize and sorghum variety trials by seed companies.

The method for the evaluation of summer annual species (other than hemp) from Table 3 was a formal field trial in two of New Zealand's warmer regions, at sites with 'marginal' soil/rainfall features. Perennial species could only be assessed where mature stands of plants existed or by analysis of yield in the New Zealand

Table 3 Herbaceous species pre-selected for use in the study and the type/source and number of crop plots of each

Common name	Scientific name	Commercial field data	New Zealand literature data	Crop Category
Lucerne	*Medicago sativa*	0	12	P
Harding grass	*Phalaris aquatica*	0	2	P
Miscanthus	*Miscanthus x giganteus*	0	0	P
Rapu	*Typha orientalis*	6	1	P
Gorse	*Ulex europaeus*	0	3	P
Jer. artichoke	*Helianthus tuberosus*	0	0	P or S
Maize	*Zea mays*	1054	7	S
Sorghum	*Sorghum bicolor*	64	11	S
Pearl millet	*Pennisetum glaucum*	0	0	S
Sunflower	*Helianthus annuus*	6	0	S
Hemp	*Canabis sativa*	1	4	S
Barley	*Hordeum vulgare*	0	26	W
Tickbean	*Vicia faba*	0	29	W
Triticale	*x Triticosecale*	0	8	W
Oats	*Avena sativa*	0	26	W

The three growth habit categories are Perennial (*P*), Summer annual (*S*) and Winter annual (*W*)

science literature. In some cases these results could be further refined by use of crop models. The timing of field trials for winter annual species, like perennials, did not fit within the 1 year available before a decision was required on the best four to six species, so ranking of these also relied on the New Zealand literature.

The cultivar choices in the trial with summer annuals were based on recommendations by researchers in the USA and Australia. We sourced seed of very high dry mass (usually subtropical) cultivars of C4 grass species of maize, sorghum and pearl millet. The trials also included sunflower and Jerusalem artichoke for use in cooler regions where the subtropical cultivars of C4 grasses would not be productive. A custom grower of the regulated species hemp was identified to make field measurements. The high DM clone of Miscanthus, *Miscanthus x giganteus* was also pre-selected for further investigation since its introduction to New Zealand was announced by a commercial venture (Brown 2009).

6.2 Species Selection Among the 15 Most Promising

6.2.1 Literature Review as Selection Basis

Among the species in Table 3 New Zealand literature was the basis to exclude some from the final list of three to four 'best' species for field testing as feedstock supply species for gasification. Those excluded from the short list were two perennials (rapu and gorse) and two winter annuals (barley and oats). The basis for these decisions is provided under *Issues* in each species' subsection of Sect. 4.

6.2.2 Field Measurements as Selection Basis

Preliminary field measurements led to the exclusion of Harding grass and hemp (see Sect. 4). Miscanthus was retained, even though it was not possible to establish a mature (3 year old) stand of the Mxg clone. The next best option was to calculate its biomass yield for several years with a crop model from the UK using the soil and weather data from a specific New Zealand site (see Sect. 4). The remainder of selection decisions were based on a field trial of summer annuals. The two sites were in Hawke's Bay and Northland (the warmest region, and where the trial site was irrigated due to below average rainfall). For details of the field trials see Kerckhoffs et al. (2011); other aspects will be reported in a subsequent research paper.

Table 4 provides evidence that the warmest region of New Zealand can produce very high DM yields of subtropical cultivars of maize, sorghum and pearl millet in sites with rooting depth restriction, provided rainfall is sufficient (or the crops are irrigated). All species at the Hawke's Bay site had very restricted yields in a year with an early summer water deficit, but results showed that some sorghum cultivars were much less affected than maize or pearl millet. Jerusalem artichoke was included in the trial since it can be grown as an annual.

The high yield results with tropical maize in the Northland trial confirm that maize was the correct choice as the benchmark species for summer annuals. The similarly high biomass yields with pearl millet and some sorghum cultivars also supports their consideration for use in New Zealand, although their geographic range is more restrictive than for maize. The performance of two sorghum cultivars in a drought situation is very promising for their use in marginal sites. Jerusalem artichoke also proved worthy of further investigation due to its modest nutrient and water requirements. In preliminary trials in Hawke's Bay and Canterbury the shoot dry mass yields were 15 and 17 t DM/ha, respectively (Kerckhoffs et al. 2011).

Sunflower was excluded from the final list of species for New Zealand following the field results in Table 4; the related species Jerusalem artichoke is more promising.

6.2.3 Ranking Procedures Among the Better Candidate Species

The species selection approach included making a yield estimate of species in marginal sites, in order to rank them to identify the best biomass crops for the intended use. This was done by the use of crop models, first applied to the benchmark species maize and lucerne. Their high yields in good arable sites were documented in Sect. 4. Section 7 summarises the application of APSIM models for the two benchmark species, maize and lucerne, to be presented in more detail in a research paper.

Table 4 Crop yields (t DM ha^{-1}) and dry matter percentages (DM%) at two locations

Crop	Cultivar	Northland Yield (t DM ha^{-1})	Northland DM (%)	Hawke's Bay Yield (t DM ha^{-1})	Hawke's Bay DM (%)
Maize	33 M54	33.7	45	13.2	37
Maize	38 H20	26.0	34	12	55
Sorghum	Bettagraze	19.5	27	11	44
Sunflower	Hysun 38	10.4	21	8.1	36
Sorghum	Jumbo	30.3	25	20.6	31
Pearl millet	Nutrifeed	31.2	29	13.3	29
Sorghum	Speedfeed	21.8	26	12.2	38
Sorghum	Sugargraze	28.1	24	17.7	27
Jerusalem artichoke	Inulinz	15.3[a]	21	-	-
LSD		6.1	3	5.3	8.2
F-pr		<0.001	<0.001	0.005	<0.001

Table adapted from Kerckhoffs et al. (2011). LSD: least square deviation
Jerusalem artichoke yield is shoot dry mass only, excluding tubers

It then summarises the further use of models, where available, to compare short-listed biomass species candidates to the relevant benchmark crop yield in marginal sites.

The species selection procedure described in this Section was used by the authors to advise the Biofuels to Syngas to Liquid Fuels programme of the University of Canterbury as to which species should receive further research attention (Renquist and Shaw 2010).

7 Modelled Crop Dry Mass in Marginal Conditions

7.1 Models for Benchmark Species: Maize and Lucerne

It would be a multi-year task using field trials to estimate the dry mass yield of maize and lucerne under marginal conditions. Crop models are very useful to estimate yields under such conditions. Both species have crop models in the Australian crop model package APSIM (Keating et al. 2003) and each of them also has New Zealand calibrations.

New Zealand scientists have developed calibrations of APSIM models in the main arable cropping regions using both research and commercial trial data. While these calibrations are still being refined in order to use the models in very precise crop physiological applications, our use of APSIM was less demanding. The requirement was just for accurate enough yield estimates to rank species as having "higher, lower or similar" dry mass yields.

Soil water supply and temperature are two of the key defining parameters in the crop models, and for major crop species it is already known whether they are more sensitive to a deficit of water or warm temperatures. Marginal sites can often be categorised as yield-limiting due to one or the other of these two environmental factors.

The details of the APSIM model output graphs and the tables that illustrate whether or not an acceptable DM yield is achieved in each combination of site conditions are not shown in this review, but will be more fully explored in a research paper on this topic.

7.1.1 Maize (Summer Annual Benchmark Species)

The target yield for the summer annual benchmark, maize, was set at 15 t DM/ha, a somewhat arbitrary value but a yield that is likely to prove economically viable for biomass production. This is well below the maximum yield achieved in major North Island regions (see Sect. 4). The APSIM maize model creates an output graph for each region with a range of yields in response to soil water-holding (a combination of soil depth and texture) and rainfall and temperature (relative to the regional mean values). The target yield value may appear once or more in an output graph, which is examined to identify what combinations of non-optimal temperature and water deficit (a function of soil water-holding capacity and rainfall) are associated with yields reduced to that target level, but not below.

From the APSIM output graph and a table that is populated with the site conditions associated with the target yield of the benchmark species maize an appropriate conclusion can be drawn. One example is: "a 15 t DM/ha maize yield in Hawke's Bay region could be achieved without irrigation in a high water-holding soil even with 40% below-average rainfall when mean temperature is average or 1° above average." The site situations that are 'marginal' for maize in that soil category (having a dry mass yield equal to or less than 15 t/ha) are those with lower relative rainfall and/or with relative mean temperatures outside the optimum values shown. Non-optimal temperatures in New Zealand are usually lower than the optimum, but are in some cases higher.

In contrast to Hawke's Bay, a 15 t DM/ha yield of maize grown in the same soil texture in Canterbury requires 50% above-average rainfall and a temperature mean 1–2° above average. This comparison of climatic regions indicates that for a marginal site (without irrigation) in the shorter growing season of the South Island, the benchmark species maize will yield less than its North Island target yield under average rainfall and temperature conditions. This increases the chances that another species (adapted to a lower seasonal heat requirement) could match maize at these higher latitudes.

7.1.2 Lucerne (Perennial Benchmark Species)

For the perennial species growth habit category the benchmark species lucerne was assessed in the same manner as with maize. The shape of the biomass yield response graph looked similar to the one for maize (neither shown here), since the yields from all hay cuttings were combined.

While the regional differences in simulated yield for non-irrigated lucerne were not as great as the regional differences in maize dry mass production, the difference between the Hawke's Bay and Canterbury regions was still notable. The Hawke's Bay simulation indicated a yield of 15 t DM/ha could be achieved in high water holding capacity soils in a season with a temperature mean equal to the long-term average, even when rainfall was 50% below average. To achieve an equivalent yield in Canterbury required temperatures 1–3° above normal, with rainfall 20% above the long-term mean.

Lucerne is nevertheless a proven and well adapted crop to use as a perennial benchmark in the South Island, to allow comparisons with new perennial biomass species that are likely to be important for marginal sites. Lucerne could also serve as a benchmark for summer annuals in sites where the benchmark species maize may not be well suited.

7.2 Models for Comparing Candidates to Benchmark Species

The target yield for the summer annual benchmark species, maize, is 15 t DM/ha, as described in the previous subsection. Expressing the target yield in terms of yield-limiting environmental conditions enables a comparison to other summer annual biomass species, if their yields can be observed in conditions that would also limit the benchmark species maize to a similar yield (as simulated in the model). A summer annual biomass species that yielded 15 t DM/ha or greater under marginal (target yield) conditions would be well-ranked to receive more detailed further assessment.

7.2.1 Sorghum Versus Maize

There is an APSIM model for sorghum which allows a direct comparison to simulated maize yields. We calibrated the model for the Waikato region (the region closest to Northland where there were data to calibrate APSIM) in order to compare simulated yield results to those of the 2010–2011 field trial in Northland that contained both sorghum and maize. The maximum yields simulated in favourable conditions in the Waikato region were much higher for maize than sorghum (>25 t DM/ha versus 18 t DM/ha). But using the maize 'marginal' target yield of 15 t DM/ha (where the required Waikato region site conditions for maize were medium soil water-holding, rainfall 20% below average, and temperature 1° below average) the differences with sorghum were smaller.

The conditions in which sorghum achieved the 15 t DM/ha target yield were 50% less rainfall than average and mean temperature 1° above normal. Those conditions were wet enough for sorghum to match the 15 t DM/ha yield of maize, but they were

2° cooler than the sorghum optimum mean temperature. This was quite limiting and the sorghum model predicted a yield of only 12.8 tDM/ha. So in average Waikato weather the benefit of the drought resistance of sorghum is more than offset by its sensitivity to low temperature, even just 1 °C below average. If the models are accurate in this respect, it may explain the variable results with sorghum in most North Island districts. For example, the model predicted a large yield decrease (yield <50%) in sites that are 3° cooler than the optimum. This supports the findings of our 2009 field observations in the lower North Island (unpublished), where dry mass yield was less than half of the predicted Waikato yield.

The model analysis also highlights the importance of defining 'marginal' appropriately for the crop species and regional climate.

7.2.2 Sunflower Versus Maize

There is a sunflower model in the APSIM software, so it was possible to do a New Zealand calibration and directly compare sunflower to maize and sorghum yield simulations in the Hawke's Bay region. Even under the most favourable conditions maximum simulated sunflower dry mass yield was 11.4 tDM/ha. This underestimate is probably due to the model being developed using oilseed cultivars, which are more compact with lower dry mass than forage cultivars. But it also excludes the effect of bird predation on seed. This finding supports the field trial results (Kerckhoffs et al. 2011) where dry mass yield of a forage sunflower cultivar was lower than either maize or sorghum in both irrigated and non-irrigated trials.

7.2.3 Giant Miscanthus Versus Lucerne

The Mxg clone of Miscanthus is a very promising biomass species, so it was a priority to assess it as fully as possible during the species selection process. While there is no APSIM crop model, there is a Miscanthus model in the UK that was available to utilise for yield simulation (Hastings et al. 2009). The APSIM lucerne model also had a benchmarking role, to simulate the perennial species target yield in the appropriate climatic region for a yield comparison to the simulated average yield of Mxg at a particular site in that region.

The UK model for Mxg was applied to a specific site near Huntly in the Waikato region. Using soil information and meteorological records from the recommended station, the first author of the paper on the Miscanfor21SP model (Hastings et al. 2009) simulated the 13-year annual crop dry mass. The simulated peak dry mass in early winter averaged 27 tDM/ha, while late winter mean yield (the preferred time of harvest) was 18.7 tDM/ha.

The APSIM lucerne model, to compare to the Miscanfor21SP output, was calibrated for the Waikato using very similar but longer-term met data. The benchmark target yield is 15 tDM/ha in site conditions with 20% below average rainfall and a temperature right at the long-term mean. This is probably wetter than the 13-year data set used for the Miscanthus model (which included six dry years). The apparent conclusion is that Mxg is reasonably likely to exceed the yield of lucerne and is certainly worth undergoing further research.

7.2.4 Jerusalem Artichoke Versus Lucerne

An assessment was made to compare the promising new biomass species, Jerusalem artichoke, with lucerne. Informal yield comparisons in the Hawke' Bay region, where the most field data has been collected, have shown shoot dry mass yields near 15 tDM/ha in good arable soils (Kerckhoffs et al. 2011). This is higher than first-year yields of Hawke's Bay lucerne, but lower than the best lucerne yields in the second and third years (Shaw et al. 2005b).

Using the APSIM lucerne model, a target yield of 15 tDM/ha in Hawke's Bay region was associated with the following site conditions: High soil water-holding, rainfall 50% below average and temperature 1–2° above average. Jerusalem artichoke is, based on several studies (Kays and Nottingham 2008), able to grow well despite some water deficit and has high water use efficiency. It is also able to grow at cooler temperatures than many major crop species, having a heat unit base temperature of 0° for growth (Kays and Nottingham 2008).

While this is a new biomass crop in New Zealand, the preliminary conclusion is that shoot dry mass yield is similar to lucerne in Hawke's Bay region (Kerckhoffs et al. 2011). In the South Island, where non-irrigated lucerne yields in most soils are lower, Jerusalem artichoke has the potential to out yield lucerne. Field trials in the South Island, assisted by use of the APSIM model for lucerne, should clarify this matter.

7.2.5 Triticale Versus Wheat

The benchmark species chosen for the winter annual crop growth category is wheat. This crop is well-modelled in New Zealand using APSIM (Keating et al. 2003). While winter wheat cultivars have fairly high whole crop dry mass, e.g., 15.3 tDM/ha (Stephen et al. 1977), it is such a key direct human food crop that there would be market resistance to using it as a bioenergy crop species. Among the cereal grains triticale produces the highest dry mass yields in New Zealand, e.g., 22 tDM/ha (Scott and Hines 1991) and has the further advantage that it is not used as human food.

There is not yet an APSIM model for triticale, so a direct measure of its yield potential on marginal sites will require field tests. Wheat yields under marginal site conditions similar to those in the field trial can be estimated using the wheat APSIM model, or by including interspersed plots of wheat with the triticale.

8 Conclusions

This review of biomass species aimed to screen and rank candidate species in terms of high dry mass production in the climates found in New Zealand. The temperate rain-fed climate, with only mild winter frosts in most arable districts, could support the growth of an abundance of species. However, the review was not designed to be all-inclusive but 'nevertheless did consider most non-woody high dry mass species likely to meet the relevant criteria for use as bioenergy crops.'

8.1 The Methodological Tool for Species Selection

The engineering project research aim was to assess the best three New Zealand biomass species in a gasification plant supply model. Our sub-contracted aim for the crop research was to identify three species that are highly suitable to serve this purpose. That ultimate decision will be based on research that includes the species selection phase (with the species short list reported here) and further acquisition of agronomic knowledge on successful culture of the new species, as the basis for selecting the three most suitable species. This information will be applied as inputs to an engineering model on gasification of the biomass.

The review findings should, however, have wider interest and applicability to new energy crops research in other countries. Where there is an urgency to develop renewable fuels, the option of biofuels is likely to be assessed. The first step, screening of biomass species for regional suitability, could easily take a decade but may (as in this case) need to be completed more quickly. This review includes a section on the elements (in addition to a science literature review) of a species selection approach that was able to deliver species selection results in 2 years.

To summarise our 2-year species selection procedure, we:

1. began with a standard international literature review;
2. grouped prospective species into three categories based on growth habit (summer annual, perennial and winter annual);
3. sourced NZ-specific expertise on biomass and weed species, including identification of three well-studied New Zealand crop species to act as a benchmark for each growth habit category;
4. gathered new field data to update findings and fill gaps in New Zealand knowledge;
5. utilised APSIM crop models to simulate dry mass yield of benchmark species in 'marginal' site conditions; and
6. used models of the pre-selected candidate species (where they existed) to simulate dry mass yield in marginal conditions to compare to marginal yield of their benchmark species.

The combined use of these elements represents a methodological tool to quantify species screening and ranking. The first two steps shortened the biomass species candidate list from 'all world species that were reported to have high dry mass yield' to a manageable number for closer examination. The third and fourth steps made use of information particular to New Zealand to further narrow the field to the 15 most promising species. The benchmark species for each growth habit category were the highest yielding arable crop species that also had crop models well calibrated in multiple climatic regions of New Zealand. These were silage maize as the summer annual benchmark, lucerne as the benchmark perennial and winter wheat as the winter annual benchmark.

The final steps involved use of crop models (the Australian APSIM models) to calculate the most relevant 'target yield' to compare candidate species to. This was the simulated dry mass yield of the appropriate benchmark species in the 'marginal' site conditions under which it can still produce an acceptable yield, nominally set at 15 t DM/ha. A species that requires optimal conditions to yield this dry mass would fail the comparison, along with all low dry mass species. When an APSIM crop model also existed for a candidate biomass species then its marginal site yield was also simulated, to allow a direct comparison with the target yield.

These selection procedures will feed into the next stage: agronomic research on crop growth and meeting the year round supply to the fuel plant using a combination of species and biomass storage. No new biomass species came to our attention in the following 18 months that would have met the criteria and whose reported dry mass yield in the climatic range of New Zealand would have ranked it ahead of the species selected (listed in the next section).

8.2 *The Selected 'Best' Species for New Zealand*

We have identified seven species suitable as gasification feedstock in terms of high yield and adaptation to marginal sites. Any three of these can be used in an engineering supply model for gasification. Two of the benchmark species are among this short list. These are lucerne (*Medicago sativa*) and silage maize (*Zea* mays). The other two suitable arable crops are triticale (*x Triticosecale*) and tickbean (*Vicia faba*). The new or less well-known species identified as the most promising biomass crops in the relevant New Zealand climates were subtropical cultivars of sorghum (*Sorghum bicolor*), Jerusalem artichoke (*Helianthus tuberosus*) and the Mxg clone of Miscanthus (*Miscanthus x giganteus*).

To compare the features of the species we have selected it will be useful to refer back to the Sect. 4 list of the key attributes an ideal New Zealand biofuel crop should possess.

8.2.1 General Features of the Best Biomass Species

Note that three of the 'best' seven species are perennials; these have inherent advantages in terms of sustainability and greenhouse gas minimisation (for both biomass and food crops). Less cultivation lowers CO_2 losses from soil and reduces erosion and nutrient loss to surface waterways.

Biomass species that are somewhat drought resistant but have a good yield response to greater rainfall could be used in ‘marginal’ sites defined as areas where soil water supply is not dependable. That would reserve the most reliable sites for food crops or livestock forage/grazing. The biomass sites would have very high yields in years when they had ample rainfall and could therefore have an overall average dry mass yield that may still prove to be economically viable. This could apply to sorghum in the warmer regions of New Zealand and to Jerusalem artichoke and Miscanthus in many regions.

Special considerations apply to the cooler climate of the lower South Island, although the advantages of using perennial species remain the same. Whether winter annual or summer annual species have a more favourable cropping season depends to some extent on what second crop can be grown to maximise annual biomass. The cooler South Island weather is a disadvantage for many summer annuals, but cereal grains now have high-yielding summer cultivars. This enables both types of rotations: a winter cereal plus a partial short-season crop such as sunflower or a spring cereal followed by a winter legume such as tickbean.

8.2.2 Specific Features of the Best Biomass Species

Jerusalem artichoke. As a new biomass species in New Zealand not all of its features are definitively known. However, its ability to rapidly establish a canopy following spring or early summer planting is a strong point compared to any other perennial crops tested. If each species is rated by the Sect. 4 list of criteria for the ideal biomass crop (repeated at the start of Sect. 8.2), Jerusalem artichoke receives more ticks than any other species. If the European evidence for higher shoot dry mass at higher latitudes is duplicated in New Zealand then Jerusalem artichoke will be a particularly useful species for South Island biomass production.

While shoot dry mass yields are lower than some species, no issues noted to date appear to seriously detract from this species’ potential in the majority of New Zealand (but not the northernmost regions due to lack of chilling of tuber buds). Information is still lacking on the costs and protocols for procedures such as planting and storing tubers.

Miscanthus. The clone Mxg is now planted in research trials and at least three commercial plantings in New Zealand. These have documented the species’ ability to establish and grow well in this country. In terms of the Sect. 4 list of criteria for the ideal biomass crop Mxg satisfies a large number of the criteria and is likely to have a very high dry mass yield. Miscanthus has low nitrogen content in its mass, so it requires less nitrogen fertiliser and its combustion produces less reactive nitrogen.

Some issues to resolve include achieving easy crop establishment using tissue cultured plants. Weed competition has been a major difficulty, particularly in crops being established in the autumn since there are weed species that grow better than Mxg in the mild New Zealand winter climate. Potential issues, such as high moisture content at harvest if regrowth occurs in late winter, can only be resolved once the New Zealand test plantings are old enough.

Lucerne. This perennial benchmark species is well proven for its moderate to high dry mass productivity and adaptability to a wide range of environments, including those that are considered marginal. While the yield is rather sensitive to low temperature and low soil water supply that may also prove to be true of newer species. Another feature of lucerne is past experience using the leaves for higher value feeds; this would leave the stems as a lower-cost energy feedstock. Whole crop lucerne has the same issue as maize silage: a fuel plant will need to compete with its high value as livestock forage. Lucerne ticks many of the criteria of an ideal biomass species, but not low nitrogen content and ease of establishment without irrigation or by minimum tillage. On the other hand, management practices to deal with issues are well developed.

Triticale. This high-yielding cereal grain was selected as the winter annual with the best biomass potential, particularly for use in the cooler parts of New Zealand. Since summer rainfall is much less likely to be limiting in the Southland region the crop land would rarely be 'marginal' for soil water supply. However, there are other yield-limiting factors such as low temperature that could be used to define marginal sites.

Since both winter annual and spring (summer annual) cultivars of triticale produce good yields in the Southland region, there are two cropping scenarios that could use triticale to maximise annual dry mass yield from a site. If used as a winter annual (harvested midsummer) the late summer rainfall may be sufficient for a fast-growing species between the harvest of one winter annual and the planting of the next one, such as sunflower or hemp. This would make use of the solar energy that would be missed between two triticale crops and increase the total biomass yield. Alternatively, with triticale grown as a summer annual the second species would need to be winter hardy and preferably a legume, such as tickbean (*Vicia faba*) or crimson clover (*Trifolium incarnatum*). Triticale ticks a number of criteria of an ideal biomass species, but not the ones to be a perennial, to have low nutrient requirement, easy pest control, and low nitrogen and ash content.

Maize. This benchmark summer annual is the leading biomass crop in Europe, mainly for use in anaerobic digesters for methane. So there are clearly some circumstances where it is justified to grow as biomass, even on regular arable crop land. The use of maize grain for ethanol would be in direct competition with world food supply, but the use of silage maize is only in competition with livestock forage. If subtropical cultivars are grown in a crop rotation and treated differently than forage (fertilised less and harvested prior to seed development) it would increase sustainability relative to a continuous forage maize rotation.

Regarding its production on marginal land, the type of site would differ from sorghum. Maize is too sensitive to water deficit for sites in the higher probability range of drought. On the other hand, sites that are 2 °C too cool for sorghum are still optimal for maize, so sites that are more prone to even cooler seasons may be considered marginal for maize. The same argument as applied to soil water supply in Sect. 8.2.1 may apply to maize regarding cool season risk. Maize ticks the ideal species criteria for high dry mass but not the ones to be a perennial, to have low nutrient requirement, easy pest control, and low nitrogen and ash content.

Sorghum. Subtropical sorghum cultivars showed considerable promise for use in the warmer regions of New Zealand, especially Northland. The trial was irrigated due to an extended drought, so further tests in rain-fed marginal sites are required. The criterion that biomass be produced on 'marginal' sites does create an argument for excluding a summer annual grass like sorghum due to its high nitrogen fertiliser requirement (see Sect. 4.2.3). However, use of a winter legume crop such as tickbean between sorghum crops could address this drawback. Sorghum ticks the ideal species criteria for high dry mass and tolerance of water deficit but not the ones to be a perennial, to have low nutrient requirement, early spring growth, and low nitrogen and ash content.

Species ranking: There is a good chance that both Miscanthus and Jerusalem artichoke will be ranked in the top three biomass species once the agronomic studies characterise the two species' potential in New Zealand. Quantifying the yield of triticale in marginal site conditions also requires added field data or modelling. However, we estimate it will rank in the first four species based on current knowledge. Among the four better-known species the current ranking for use by gasification using the criteria in this review is: (1) maize, (2) lucerne, (3) tickbean and (4) sorghum. For discussion that includes preliminary findings from our 2012 field trials with Jerusalem artichoke see the review article by Kerckhoffs and Renquist (Kerckhoffs and Renquist 2012).

References

Alexopoulou E, Christou M, Mardikis M, Chatziathanasiou A (2000) Growth and yields of kenaf varieties in central Greece. Ind Crop Prod 11:163–172

Amaducci S, Amaducci MT, Benati R, Venturi G (2000) Crop yield and quality parameters of four annual fibre crops (hemp, kenaf, maize and sorghum) in the north of Italy. Ind Crop Prod 11:179–186

Amon T, Amon B, Kryvoruchko V, Machmueller A, Hopfner-Sixt K, Bodiroza V, Hrbek R, Friedel J, Poetsch E, Wagentristl H, Schreiner M, Zollitsch W (2007) Methane production through anaerobic digestion of various energy crops grown in sustainable crop rotations. Bioresour Technol 98(17):3204–3212

Angelini LG, Ceccarini L, Nassi o Di Nasso N, Bonari E (2009) Long-term evaluation of biomass production and quality of two cardoon (*Cynara cardunculus* L.) cultivars for energy use. Biomass Bioenergy 33:810–816

Archontoulis SV (2011) Analysis of growth dynamics of Mediterranean bioenergy crops. Dissertation, Wageningen University

Archontoulis SV, Vos J, Yin X, Bastiaans L, Danalatos NG, Struik PC (2011) Temporal dynamics of light and nitrogen vertical distributions in canopies of sunflower, kenaf and cynara. Field Crop Res 122:186–198

Banse M, van Meijl H, Tabeu A, Woltjer G, Hellmann F, Verburg PH (2011) Impacts of EU biofuel policies on world agricultural production and land use. Biomass Bioenergy 35:2385–2390

BANZ (2011) New Zealand bioenergy strategy. Bioenergy, 20 pp. http://www.bioenergy.org.nz/NZBioenergyStrategy2010.pdf. Retrieved 20 July 2011

Bauen A, Berndes G, Junginger M, Londo M, Vuille F (2009) Bioenergy—a sustainable and reliable energy source: a review of status and prospects. IEA Bioenergy ExCo 2009:06
Beringer T, Lucht W, Schaphoff S (2011) Bioenergy production potential of global biomass plantations under environmental and agricultural constraints. Glob Chang Biol Bioenergy 3:299–312
Berndes C (2002) Bioenergy and water—the implication of large scale bioenergy production for water use and supply. Glob Environ Change 12:253–271
Bessou C, Ferchaud F, Gabrielle B, Mary B (2010) Biofuels, greenhouse gases and climate change. Agron Sustain Dev 31:1–79
Biosecurity NZ (2011a) Pampas grass (Cortaderia sellowana) in New Zealand. NZ Ministry of Agriculture & Forestry. http://www.biosecurity.govt.nz/pests/pampas-grass. Accessed 28 Oct 2011
Biosecurity NZ (2011b) Elephant grass (Pennisetum purpureum) is in New Zealand. Pests and diseases webpage. http://www.biosecurity.govt.nz/pests/elephant-grass. Accessed 28 Oct 2011
Biosecurity NZ (2012a) Giant reed (Arundo donax) in New Zealand. NZ Ministry of Agriculture, Forestry and Fisheries. http://www.biosecurity.govt.nz/pests/giant-reed. Accessed 12 Jan 2012
Biosecurity NZ (2012b) Water hyacinth factsheet. NZ Ministry of Agriculture, Forestry and Fisheries. http://www.biosecurity.govt.nz/files/pests/water-hyacinth/water-hyacinth-fact-sheet.pdf. Accessed 27 Feb 2012
Blanco-Canqui H, Lal R (2009) Corn stover removal for expanded uses reduces soil fertility and structural stability. Soil Sci Soc Am J 73(2):418–426
Börjesson P, Tufvesson L, Lantz M (2010) Life cycle assessment of biofuels in Sweden. Report No. 70—Environmental and energy systems studies. Lund University, Lund, 85 pp
Booker JW (2008) Production, distribution and utilisation of maize in New Zealand. M. App. Sc. Thesis, Lincoln University
Boundy B, Davis SC, Wright L, Badger PC, Perlack B (2010) Biomass energy data book 2010. US Dept of Energy, 230 pp. http://cta.ornl.gov/bedb
Brito Cruz CH (2009) Bioenergy in Brazil. On-line presentation by the Brazilian Academy of Sciences. http://www.abc.org.br/IMG/pdf/doc-11.pdf. Accessed 17 Jan 2012
Brown PC (2009) Miscanthus—New Zealand experience. In: Proceedings of "Linking technology and biomass" conference, New Zealand. IEA Bioenergy Task 30, short rotation crops for bioenergy. Website www.shortrotationcrops.org
Brown HE, Moot DJ, Pollack KM, Inch C (2000) Dry matter production of irrigated chicory, lucerne and red clover in Canterbury. Agron N Z 30:129–137
Brown HE, Moot DJ, Pollack KM, Inch C (2003) Long term growth rates and water extraction patterns of dryland chicory, lucerne and red clover. Legumes for dryland pastures. In: Proceedings of the New Zealand Grassland Association Symposium 2003:91–99
Brown HE, Moot DJ, Pollack KM (2005a) Herbage production, persistence, nutritive characteristics and water use of perennial forages grown over 6 years on a Wakanui silt loam. N Z J Agric Res 48(4):423–439
Brown HE, Moot DJ, Teixeira EI (2005b) The components of lucerne (Medicago sativa) leaf area index respond to temperature and photoperiod in a temperate environment. Eur J Agron 23(4):348–358
Brown HE, Moot DJ, Teixeira EI (2006) Radiation use efficiency and biomass partitioning of lucerne (Medicago sativa) in a temperate climate. Eur J Agron 25(4):319–327
Bryan BA, King D, Wang E (2010) Biofuels agriculture: landscape-scale trade-offs between fuel, economics, carbon energy, food and fiber. Glob Chang Biol Bioenergy 2:330–345
Campbell JE, Lobell DB, Genova RC, Field CB (2008) The global potential of bioenergy on abandoned agriculture lands. Environ Sci Technol 42:5791–5794
Carter KE, Stoker R (1985) Effects of irrigation and sowing date on yield and quality of barley and wheat. N Z J Exp Agric 13:77–83
Causley DC (1990) Effect of minimum tillage, sowing rate, and sowing time on the yield of a sorghum-sudangrass hybrid in the Manawatu. N Z J Agric Res 33(1):15–20

Ceotto E (2005) The issues of energy and carbon cycle: new perspectives for assessing the environmental impact of animal waste utilization. Bioresour Technol 96:191–196
Ceotto E, Di Candilo M (2010) Sustainable bioenergy production, land and nitrogen use. In: Lichtfouse E (ed) Biodiversity, biofuels, agroforestry and conservation agriculture. Springer Science+Business Media B.V., New York, pp 101–122
Christian DG, Riche AB, Yates NE (2008) Growth, yield and mineral content of Miscanthus x giganteus grown as a biofuel for 14 successive harvests. Ind Crop Prod 28:320–327
Chu ACP, Tillman RF (1976) Growth of a forage sorghum hybrid under two soil moisture regimes in the Manawatu. N Z J Exp Agric 4(3):351–355
Clifton-Brown JC, Lewandowski I, Andersson B, Basch G, Christian DG, Bonderup-Kjeldsen J, Jørgensen U, Mortensen J, Riche AB, Schwarz K-U, Tayebi K, Teixeira F (2001) Performance of 15 Miscanthus genotypes at five sites in Europe. Agron J 93(5):1013–1019
Clifton-Brown JC, Stampfl PF, Jones MB (2004) Miscanthus biomass production for energy in Europe and its potential contribution to decreasing fossil fuel carbon emissions. Glob Chang Biol 10:509–518
Connor DJ, Jones TR, Palta JA (1985) Response of sunflower to strategies of irrigation. 1. Growth, yield and the efficiency of water-use. Field Crop Res 10(1):15–36
Connor DJ, Hall AJ, Sardas VO (1993) Effects of nitrogen content on the photosynthetic characteristics of sunflower leaves. Aust J Plant Physiol 20:251–263
Cosentino SL, Patane C, Sanzone E, Copani V, Foti S (2007) Effects of soil water content and nitrogen supply on the productivity of *Miscanthus x giganteus* Greef et Deu. in a Mediterranean environment. Ind Crop Prod 25:75–88
Cottier K (1973) Experiments with warm-zone crops for summer green feed in Waikato. Proc Agron N Z 3:25–31
Dale VH, Kline KL, Wiens J, Fargione J (2010) Biofuels: implications for land use and biodiversity. Biofuels and sustainability reports, January 2010. Ecological Society of America biodiversity. Biofuels Sust Reps Series, Ecological Society of America. http://www.esa.org/biofuelsreports. Accessed 12 Aug 2012
Danalatos NG, Gintsioudis II, Skoufogianni E et al (2006) Three years kenaf cultivation in central Greece: assessment and future perspectives. In: Proceedings of the international conference on information systems in sustainable agriculture, agro-environment food and technology, Volos, Greece, pp 382–386
Danalatos NG, Archontoulis SV, Mitsios I (2007) Potential growth and biomass productivity of Miscanthus x giganteus as affected by plant density and N fertilization in central Greece. Biomass Bioenergy 31:145–152
Danalatos NG, Archontoulis SV, Tsibukas K (2009) Comparative analysis of sorghum vs. corn growing under optimum and water/nitrogen limited conditions in central Greece. In: Proceedings of the 17th European biomass conference, Germany, pp 538–544
Davis SC, Anderson-Teixeira KJ, Delucia EH (2009) Life-cycle analysis and the ecology of biofuels. Trends Plant Sci 14(3):140–146
de Fraiture C, Berndes G (2009) Biofuels and water. In: Proceedings of the scientific committee on problems of the environment (SCOPE) international biofuels project reference 199 rapid assessment, Gummersbach, Germany. Cornell University, Ithaca, pp 139–153. http://cip.cornell.edu/biofuels/
de Ruiter J (2004) Performance indicators for harvest timing of whole crop cereals for silage. New directions for a diverse planet: Proceedings of the 4th international crop science congress, Brisbane, Australia, 26 Sep–1 Oct 2004.ISBN 1 920842 20 9. www.cropscience.org.au
DEFRA (2001) Planting and growing miscanthus. Best practice guidelines. Department for Environment, Food & Rural Affairs, UK, 20 pp
Densley RT, Kolver ES, Miller DB, Williams ID, Tsimba R (2005) Comparison of conventional and leafy maize silage hybrids in New Zealand dairy farm systems. Proc N Z Grassl Assoc 67:163–168
Dercas N, Liakatas A (2007) Water and radiation effect on sweet sorghum productivity. Water Resour Manage 21:1585–1600

Douglas JA (1986) The production and utilization of lucerne in New Zealand. Grass Forage Sci 41(2):81–128

Douglas JA, Follett JM, Douglas MH, Deo B, Scheffer JJC, Littler RA, Manley-Harris M (2007) Effect of environment and time of planting on the production and quality of yacon (*Smallanthus sonchifolius*) storage roots. N Z J Crop Hortic Sci 35:107–116

Duer H, Christensen PO (2010) Socio-economic aspects of different biofuels development pathways. Biomass Bioenergy 34:237–243

EEA (2006) Environmental Energy Agency, How much bioenergy can Europe produce without harming the environment? Report 7, ISBN 92–9167–849-X c_ EEA, Copenhagen, p 72

Eerens JPJ (2003) Potential economic viability of growing industrial hemp (Cannabis sativa) at the Taupo, New Zealand effluent disposal site. N Z J Crop Hortic Sci 31:204–208

Egunjobi JK (1971) Ecosystem processes in a stand of *Uleux europaeus* L. 1. Dry matter production, litter fall and efficiency of solar energy utilization. J Ecol 59:31–38

Environment Canterbury (2011) Reed canary grass, weed of the month. http://ecan.govt.nz/publications/General/weed-of-the-month-reed-canary-grass-000710.pdf. Accessed 28 Oct 2011

Fischer G, Prieler S, van Velthuizen H, Berndes G, Faail A, Londo M, de Wit M (2010a) Biofuels production potential in Europe: sustainable use of cultivated land and pastures. Part II. Land use scenarios. Biomass Bioenergy 34:173–187

Fischer G, Prieler S, van Velthuizen H, Lensink SM, Londo M, de Wit M (2010b) Biofuels production potential in Europe: sustainable use of cultivated land and pastures. Part I. Land productivity potentials. Biomass Bioenergy 34:159–172

Franco C, Lopes H, Pinto F, Andre R, Cabrita GI (2009) Gasification study of *Cynara cardunculus* to produce hydrogen rich gas. In: Proceedings of the 17th European biomass conference, Hamburg, Germany, pp 629–634

Fritsche UR, Kampman B, Bergsma G (2009) Better use of biomass for energy. Position paper of IEA RETD and IEA bioenergy, International Energy Agency (IEA), 151 pp

Galloway JN, Cowling EB, Seitinger SP, Socolow RH (2002) Reactive nitrogen: too much a good thing? Ambio 31:60–63

Gibson AR (2007) Growth studies with hemp (*Cannabis sativa* L.). M.S. thesis, Massey University, 45 pp

Goksoy AT, Demir AO, Turan ZM, Dagustu N (2004) Response of sunflower (*Helianthus annuus* L.) to full and limited irrigation at different growth stages. Field Crop Res 87:167–178

Gominho J, Lourenco A, Palma P, Lourenco ME, Curt MD, Fernandez J, Pereira H (2011) Large scale cultivation of *Cynara cardunculus* L. for biomass production – a case study. Ind Crop Prod 33:1–6

Gunnarson S, Malmberg A, Mathisen B, Theander O, Thyselius L, Wunsche U (1985) Jerusalem artichoke (Helianthus tuberosus) for biogas production. Biomass 7:85–97

Hall P, Gifford J (2007) Bioenergy options for New Zealand: a situation analysis of biomass resources and conversion technologies. Scion Research, Rotorua

Hastings A, Clifton-Brown J, Wattenbach M, Mitchell CP, Smith P (2009) The development of MISCANFOR, a new Miscanthus crop growth model: towards more robust yield predictions under different climatic and soil conditions. Glob Chang Biol Bioenergy 1:154–170

Heaton EA, Dohleman FG, Long SP (2008) Meeting US biofuel goals with less land: the potential of Miscanthus. Glob Chang Biol 14:2000–2014

Howarth RW, Bringezu S (eds) (2009) Biofuels: environmental consequences and interactions with changing land use. Proceedings of the scientific committee on problems of the environment (SCOPE) international biofuels project rapid assessment, Gummersbach, Germany. Cornell University, Ithaca. http://cip.cornell.edu/biofuels/

IEA Bioenergy Executive Committee (2009) Bioenergy—the impact of indirect land use change. May 2009 Workshop ExCo63, IEA Bioenergy: ExCo: 2009: 04

Jatropha World (2012) The national mission on Jatropha biodiesel. Centre for Jatropha promotion. http://www.jatrophabiodiesel.org/indianPrograms.php. Accessed 17 Jan 2012

Johnson B (2005) Silage: forage crop and pasture-darling downs. http://www2.dpi.qld.gov.au/pastures/4084.html. Accessed 23 Mar 2009

Jones AV, Andrews M, Foorde JDA (1989) Seasonal growth and final yield of autumn sown spring and winter field bean (Vicia faba L.) cultivars. Proc Agron Soc N Z 19:71–75

Karpenstein-Machan M (2001) Sustainable cultivation concepts for domestic energy production from biomass. Crit Rev Plant Sci 20(1):1–14

Katola T, Salmi T (2010) Sustainability life cycle comparison of biofuels: sewage the saviour? Manage Environ Qual 21(6):796–811

Kays SJ, Nottingham SF (2008) Biology and chemistry of Jerusalem artichoke: *Helianthus tuberosus* L. CRC Press, Boca Raton, 478 pp

Keating BA, Carberry PS, Hammer GL, Probert ME, Robertson MJ, Holzworth D, Huth NI, Hargreaves JNG, Meinke H, Hochman Z, McLean G, Verburg K, Snow V, Dimes JP, Silburn M, Wang E, Brown S, Bristow KL, Asseng S, Chapman S, McCown RL, Freebairn DM, Smith CJ (2003) An overview of APSIM, a model designed for farming systems simulation. Eur J Agron 18:267–288

Kerckhoffs LHJ, Renquist AR (2012) Selecting biomass species for temperate climates. Agron Sust Dev 32 (in press). doi: 10.1007/978-94-007-5449-2_5

Kerckhoffs LHJ, Shaw S, Trolove S, Astill M, Heubeck S, Renquist R (2011) Trials for producing biogas feedstock crops on marginal land in New Zealand. Agron N Z 41:109–124

Kerr JP, Menalda PH (1976) Cool season forage cereal trials in Manawatu and Wairarapa. Proc Agron Soc N Z 6:27–30

Kim H, Kim S, Dale BE (2009) Biofuels, land use change, and greenhouse gas emissions: some unexplored variables. Environ Sci Technol. http://pubs.acs.org. Downloaded 19 Jan 2009

Kiniry JR, Blanchet R, Williams T, Jones C (1992) Sunflower simulation using the epic and almanac models. Field Crop Res 30(3–4):403–423

Lambert MG, Jung GA, Costal DA (1989) Forage shrubs in North Island hill country. 1. Forage production. N Z J Agric Res 32(4):477–483

Lapola DM, Schaldach R, Alcamo J et al (2010) Indirect land-use changes can overcome carbon savings from biofuels in Brazil. Proc Natl Acad Sci 107:3388–3393

Lemus R, Lal R (2005) Bioenergy crops and carbon sequestration. Crit Rev Plant Sci 24:1–21

Lewandowski I, Schmidt U (2006) Nitrogen, energy and land use efficiencies of miscanthus, reed canary grass and triticale as determined by the boundary line approach. Agric Ecosyst Environ 112:335–346

Lewandowski I, Clifton-Brown JC, Scurlock JMO, Huisman W (2000) Miscanthus: European experience with a novel energy crop. Biomass Bioenergy 19:209–227

Li S-Z (2010) Meeting the demands of food, feed, and energy by sweet sorghum. FAO meeting, 14–15 July 2010. http://www.fao.org/bioenergy/26369-0887fe6c2880f4aa44c69d0a48457cb6e.pdf. Accessed 17 Jan 2012

Li FY, Jamieson PD, Pearson AJ (2006) AmaizeN: developing a decision-support tool to optimise nitrogen management of maize. Agron N Z 36:61–70

Logan LA, Radcliffe JE (eds) (1985) Fodder trees: a summary of current research in New Zealand. Report 106, ISSN: 0110 3725. New Zealand Department of Scientific & Industrial Research

Massignam AM, Chapman SC, Hammer GL, Fukai S (2009) Physiological determinants of maize and sunflower grain yield as affected by nitrogen supply. Field Crop Res 113:256–267

McDonald RC, Stephen RC (1979) Effect of sowing and harvesting dates on dry matter production of autumn-sown Tama ryegrass, ryecorn and oats. N Z J Exp Agric 7:271–275

McIntosh DJ (1998) The 5 minute guide to industrial hemp in New Zealand. New Zealand Hemp Industries Association, Auckland, pp 62

McLaughlin SB, Kszos LA (2005) Development of Switchgrass (*Panicum virgatum*) as a bioenergy feedstock in the United States. Biomass Bioenergy 28:515–535

McLaurin WJ, Somda ZC, Kays SJ (1999) Jerusalem artichoke growth, development, and Field storage. I. Numerical assessment of plant part development and dry matter acquisition and allocation. J Plant Nutr 22:1303–1313

McPartland JM, Cutler S, McIntosh DJ (2004) Hemp production in Aotearoa. J Ind Hemp 9:105–115

Monti A, Di Virgilio N, Venturi G (2008) Mineral composition and ash content of six major energy crops. Biomass Bioenergy 32:216–223
Monti A, Fazio S, Venturi G (2009) Cradle-to-farm gate life cycle assessment in perennial energy crops. Eur J Agron 31:77–84
Mueller SA, Anderson JE, Wallington TJ (2011) Impact of biofuel production and other supply and demand factors on food price increase in 2008. Biomass Bioenergy 35:1623–1632
New Zealand Biosecurity Institute (2009) Giant reed control measures demonstration in Dargaville. Northland/Auckland branch meeting, New Zealand Biosecurity Institute, 25 Nov 2009
New Zealand Energy Data File (2011) New Zealand annual report by Energy and Communications Branch Ministry of Economic Development, Wellington, NZ, pp 172
Newton SD, Hill GD (1987) Response of field beans (Vicia faba L. cv. Maris Bead) to time of sowing, plant population, nitrogen and irrigation. N Z J Exp Agric 15:411–418
Pacific Seeds (2009) Nutrifeed: summer forage with the feed quality of oats. Pacific Seeds Ltd Variety information Technote, Australia. http://www.pacificseeds.com.au/nutrifeed.html. Accessed 8 Feb 2009
Pang S (2011) Biomass to hydrogen-rich syngas and liquid fuels through thermochemical processing. IEA Bioenergy, Task 33: thermal gasification of biomass. Workshop 14 April 2011, Christchurch
Patterson T, Dinsdale R, Esteves S (2008) Review of energy balances and emissions associated with biomass-based transport fuels relevant to the United Kingdom context. Energy Fuel 22:3506–3512
Pegman APM, Ogden J (2005) Productivity-decomposition dynamics of Typha orientalis at Kaitoke Swamp, Great Barrier Island, New Zealand. N Z J Bot 43(4):779–789
Perlack RD, Wright LL, Turhollow AF, Graham RL, Stokes BJ, Erbach DC (2005) Biomass as feedstock for a bioenergy and bioproducts industry: the technical feasibility of a billion-ton annual supply. DOE/GO-102995-2135 or ORNL/TM- 2005/66. Oak Ridge National Laboratory, Oak Ridge, 60 pp
Pierce FJ, Rice CW (1988) Crop rotations and its impact on efficiency of water and nitrogen use. In: Hargrove WL (ed) Cropping strategies for efficient use of water and nitrogen, ASA special publication 51. ASA/CSSA/SSSA, Madison, pp 21–42
Piggot GJ, Farrell CA (1980) Sweet sorghum and beet crops for energy in northern North Island. Proc Agron Soc N Z 10:3–4
Piggot GJ, Farrell CA (1984) The culture and yield of sorghum for forage and sugar in Northland. Proc Agron Soc N Z 14:105–109
Propheter JL, Staggenborg S (2010) Performance of annual and perennial biofuel crops: nutrient removal during the first two years. Agron J 102(2):798–805
Propheter JL, Staggenborg S, Wu X, Wang D (2010) Performance of annual and perennial biofuel crops: yield during the first two years. Agron J 102(2):806–815
Queensland Primary Industries and Fisheries (2005) Pearl millet: a new feed grain for northern Australia. Queensland Dept of Primary Industries. http://www2.dpi.qld.gov.au/cropresearch/15402.html. Accessed 7 Feb 2012
Radcliffe JE (1986) Gorse—a resource for goats. N Z J Exp Agric 14(4):399–410
Rauch R (2011) Conversion of biomass over steam gasification to biofuels and chemicals—status of VUT work at Gussing. IEA Bioenergy, Task 33: thermal gasification of biomass. Workshop 14 April 2011, Christchurch
Rawate PD, Hill RM (1985) Extraction of a high-protein isolate from Jerusalem artichoke (Helianthus tuberosus) tops and evaluation of its nutritional potential. J Agric Food Chem 33:29–31
Reid JB, Stone PJ, Pearson AJ, Wilson DR (1999) Yield response to nutrient supply across a wide range of conditions: 2. Analysis of maize yields. Field Crop Res 77:173–189
Rengasamy JI, Reid JB (1993) Root system modification of faba beans (Vicia faba L.), and its effects on crop performance. 1. Responses of root and shoot growth to subsoiling, irrigation and sowing date. Field Crop Res 33:175–196

Renquist R, Shaw S (2010) Preferred herbaceous crops for gasification. NZ Institute of Plant & Food research report 4668, 46 pp

Rettenmaier N, Koppen S, Gartner SO, Reinhardt GA (2010) Life cycle assessment of selected future energy crops for Europe. Biofuels Bioprod Biorefin 4:620–636

Rhodes PJ (1977) Summer and early autumn forage yields of maize, sorghums and millets in Nelson and Marlborough. Proc Agron Soc N Z 7:31–35

Robertson GP, Hamilton SK, Del Grosso SJ, Parton WJ (2010) Growing plants for fuel: predicting effects on water, soil, and the atmosphere, Biofuels and Sustainability reports. Ecological Society of America, Washington, DC

Sadras VO, Calvino PA (2001) Quantification of grain yield response to soil depth in soybean, maize, sunflower, and wheat. Agron J 93(3):577–583

Scott WR, Hines SE (1991) Effects of grazing on grain-yield of winter barley and triticale—the position of the apical dome relative to the soil surface. N Z J Agric Res 34:177–184

Seiler GJ (1993) Forage and tuber yields and digestibility of selected wild and cultivated genotypes of Jerusalem Artichoke. Agron J 85:29–33

Shahbazi G (2009) Potential of cattails as energy crop for biofuel production. Seminar abstract. From http://www.bioenergy.psu.edu/announcements/ghasem.pdf. Accessed 16 Apr 2009

Shaw SR, Pearson AJ, Rogers B (2005a) Effect of winter land management on maize production—2002–04. NZ Institute of Crop & Food research report 46 to Foundation Arable research

Shaw SR, Pearson AJ, Rogers B (2005b) Crop rotations for maize production in New Zealand. Institute of Crop & Food research report 51 to Foundation Arable research

Shaw S, Rogers B, Reid J (2007) Silage, maize grain and total annual forage production in maize intercropped with various forage crops sown at different times. NZ Institute of Crop & Food Research confidential report 1978, 29 pp

Simmons BA, Loque D et al (2008) Next-generation biomass feedstocks for biofuel production. Genome Biol 9(12):6

Sims R, Taylor M, Saddler J, Maybee W (2008) From 1st to 2nd generation biofuel technologies—extended executive summary. IEA Bioenergy, pp 12. http://www.iea.org/textbase/papers/2008/2nd_Biofuel_Gen.pdf. Accessed 15 Apr 2009

Singh BR, Singh DP (1995) Agronomic and physiological responses of sorghum, maize and pearl millet to irrigation. Field Crop Res 42(2/3):57–67

Standish RJ, Robertson AW et al (2001) The impact of an invasive weed Tradescantia fluminensis on native forest regeneration. J Appl Ecol 38(6):1253–1263

Steer BT, Milroy SP, Kamona RM (1993) A model to simulate the development, growth and yield of irrigated sunflower. Field Crop Res 32(1–2):83–99

Stephen RC, McDonald RC, Kelson A (1977) Influence of cutting date and frequency on dry matter production and nitrogen content on autumn-sown greenfeeds. N Z J Exp Agric 5:227–231

Stewart DJ (1983) Methane from crop-grown biomass. In: Wise DL (ed) Fuel gas systems. CRC Press, Boca Raton

Stone LR, Goodrum DE, Schlegel AJ, Jaafar MN, Khan AH (2002) Water depletion depth of grain sorghum and sunflower in the central High Plains. Agron J 94(4):936–943

Struik PC, Amaducci S, Bullard MJ, Stutterheim MC, Venturi G, Cromack HTM (2000) Agronomy of fibre hemp (Cannabis sativa L.) in Europe. Ind Crop Prod 11(2–3):107–118

Swanton CJ, Cavers PB (1989) Biomass and nutrient allocation patterns in Jerusalem artichoke (*Helianthus tuberosus*). Can J Bot 67:2880–2887

Taylor AO (1973) An environmental problem with grain sorghums. Proc Agron Soc N Z 3:57–62

Taylor AO, Rowley JA, Esson MJ, Easton JD, Wallace R (1974) Sorghums for conserved feed in Northland. Proc Agron Soc N Z 4:74–78

Teixeira EI, Moot DJ, Brown HE, Fletcher AL (2007a) The dynamics of lucerne (Medicago sativa L.) yield components in response to defoliation frequency. Eur J Agron 26(4):394–400

Teixeira EI, Moot DJ, Brown HE, Pollock KM (2007b) How does defoliation management impact on yield, canopy forming processes and light interception of lucerne (Medicago sativa L.) crops? Eur J Agron 27(1):154–164

Teixeira EI, Moot DJ, Mickelbart MV (2007c) Seasonal patterns of root C and N reserves of lucerne crops (Medicago sativa L.) grown in a temperate climate were affected by defoliation regime. Eur J Agron 26(1):10–20

Teixeira EI, Moot DJ et al (2008) Defoliation frequency and season affected radiation use efficiency and dry matter partitioning to roots of lucerne (Medicago sativa L.) crops. Eur J Agron 28(2):103–111

U.S. Dept of Energy (2011) U.S. billion-ton update: biomass supply for a bioenergy and bioproducts industry. Leads: Perlack RD, Stokes BJ, ORNL/TM-2011/224. Oak Ridge National Laboratory, Oak Ridge, pp 227

van der Drift A, van Doorn A, Vermeulen JW (2000) Ten residual biomass fuels for circulating fluidized-bed gasification. Biomass Bioenergy 20:45–56

van Oosterom EJ, Borrell AK, Chapman SC, Broad IJ, Hammer GL (2010) Functional dynamics of the nitrogen balance in sorghum: I. N demand of vegetative plant parts. Field Crop Res 115:19–28

West TO, Marland G (2002) A synthesis of carbon sequestration, carbon emissions, and net carbon flux in agriculture: compare tillage practices in the United States. Agric Ecosyst Environ 9:217–232

Withers NJ (1973) Kenaf under temperate conditions. N Z J Expt Agric 1:253–257

Wortmann CS, Liska AJ, Ferguson RB, Lyon DJ, Klein RN, Dweikat I (2010) Dryland performance of sweet sorghum and grain crops for biofuel in Nebraska. Agron J 101(1):319–326

Wright LL, Gunderson CA, Davis EB, Perlack RD, Baskaran LM, Eaton LM, Downing ME (2009) Switchgrass production potential and use for bioenergy in North America. Taupo New Zealand Workshop 'Linking technology and biomass,' IEA Bioenergy Task 30: short rotation crops for bioenergy. International Energy Agency

Wunsche U (1985) Energy from agriculture: some results of Swedish energy cropping experiments. In: Palz W, Coombs J, Hall DO (eds) Energy from biomass. Elsevier, Amsterdam, pp 295–300

Zegada-Lizarazu W, Monti A (2011) Energy crops in rotation: a review. Biomass Bioenergy 35:12–25

Zegada-Lizarazu W, Elbersen HW, Cosentino S, Zatta A, Alexopoulou E, Monti A (2010) Agronomic aspects of future energy crops in Europe. Biofuels Bioprod Biorefin 4:674–691

Biodiesel Production for Sustainable Agriculture

Varsha Sharma, Kishan G. Ramawat, and B.L. Choudhary

Abstract Biodiesel is produced by transesterification of edible and non-edible oils obtained from a wide range of plants. Biodiesel has developed rapidly as an eco-friendly, renewable, alternative source of energy compared to the limited resources of fossil fuels. There are wide ranging socio-economic implications of biodiesel for rural population in developing countries because biodiesel is an agriculture-based industrial product from plants. Diversion of edible oil for biodiesel, land use for biodiesel crops, and technology for biodiesel production are issues to be addressed. The use of edible and non-edible crops for biodiesel production and sustainability are discussed in this article. Biodiesel, amounting to about 300,000 barrels per day is mainly produced from edible oils and small quantities from non-edible oils from *Jatropha curcas*. *Jatropha curcas* is a multipurpose, drought-resistant, biofuel tree originating from Central and South America, though now growing pantropic. *Jatropha* plants produce up to 6.5 t seeds and yield about 2,000 L oil per hectare. Therefore agrotechnologies have to be developed towards mechanization and cost reduction to make an industrial product. A debate has already been started about use of edible oil for the production of biodiesel considering impact on land usage, prices of edible oil and benefits to farmers. The other potential non-edible oil crops are castor, neem and karanj (*Pongamia*). Therefore, a rational policy about biodiesel crops has to be evolved looking to the various needs of a particular country. Use of edible oil crops like soybeans, rapeseeds, sunflower and palm oil for non-edible purpose is a matter of debate from socio-economic aspect as population of many countries is malnourished. The Food and Agriculture Organization (FAO) of the United Nations emphasizes that the versatility of *Jatropha* may make it useful in poor, remote areas of the world.

Keywords Biodiesel • Edible oil • *Glycine max* • *Jatropha* • Non-edible oils

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DOI 10.1007/978-94-007-5449-2_6,

Abbreviations

EU	European Union
FAO	Food and Agriculture Organization
UN	United Nations
USA	United States of America

1 Introduction

The crops that are used for biofuel production are called energy crops and the utilization of energy crops as a source of renewable fuels is a concept with great relevance to current ecological and economic issues at both national and international scales. The relationship between biofuels, oil, and food markets is complex. Biofuel is liquid fuel obtained from plants through specific chemical processes. It can either be biodiesel or ethanol. In energy markets, biofuels are complementary with petroleum-based petrol and diesel. The increasing price of petroleum products and concerns about oil production are likely to have serious implications for the automobile industry in the forthcoming era. Strong global growth in demand has caused food crop based biofuels to become a significant consumer of food crops. Due to this shift in utilization of food crops for biofuels production has resulted in increasing prices of edible oils in affected markets (Collins 2008). Until now, biofuels have been produced by processing agricultural crops using available technologies.

These so called first-generation biofuels can be used to blend with conventional fuels in most vehicles and can be distributed through existing infrastructure. Advanced conversion technologies are needed for a second generation of biofuels. The second generation will use a wider range of biomass resources-agriculture, forestry and waste materials and promise to achieve higher reductions in greenhouse gas emissions and the costs of fuel production (Smeets et al. 2006; Hoogwijk et al. 2005). Agrotechnology for biodiesel crops is developing gradually to produce renewable and environmentally friendly fuel. Plant based fuels are best renewable sources, and its use can lead to a better balance of carbon dioxide and other green house gases formation responsible for global warming. The bio-energy system makes a significant contribution to the world's growing energy needs. Brazil was the first country to start a major biofuel program in the 1970s. The basis was ethanol, produced from the country's massive cane-sugar program. Biofuels are renewable, non-toxic, biodegradable and they contribute to energy security and reducing environment pollution. Biofuels can be produced from selected agricultural biomass of crops they offer opportunities to improve the income levels of small holder farmers. At a community level, farmers can cultivate energy crops that fetch an income while also meeting their food needs (Reddy et al. 2008). Further, due to high substitutability among major food crops and globally integrated markets, biofuels production also affects non-biofuels crops. Higher food crop prices in turn increase the feedstock cost for biofuels production. The renewable sources would only be able to compete with the fossil fuel resources if special plant crops containing energy producing hydrocarbon like material are breed and cultivated (Kalita 2010). The world-wide impetus for promoting biofuels has led to an increased focus on technology development.

Efforts are being made to develop biofuels from microbes like *Escherichia coli* and *Saccharomyces cerevisiae* (Kalscheuer et al. 2004, 2006). The first generation biofuels are obtained from edible crop plants like corn, wheat, maize, soybean, sunflower, safflower, sugarcane, rapeseed and palm oil (Pinto et al. 2005; Demirbas 2006). The second generation biofuels are obtained from non-edible crop plants like *Miscanthus, Panicum virgatum* or switchgrass, *Hevea brasiliensis* or rubber seeds, *Calotropis gigantia*, *Euphorbia tirucalli*, *Jatropha curcas*, *Pongamia pinnata*, and *Calophyllum inophyllum*. The third generation biofuels are likely to be obtained from microalgae like *Botryococcus braunii*, *Chlorella* sp., *Dunaliella tertiolecta*, *Gracilaria, Pleurochrysis carterae, Sargassum* (Christi 2007; Miao and Wu 2004; Qin 2005).

It is evident from available information that food crops comprise about 30–40% of the biofuel production. This has consequence in edible oil's price (Mukherjee et al. 2011). The current enthusiasm for biofuels has resulted in private venture capital funding for startup biotechnology companies devoted to developing these species. This is coupled with government sponsored research to improve the crops and develop techniques for profitably processing the biomass into fuel (Waltz 2008). Agrotechnology for biodiesel crops are the subject of improvements in crop productivity, crop suitability and biofuels processing are all within the realm of proven biotechnological approaches. The production of ethanol from starch crops and biodiesel from oil crops is based on established technologies. In this review we consider biodiesel as alternative source of energy with the current traits that make crops more valuable for biofuel use in imminent era.

2 Biodiesel as Alternative Source of Energy

Nowadays, biodiesel fuel is an important energy resource because it is a secure, renewable, and environmentally safe alternative to fossil fuels (Sadano et al. 2010). In pursuit of better life, biofuels and bio-energy are being used as alternatives to depleting resources in particular to "petroleum products". These renewable energy sources offer prospects of increasing energy supplies in a self reliant way in developing countries like India and also work as checkpoint for aggravating green house gases (Fairless 2007). There are numerous eco-benefits to replacing oil with biofuels like ethanol and biodiesel. For one, since such fuels are derived from agricultural crops, they are inherently renewable. Emissions from burning biodiesel in a conventional diesel engine have significantly lower levels of unburned hydrocarbons, carbon monoxide, carbon dioxide, particulate matter, sulfur oxides, odor, and noxious smoke compared to emissions from petrodiesel (Razon 2009). Carbon dioxide emissions from combustion of oxidized neat biodiesel produced 15 and 16% lower exhaust carbon monoxide and hydrocarbons, respectively when compared to

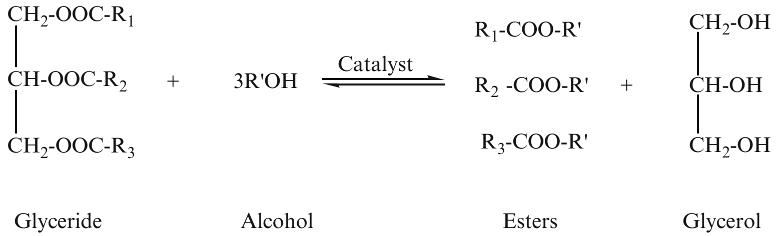

Fig. 1 General transesterification reaction of triglycerides for biodiesel production

petrodiesel (Abdul and Gerpen 2001), but there is a more significant carbon dioxide benefit with biodiesel made from plant oils, i.e., growing biodiesel crops (Singh and Singh 2010). During the photosynthesis process as the plants are growing and developing, carbon dioxide is drawn from the environment into the plant tissues; the plants are really carbon dioxide scrubbers. Biodiesel processed from plant oils is carbon dioxide neutral. In addition, the plants release beneficial oxygen into the environment. Accidental spills of biodiesel are far less of a problem compared to petrodiesel. Pure biodiesel is fully biodegradable; in fact, about 98% of biodiesel degrades in about 3 weeks (Leung et al. 2006). The biodegradable property of biodiesel makes it an especially attractive fuel choice for environmentally sensitive areas such as national parks, forests, and marinas (Singh and Singh 2010).

Biodiesel is defined as monoalkyl esters of long-chain fatty acid derived from triglycerides or renewable lipid resources e.g. rapeseed oil, soybean oil, palm oil with short-chain alcohols by transesterification or esterification (Fig. 1.) (Xu and Hanna 2009). Glycerol, as the principal by-product from biodiesel production units, is a low-cost renewable resource.

Several methods for the production of biodiesel have been developed using chemical and biological catalysts, such as alkali and acidic compounds, and lipase (Kim et al. 2007). Recently, biodiesel production with enzymatic transesterification gained attention. Compared with chemical approaches, it conserves energy and easy to recover the by-product glycerol, as no acidic or alkali catalysts have to be removed from the product, and free fatty acid and water would not interfere with the reaction (Meher et al. 2006). Cost effective and eco-friendly development is required for biodiesel production. Involvement of private sector in this venture has accelerated the process of technology development. Biodiesel is gaining importance as an alternative source of energy but technology for biodiesel production from plants as well as combustion engine technology has to be refined in near future.

3 World Demand and Production of Biodiesel

World-wide production of biofuels is rapidly growing. Apart from the European Union (EU) and the USA other countries like Canada, Brazil, Australia, India and China also implemented targets for biofuels volumes and market shares. The demand

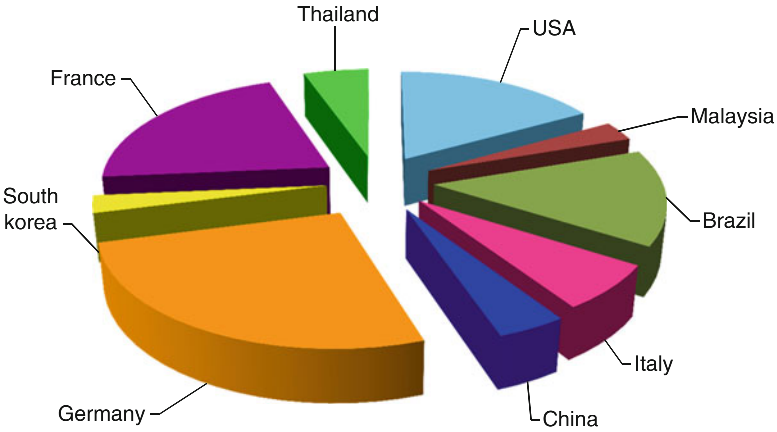

Fig. 2 Biodiesel production share of major producer countries

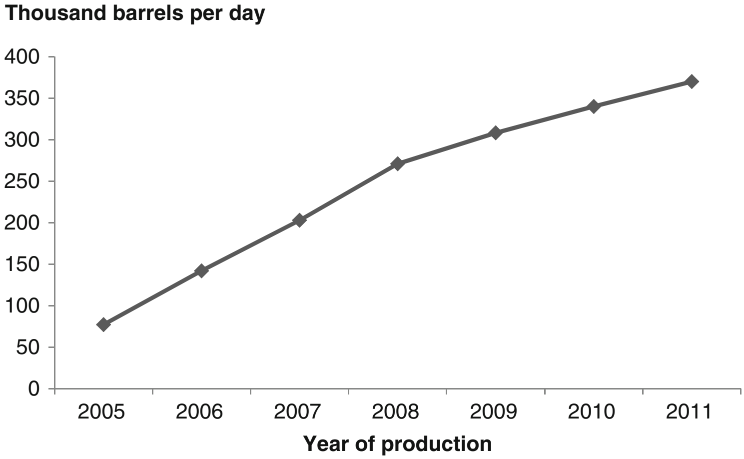

Fig. 3 Biodiesel production in the world up to 2009 and projected production up to 2011

for biofuels in the USA, Europe, and other developed nations is partly driven by the need to replace fossil fuels and lessen dependence on high-priced imported oil. Biodiesel production in different countries is presented in Fig. 2. World biodiesel production has grown considerably during the last decade and likely to reach 350,000 barrel per day in 2011–12 (Fig. 3). Recently, in the EU more than 20% of edible oilseed production was processed into biodiesel. Biofuel demand depends on economic profitability and on government subsidies and mandates for biofuel use. In India, the energy demand is increasing at a rate of 6.5% per annum. The crude oil demand of the country is met by import of about 80%. Thus the energy security has become a key issue for the nation as a whole (Jain and Sharma 2010). Although an increased demand for biofuels could help farmers by raising the price of their crops; the higher prices could also hurt those same farmers, most of whom spend most of their income on food. The federal Energy Independence and Security Act of 2007 as amended in March of 2008 mandates 34.6 million tons (MT) of vehicle ethanol fuel in 2008, 49.96 MT in 2012, and 56.78 MT in 2015 (Tweeten and Thompson 2008). According to estimates published by the European Commission (2006), which are based on the same partial equilibrium model, oilseed area would be 0.75 million hectares (Mha) higher than without biofuel policies and reach 8 Mha as compared to 7.25 Mha without the expected increase in biodiesel demand. Sunflower seed area would expand by 0.2 to 1.7 Mha as compared to unchanged biofuel policies. Rapeseed area would increase by 0.55 Mha. Also cereal area could expand by 2.5–52.5 Mha as compared to the baseline.

The economic literature on the impacts of biofuels on agricultural markets is scarce, as the biofuel boom appeared only recently. The expansion of biofuel production and consumption is not limited to the United States. Increased crop-based production took place in Brazil over the last several decades, as Brazil used sugarcane as a feedstock to produce ethanol, and then use ethanol on a large scale to fuel vehicles. Canada has mandated that biofuels make up 5% of all transportation vehicle fuel by 2010. Meanwhile, Argentina has a system of differential export taxes resulting in a lower tax rates for biodiesel exports than the tax rates on feedstock exports such as corn or soybean oil. With ever increasing world demand for fuels, alternative sources have to be developed which are cost effective and eco-friendly. Still biodiesel is mainly produced from edible oils (cultivated) and small quantities from non-edible oils like *Jatropha*. Many issues need to be resolved before making a commercially viable fuel, such as area under cultivation for edible versus non- edible crops, production costs and issue related to subsidy by governments. If biodiesel has to blend in fossil diesel all over the world, still there would be a big gap between demand and supply.

4 Role of Food and Agriculture Organization and Governments

The encouragements to biodiesel fuel programs by the governments in many countries have expedited the efforts for the development of different production technologies, the evaluation and performance testing of different biodiesel fuels in various types of automotive vehicles, and the assessment of their impact on the environment, economy and society are current themes of interest in biodiesel production (Tyagi et al. 2010). The OECD-FAO Agricultural Outlook 2007–2016 projection states that structural changes, such as increased feedstock demand for biofuel production, and the recent reduction of surpluses due to past policy reforms, may keep prices above historic equilibrium levels during the next 10 years (OECD-FAO 2007).

In the BRIC nations (Brazil, Russia, India and China), key government initiatives are spawning hundreds of new opportunities for feedstock developments, biodiesel production and export. At present, EU accounts for 66% of the world's total biodiesel production, followed by the United States at 1.5 MT. The world biodiesel production and capacity have increased from 7.1 MT in 2006 to 9.0 MT in 2007, and 12.2 MT in 2006 to 23.1 MT in 2007, respectively. Currently, more than 200 nations will become biodiesel producing nations and suppliers (Xu et al. 2009). The Indian draft policy, Government of India (2008) appeared to have backed off the country's exclusive promotion of *Jatropha* and instead called for the use of any non-edible oilseeds grown on marginal, degraded or wastelands. The draft policy also recommended establishing 20% blending targets by 2017 for both ethanol and biodiesel. Government policies are also influencing biofuel industries in Canada, Argentina, China, countries of the Former Soviet Union, Malaysia, and Indonesia. The Government of India (GoI) has given a top priority for promotion and use of non-edible oils such as *Jatropha curcas*, *Pongamia pinnata*, etc., for production of biodiesel. Policies regarding use of crop plants for biofuel production and land use for such crops are being refined in the developing countries, which are main producers of edible and non-edible crops (FAOSTAT 2009). However, biofuels have to compete with petroleum products when subsidies are withdrawn. Governments are supporting research and developments efforts to make biodiesel a success, including subsidy on its production to reduce pollution and dependency on fossil diesel. Private industrial participation is necessary to further develop the various aspects of technology.

5 Agrotechnology Traits for Biodiesel Crops

Agrotechnological traits play an important role in the development of new biofuel crops and improve the efficiency and cost effectiveness of these crops. Agrotechnology is being used to improve the crops making them more productive or suitable to biofuels use. *Jatropha curcas* oil contains about 14% free fatty acid which is beyond the limit of 1% level which can be efficiently converted into biodiesel by transesterification using an alkaline catalyst (Tiwari et al. 2007). The fatty acids reported in a study of *J. curcas* oil are palmitic acid (11.3%), stearic acid (17%), arachidic acid (4.7%), oleic acid (12.8%), and linoleic acid (47.3%) (Adebowale and Adedire 2006). A potential major constraint in the widespread acceptance of *Jatropha* as a source of biodiesel is the presence of phorbol esters, which, when consumed by man and animal, are toxic and are also co-carcinogens (Makkar et al. 2009). So far sporadic efforts are made to improve *J. curcas* for biodiesel. Followings are the important traits required for the pertinent biodiesel crop research.

- Improvement in the crop productivity.
- Improvement in the efficiency of photosynthesis.
- Development of hybridization methods for increasing the yield.
- Improvement in abiotic stress tolerance.
- Improvement in the efficiency to utilize nutrients.
- Enhanced biomass production.
- Preparing the crop for biofuel processing.
- Economic exploitation of cake and waste

Jatropha seeds are manually collected, mainly from plants growing in wild. Therefore, above mentioned agrotechnologies have to be developed towards mechanization and cost reduction to make it an industrial product.

6 Crops for Biodiesel

Biodiesel can be produced from edible oilseed crops such as soybean (*Glycine max* L.), rapeseed (*Brassica* spp.) or sunflower (*Helianthus annuus* L.). Other vegetable oils that have been used in biodiesel production include corn, cotton seed and peanuts. However, given the large gap between the demand and supply of edible oils, many countries cannot afford to use vegetable oils for biodiesel production. In addition, the use of vegetable oil as fuel is less polluting than petroleum fuels (Jain and Sharma 2010). Fortunately, bio-diesel can also be produced from non-edible oil seeds from shrubs such as Ratanjot (*J. curcas*), karanj (*Pongamia pinnata*) and neem (*Azadirachta indica*) (Reddy et al. 2008; Vasudevan and Briggs 2008). The production of biodiesel from different crops and testing its efficacy was carried out using internal combustion engine. The results are presented in Table 1.

The *Jatropha* seed is considered to be toxic to animals, one of the reasons it is used as a natural hedge in developing nations. Recognition, that *Jatropha* oil can yield an exceptional biodiesel has led to a surge of interest in *Jatropha* across the globe, more so in view of the potential for avoiding the dilemma of "food vs. fuel" (Reddy and Pamidimarri 2010; Mandpe et al. 2005). In the United States, biodiesel is made from soybean oil, while in Europe; rapeseed (or canola) oil is a common feedstock. In developing countries like India, *Jatropha* is a viable source of non-edible oil. Crops currently using for biodiesel production and agrotechnology efforts to improve the production and reduce the cost of biofuel in these species are discussed. A debate has already been started about use of edible oil for the production of biodiesel considering impact on land usage, prices of edible oil and benefits to farmers. Therefore, a rational policy about biodiesel crops has to be evolved looking to the various needs of a particular country.

6.1 Non–edible Oil Crops

6.1.1 *Jatropha curcas*

Biology

Jatropha curcas is a small tree belongs to the Euphorbiaceae family (Figs. 4, 5, 6, 7, 8 and 9). The leaves are cordate, 3–5 lobed and 10–15 cm long; stomata are hypostomatic and paracytic (Rubiaceous) type. It flowers twice in a year in India, during May–June and September–November. Seeds usually mature within a month.

Table 1 Alternative and traditional biodiesel crops which have been engine-tested and their estimated oil yields in kg/ha

Scientific name	Common name	Plant type	Plant part	Oil yield (kg/ha)	References
Azadirachta indica	Neem	Tree	Seed	2,670	Nabi et al. (2006) and Azam et al. (2005)
Balanites aegyptiaca	Desert date	Tree	Kernel	1,600	Chapagain et al. (2009) and Deshmukh and Bhuyar (2009)
Brassica carinata	Ethiopian mustard	Herb	Seed	900–1,300	Bouaid et al. (2005, 2009)
Calophyllum inophyllum	Polanga	Tree	Seed	4,680	Banapurmath et al. (2008), Sahoo et al. (2007), and Sahoo and Das (2009)
Camelina sativa	Camelina	Herb	Seed	1,100–1,400	Frohlich and Rice (2005)
Carthamus tinctorius	Safflower	Herb	Seed	200	Rashid and Anwar (2008) and Xin et al. (2009)
Corylus avellana	Hazelnut	Tree	Kernel	1,000	Gumus (2008) and Xu and Hanna (2009)
Eruca vesicaria ssp. *sativa*	Rocket	Herb	Seed	420–590	Li et al. (2009)
Hevea brasiliensis	Rubber	Tree	Seed	50	Ramadhas and Jayaraj (2005)
Jatropha curcas	Physic	Tree/shrub	Seed	1,900–2,500	Abhilash et al. (2011), Khemkladngoen et al. (2011), Mukherjee et al. (2011), Makkar et al. (2009), and Achten et al. (2008)
Linum usitatissimum	Linseed	Herb	Seed	300	Agarwal et al. (2008) and Sendzikiene et al. (2005)
Pongamia (Millettia) pinnata/Pongamia glabra	Koroch, karanja	Tree	Seed	225–2,250	Kesari and Rangan (2010), Das et al. (2009), Sahoo and Das (2009), and Sarma et al. (2005)
Ricinus communis	Castor	Tree/shrub	Seed	450	Scholz and Silva (2008)

(continued)

Table 1 (continued)

Scientific name	Common name	Plant type	Plant part	Oil yield (kg/ha)	References
Sesamum indicum	Sesame	Herb	Seed	220	Banapurmath et al. (2008) and Saydut et al. (2008)
Simarouba glauca	Paradise tree	Tree	Seed	900–1,200	Devan and Mahalakshmi (2009a, b) and Dash et al. (2008)
Thevetia peruviana	Yellow oleander	Shrub	Seed	1,575	Balusamy and Marappan (2007) and Oluwaniyi and Ibeyimi (2007)

Fig. 4 *Jatropha curcas* plant, a broad leaved bush

Fig. 5 Flowering stage in *Jatropha* plant. Under Indian conditions, the plant flowers twice in a year

The inflorescence is axillary paniculate polychasial cyme (Fig. 5). The flowers are unisexual, monoecious yellowish green in glabrous or pubescent cymes at the end of the branches. Female flower produce higher amount of nectar than male flower. Fifty percent of female flowers set fruit with 53% fertilization rate, 32% apomixes rate and 2:3 seed-ovule ratio. Fruits are trilocular capsules (Fig. 6), 1.5–3.0 cm long.

Jatropha trees produce many seeds that are very rich with oils, the seed contain 30–40% of oil with 21% saturated fatty acids and 79% unsaturated fatty acids. Oil extracted from the seeds has traditionally been used as lamp oil and also in soap manufacture (Reddy and Pamidimarri 2010; Gübitz et al. 1999).

Habitat

Jatropha is a vigorous, drought- and pest-resistant plant, and can grow under a wide range of rainfall regimes ranging from 200 to over 1,500 mm per annum. *Jatropha* trees will grow under a variety of conditions, withstanding high temperatures, drought, slope, and varied pH levels. Most data comes from *Jatropha* production in regions where water is either too scarce or too expensive with which to irrigate. The rooting system is composed of three or four primary lateral roots spreading near the soil surface and one vertical root that can reach down more than 5 m, allowing it to survive in very dry climates. *Jatropha* has shown high demands for nitrogen and phosphorous, the demands for nitrogen and phosphorous can be easily met, as *Jatropha* is highly efficient at adsorbing nutrients from marginal soils.

Occurrence

Jatropha curcas is a multipurpose, drought-resistant, biofuel tree originating from Central and South America, but now growing pantropic. It is indigenous to the Caribbean, but it is used around the world in Central and South America, India, Africa, and Southeast Asia to provide natural hedges and fence-rows, as it is not browsed by most livestock.

Fig. 6 Fruiting stage in *Jatropha* plant; under cultivation the plant bears heavy fruiting

Fig. 7 Nursery of *Jatropha* plants, ready for transplantations

Fig. 8 *Jatropha* plantation at Dariba, near Udaipur

Plantation

Propagation of *Jatropha* can be done by seed or by cuttings, with trees grown from seeds (Fig. 7) producing harvestable seed crops within 2 years and trees grown from cuttings producing harvestable seed in the first year of growth. The estimated potential area of *Jatropha* plantation is 59–1,486 Mha worldwide, and the potential production is 56–3,613 MT dry seed per year (Li et al. 2009). It has been estimated that 1 ha plantations (Fig. 8) of *Jatropha* trees could produce up to 6.5 t of seed per

Fig. 9 Pruning in *Jatropha* plants results in multiple braches and increased yield of fruits

year and about 2,000 l of oil, which can be used for soap-making, various medical treatments, and production of diesel fuel. This kind of information can be used for policy makers and prospective countries should identify their land areas suitable for *Jatropha* plantations using geographic information system and other land suitability modeling. Although *Jatropha* is expected to ease fuel shortage and global warming potential, the large-scale cultivation has become more controversial, with growing awareness of loss of biodiversity and food security (Abhilash et al. 2011). To contribute to the fuel supply, renewable energies such as *Jatropha* appear to be an attractive resource for biodiesel production in India and other countries as it can be grown on waste land and does not need intensive water supply (Leduc et al. 2009).

Agrotechnology

J. curcas is still an undomesticated plant in which many basic agronomic properties are not yet thoroughly understood (Achten et al. 2008). *J. curcas* bears bunch of fruits at the apex of the branches. Therefore, limited branching is considered one of the major factors limiting yield in this species. Manual pruning is one of the major management practices in commercial plantations of this crop (Fig. 9), resulting in production of more branches and thus increased potential for more inflorescences leading to a higher seed yield. However, this method is time-consuming, labor-intensive and expensive, e.g., plant growth regulators caused an increase in the seed hydrocarbon content in response to hormonal application to *J. curcas* (Augustus et al. 2002). Nitrogen supply can improve the plant growth, though plant can grow in low nitrogen and adapt to drought stress (Wang et al. 2011).

A single foliar application of N6-benzyladenine at 12 millimolar (mM) significantly increased branches in both the pot (4.0) and field (13.2) trials compared to manual pruning (1.8 and 5.7, respectively) and control (no new branches) plants. In the field, a single foliar application of 1.0 mM 2,3,5-triiodobenzoic acid (TIBA) resulted in a significant increment in the number of branches (15.9) after 7 months. Of all the plant growth regulators examined, 2,3:4,6-di-O-isopropylidene-2-keto-L-gluconic acid (a pesticide, common chemical name, dikegulac) at 2.0 mM produced the maximum number of branches (18.0) in the field 7 months after application (Abdelgadir et al. 2009). The same authors reported that a single foliar application of N6-benzyladenine produced more flowers per plant, more fruits per bunch, heavier and bigger fruits and seeds with more oil compared to manual pruning. Treatment with TIBA yielded more flowers per plant and heavier fruits with a higher oil content than the control and manually pruned plants. Treatment with dikegulac yielded similar results. More fruits per bunch and more seeds per fruit were also produced. Maleic hydrazide treatment yielded more flowers per plant, heavier and bigger fruits with more, heavier, oil rich seeds compared to the control and manual pruning. This study indicated that foliar application of plant growth regulators (PGR) as chemical branching agent in *J. curcas* may have a sequential effect in boosting seed production, seed oil content and improves fruit quality (Abdelgadir et al. 2010).

Biodiesel Production

The common oilseeds as soybean, cottonseed, sunflower and rapeseed are rich sources of phospholipids (Nieuwenhuyzen and Tomas 2008). Phospholipids pose many problems for the storage and processing of the crude oil and are removed from oil during refining by a process known as degumming. The oil obtained by mechanical crushing (machines known as expeller) or solvent extraction is termed "crude" oil, as it contains a number of impurities. Some of the impurities, such as seed fragments and meal fines, are oil insoluble and thus can be readily removed by filtration. Others, including free fatty acids, hydrocarbons, ketones, tocopherols, glycolipids, phytosterols, phospholipids, proteins, pigments, and resins, are soluble or form stable colloidal suspensions in the oil. Most of these have unfavorable effects on the flavor, odor, appearance, and shelf life of the oil, and therefore have to be removed from the vegetable oils by chemical or physical refining processes (Zufarov et al. 2008). Currently, industrial-scale biodiesel synthesis relies on the chemically-catalyzed transesterification of vegetable oils with short-chain alcohols, usually methanol. Chemically-catalyzed transesterification of triglycerides to their corresponding alkyl esters provides high conversion yields with short reaction times. When acid catalysts are used, careful removal of catalyst residues from the final product, entailing large volumes of wash-effluent, is required to avoid damage to engine parts (Jachmanian et al. 2010). There are various technologies explored for the development of biodiesel process at pilot plant scale using *Jatropha* oil as the raw material with methanol and sodium hydroxide as the catalyst and evaluate the produced biodiesel as a fuel. Studies of biodiesel production from *Jatropha* oil by transesterification on both bench and pilot scales have been developed and evaluated (Diwani et al. 2009). The optimization of biodiesel production was reported in *J. curcas* oil via alkali-catalyzed methanolysis. The reaction was in the presence of NaOH as catalyst was carried out to investigate the optimum conditions and to study the effects of variables on the reaction. These variables included methanol-to-oil molar ratios of 4:1–10:1, catalyst concentrations of 0.25–2.0% w/w of oil, reaction temperatures of 32–60°C, and reaction times of 5–40 min. Due to the low price of sodium hydroxide, the short reaction time, and high methyl ester content obtained, these optimum conditions can be used in large-scale production to reduce the cost of production (Nakpong and Wootthikanokkhan 2010). From the ongoing account it is clear that technology development is in its preliminary phase and refinement for industrial process is required.

6.1.2 *Pongamia pinnata*

Pongamia pinnata (L.) Pierre tree belongs to the family Fabaceae, popularly known as 'Karanj' (in Hindi). It is known for its multipurpose benefits and as a potential source of biodiesel (Sharma and Singh 2008; Kesari et al. 2009). Its added benefits to grow on marginal lands make it a suitable candidate in agro-forestry. These properties support the suitability of this plant for large-scale production required by a sustainable biodiesel industry (Scott et al. 2008; Kesari and Rangan 2010). The seeds contain about 28–34% oil with a high percentage of polyunsaturated fatty acids. The tree can grow on unproductive land and is adaptable to wide agro-climatic conditions (Kesari and Rangan 2010). The Ministry of Agriculture through National oil seeds and vegetable oils development board (NOVOD) is promoting *Pongamia* under the scheme of Integrated Development of tree-borne oil seeds. The NOVOD board has undertaken the plantation model of *Pongamia* in an area of approximately 1,400 ha for producing parent material for large-scale plantation (NOVOD Report 2009). Biodiesel production from the non-edible oil of *P. pinnata* by transesterification of the crude oil with methanol and KOH as catalyst has been established. A maximum conversion of 92% (oil to ester) was achieved using a 1:10 M ratio of oil to methanol at 60°C. When tetrahydrofuran was used as co-solvent, the conversion increased to 95% (Karmee and Chadha 2005). Agrotechnology for systematic cultivation and oil production are still required for the plant.

6.1.3 *Ricinus communis*

Ricinus communis belongs to the family Euphorbiaceae and it is commonly known as castor oil plant. Castor oil is extracted from the seeds, which contain about 46% oil. This oil is highly viscous. Castor oil dissolves easily in alcohol, ether, glacial acetic acid, chloroform, carbon sulfide, and benzene. It's made up of triglycerides: 91–95% ricinoleic acid, 4–5% linoleic acid, and 1–2% palmitic and stearic acids. Besides being used as a laxative, castor oil is widely used in the industrial field because of its many properties. In the textile industry, castor oil is used for moisturizing and removal of grease in fabrics, and for the manufacturing of waterproof fabrics. The leftover seedcake (protein-meal) has a highly-toxic component known as ricin, a blood coagulant that can be lethal in very small doses. There are expensive means of detoxifying the seedcake after oil extraction, if the high-protein meal needed to be used as animal feed. The oil can be used in medicinal applications as well, but only in small doses. Under irrigation, yields from castor bean can exceed 1,000 kg/ha of seed regularly, and experimental tests in the U.S.A. and Brazil have given yields up to 5,000 kg/ha. Although there is potential for very high oil yield from castor bean, the fact it is considered a weed by many agricultural producers certainly limits its viability as an option to produce significant amounts of biodiesel (Poteet 2006). Castor oil is already used as automobile lubricant (industrial process) and its diversion in to biodiesel may affect market cost.

6.1.4 *Azadirachta indica*

The neem tree (*Azadirachta indica*) is an evergreen tree of the family Meliaceae and is native to India and Burma. Neem trees are fast-growing and can grow up to 35 m tall, and although evergreen, they will lose their leaves in times of severe drought. One tree can produce millions of flowers, and in one flowering cycle, a mature tree may produce many thousands of seeds. Seeds are small and round to oval in shape, with oil content ranging from 20 to 33%, depending on the variety. It is estimated that a mature neem tree may produce 30–50 kg of fruit each year. Neem trees are considered to be a sacred tree in India because of their multitudinous uses. Products derived from the neem tree include neem oils, bark, leaves, and seed cake, each of which can serve a different purpose. In India, neem oil extracted from seeds is used for soap primarily, but it is also used in medicine and as a natural pesticide, capable of repelling various harmful insects from food and fiber crops (Pal 2007). There are many uses of leaves, bark and seed oil neem plant. It is mainly used as avenue tree. How much seeds are available for biodiesel production has to be surveyed properly.

6.1.5 Algae

Algae, mainly microalgae, have recently gained attention as a new biomass/biolipid source for the production of biodiesel. It has unique benefits as an aquatic species and do not require arable land for cultivation (Wawrik and Harriman 2010). This means that algae cultivation does not need to compete with agricultural commodities for growing space (Haag 2007). Microalgae are photosynthetic microorganisms which convert sunlight, water and CO_2 to sugars, from which macromolecules, such as lipids and triacylglycerols can be obtained. These triacylglycerols are the promising and sustainable feedstock for biodiesel production (Khan et al. 2009). The algae that are used in biodiesel production are usually aquatic unicellular green algae.

Under good conditions, green algae can double its biomass in less than 24 h (Christi 2007). Additionally, green algae can have huge lipid contents, frequently over 50%. This high yield, high density the use algae as a source of biomass for fuel production is investigated, in terms of its productivity, practicality, and innovative potential to create a cost competitive, environmentally friendly, and renewable source of liquid fuel. The annual productivity and oil content of algae is far greater than seed crops, e.g., soybean produces about 450 L oil per hectare, canola produces 1,200 L/ha, and palm produces 6,000 L/ha; compare that to algae can yield 90,000 L/ha (Christi 2007). Oil content was found in different green algae on the basis of dry weight, *Chlorella* spp. 28–32%, *Nitzschia* spp. 45–47%, *Nannochloropsis* spp. 31–68% and *Schizochytrium* spp. 50–77% (Campbell 2008).

In order to efficiently produce biodiesel from algae, high productivity of fatty acid-containing lipid components is essential when the algae are grown in open culture systems (Bisen et al. 2010).

The pilot plant designs are used for microalgae culture of high oil content species. There are two methods of large-scale production of microalgae: raceway pond and tubular photobioreactor (Janssen et al. 2003). On the one hand, closed photobioreactors have had more significance, because they allow a better control of the cultivation conditions than open systems. Therefore, closed photobioreactors have some advantage: higher biomass productivities are obtained and contamination troubles are controlled (Pegallapati and Nirmalakhandan 2011). On the other hand, open ponds have easier operation and construction, although they are limited in the control of culture conditions. It is the best system for mass cultivation of microalgae, moreover, raceway ponds are the most profitable (Ugwu et al. 2008). Algal oil can be an economic source for biofuel in the future is still highly dependent on the petroleum oil price.

6.2 Edible Oil Crops

There is heated debate about whether to use edible oil crops for biofuel production or not as well as cultivation of non-edible crops like *Jatropha* on land already under agriculture use. Currently, more than 95% of the world biodiesel is produced from edible oil which is easily available on large scale from the agricultural industry. However, continuous and large-scale production of biodiesel from edible oil without proper planning may cause negative impact to the world, such as depletion of food supply leading to economic imbalance. A possible solution to overcome this problem is to use non-edible oil or waste edible oil (Gui et al. 2008). The natural issue arising from the diversion of arable land from food production to bio-energy crops is that how will this affect food production and food security? Biofuel proponents, and there is already a vocal ‘biofuel lobby’, argue that bioenergy crops would only be grown on degraded or wasteland, not fertile land. But, if the wasteland is capable of supporting *Jatropha* cultivation, should it not be used for the cultivation of selected cereal or oil crops, or if not that, then fodder grasses? India and all of South Asia have large livestock populations, which serve as additional support for local food security. The region is deficient in fodder and all kinds of non-arable land should be diverted to fodder grasses, not crops to produce agrofuels (Sahai 2010). Use of edible oil crops for non-edible purpose is a matter of debate from socio-economic aspect as population of many countries is malnourished.

6.2.1 *Glycine max*

Soybean is an annual crop grown in temperate, subtropical and tropical climates around the world, and its primary producers are the United States, Brazil, Argentina, China, and India. Soybean oil is the major edible oil in the world. Uses for soybean include as an edible vegetable, as a source for cooking oil, as a crop for pasture, fodder, or silage, and as a source for oils used in the manufacture of paints, linoleum, oil cloth, printing inks, soap, insecticides, and disinfectants. The average oil content for soybeans is 18–20%, with protein content of 40%. In favorable conditions, a high yield of soybeans seeds around the world is approximately 1,700 kg/ha. Soybean oil deodorizer distillate (SODD) is a valuable by-product in the refining of soybean oil and amounts to 0.2–0.5% of the feedstock. It is rich in triglycerides, free fatty acid, natural tocopherols (vitamin E) and sterols, which are widely used in the biofuel, food and pharmaceutical industries (Yun et al. 2010). An efficient method to recover acid oil from soybean soapstock has been investigated. This soap splitting (SS) process can be successfully operated at ambient temperature only with water and sulfuric acid. Maximum yield (97%) of acid oil was achieved based on the total fatty acids of soybean soapstock. The advantages of this process revealed a prospect option to utilize SS for biodiesel production. (Balan et al. 2009: Wang et al. 2007). Soybeans are edible and protein rich crops. Surplus oil may be diverted at present for biodiesel technology development but ultimately alternative non-edible crops have to be developed.

6.2.2 *Helianthus annuus*

Sunflower is an annual, upright, broadleaf plant with a growing cycle of approximately 4 months from planting to maturity. It is native to the western United States, and it was spread throughout North America by Native Americans. The seeds are harvested for edible oil production, commercial birdseed, and as a snack-food for human consumption.

Production of sunflower takes place all over the world, including western Russia, Ukraine, and Argentina. Average yields of approximately 1,500 kg/ha are lower than soybean yields, and the necessary inputs are greater (Pimentel and Patzek 2005). A continuous process was developed for production of biodiesel from sunflower oil in basic medium. The conditions of conducting the process were found with consideration of the kinetics of the interesterification reaction. The modeling method is widely used for obtaining reliable information on the process due to its expanded thermodynamic packages, extensive data on components, and improved methods of calculation, although it also gives some anticipated differences from the real data from conducting the process (Maceiras et al. 2010; Balan et al. 2009). Similar to soybean, it is primarily edible oil and diversion to non-edible purposes is not likely to be encouraged in near future.

6.2.3 *Elaeis guineensis*

Palm oil is derived from the fruit (both the flesh and the seed) of a tall palm species that can grow up to 20 m (over 65 ft) tall. The fruits are produced in large clusters numbering from 200 to 300 per cluster, weighing up to 10 kg. Each fruit is about the size of a plum, and the oil content of the fleshy endosperm can be anywhere from 40 to 70% in some varieties, and palm kernel oil contents are typically about 50%. The oil palm originated along the western coast of Africa, and has since spread to most tropical areas in the world. Palm oil is used as an ingredient in margarines, vegetable oils, candles, lubricants, soaps, and more recently, biodiesel production. According to the U.S. Department of Agriculture, 28 MT of palm oil were produced worldwide in 2004, and it is on pace to surpass soybean oil as the most widely produced vegetable oil in the world. Demand for palm oil is rising particularly for use as a biodiesel fuel. The demand for palm oil usage is forecast to double by 2020 (Yusof and Arif 2005). To achieve that production increase, 1,160 new square miles will have to be planted every year for 20 years. Palm oil is good candidate as its oil is not preferred over soybean and sunflower but production cost and area under cultivation are important.

6.2.4 *Brassica napus*

Rape is a cruciferous crop that produces storage root below ground and vegetation similar to kale above ground. The rapeseed is harvested for oil production, with an increasing demand for use as a biodiesel source worldwide. Rapeseed oil is also used in lubricants, illuminants, and soap manufacturing. It is primarily produced in Canada (known as canola oil) and Western Europe, but significant production also takes place in China. Oil contents for rapeseed average between 37 and 50%, making it a high-yielding oil crop. Seed yields can vary from 900 to 3,000 kg/ha and yields of 1,500 kg/ha would yield approximately 500 kg of oil and 1,000 kg of a high protein meal remaining after oil extraction. Biodiesel production from rapeseed is used in a major way using different biodiesel production technologies (Long et al. 2011). How much edible oil can be diverted in the production of biodiesel without affecting the food security has to be seen in near future.

7 Economic and Environmental Benefits

Advantages and disadvantages of using biodiesel as an alternative source of energy are summarized in Table 2. Encouragement of biodiesel is mainly because of reducing dependency on limited resources of fossil fuel, developing alternative fuels from renewable sources, benefits to farmers and less pollution in the environment or higher carbon credits. Agriculture based industrial process development is always beneficial to masses as agriculture is the prime profession in many developing

Table 2 Advantages and disadvantages of biodiesel production and usage

S.No.	Advantages	Disadvantages
1.	It is made from renewable resources	Currently, it is more expensive than fossil diesel fuel.
2.	Fossil diesel fuel is a limited resource; biofuels can be manufactured from a wide range of materials including crop waste, manure, and other by products.	Biodiesel is currently mostly produced from edible oil which could lead to shortages and increased prices. The end result of this could be more hunger in the world.
3.	It causes less pollution as compared to fossil diesel-powered engines.	It tends to reduce fuel economy.
4.	It is relatively less inflammable compared to the fossil diesel.	It is less suitable for use in low temperatures
5.	It is biologically degradable and reduces the danger of contamination of soil and underground water during transport, storage and use.	It cannot be transported in pipelines and gives out more nitrogen oxide emissions.
6.	Its refineries are comparatively simpler and environmental-friendly in design than typical petrochemical refineries.	It can cause inner fuel tubes of older vehicles to lose their long-lasting qualities.
7.	Biodiesel has very good lubricating properties, significantly better than fossil diesel which can prolong engine's life.	It can only be used in diesel-powered engines.
8.	Biodiesel, despite emitting significantly less harmful carbon emission compared to standard diesel, still somewhat contributes to global warming and climate change.	It is more likely than fossil diesel to attract moisture, which can cause problems in cold weather (fuel freezing, deposit of water in the vehicle fuel delivery system, fuel cold flow, clouding, and an increased corrosion, for example) and increase the risk of microbial growth (which can also clog engine filters).
9.	It is suitable for catalytic converter.	Suitable technology and public distribution system have to be developed.

countries. These processes can provide cost benefits to farmers. Since the plant like *Jatropha* can be grown on marginal lands, use of such land will provide additional benefits to farmers as these crops will not compete with irrigated crop land. Major impact will be on environment because of large acres cultivation of plants on land otherwise lying barren (Anonymous 2010; Achten et al. 2007). When crop like soybean is used, soybean biodiesel produces 41% less greenhouse gas emissions than diesel fuel. Soybeans have another environmental advantage over crop like corn because they require much less nitrogen fertilizer and pesticides, which get into groundwater, streams, rivers and oceans. These agricultural chemicals pollute drinking water, and nitrogen decreases biodiversity in global ecosystems. Nitrogen fertilizer, mainly from corn, causes the 'dead zone' in the Gulf of Mexico (http://www.sciencedaily.com/releases/2006/07/060710180310.htm). It is evident from

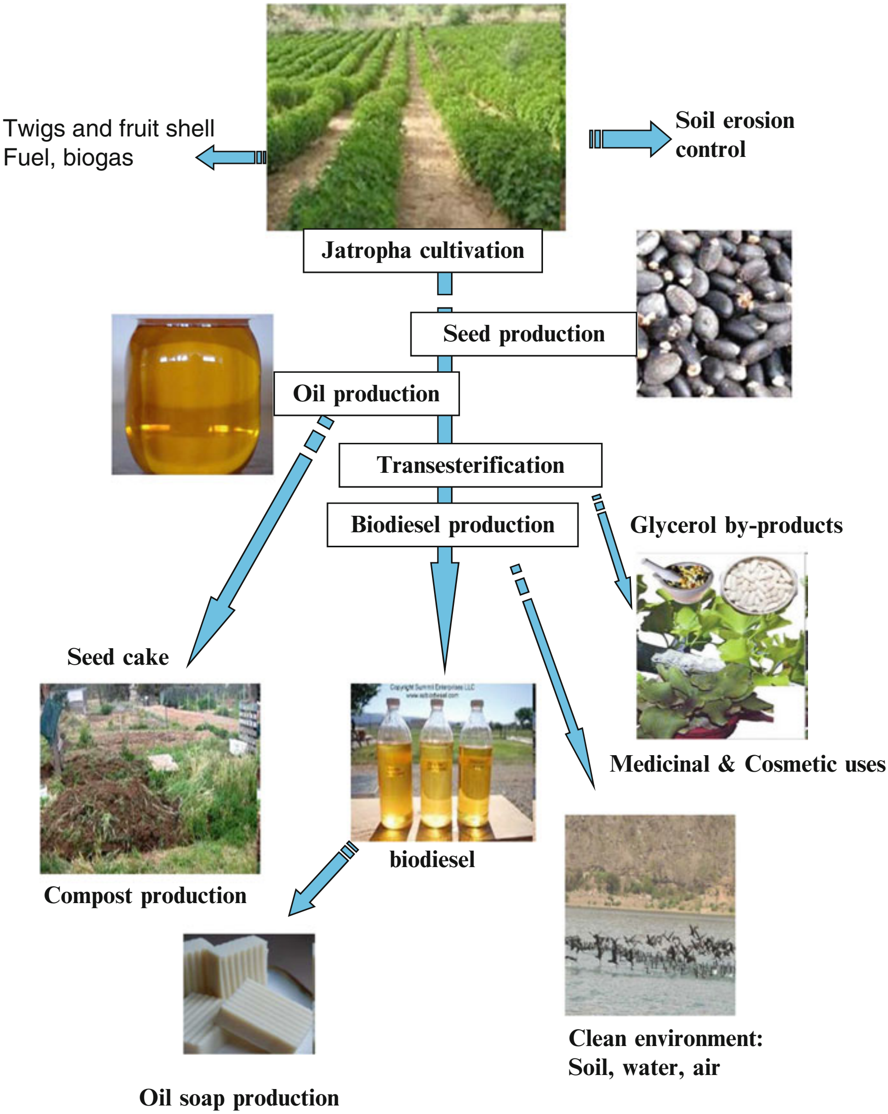

Fig. 10 Economic and environmental benefits of cultivation of *Jatropha curcas* on large waste lands for sustainable agriculture. Besides control of soil erosion and clean environment (soil, water and air), it provides seed cake and glycerol as by-products which have many uses in agriculture, cosmetic and medicinal industries

available data, usage and economic benefits that *J. curcas* is attaining significant importance in the rural economy of developing countries and will have an impact on socio-economic status of community being a labor intensive crop. The major benefits are summarized in Fig. 10.

8 Impact on Agriculture Sustainability

Biofuels have become a major issue on global commodities markets over the last years as they are increasingly put forward as politically, environmentally and economically friendly alternatives to fossil fuels. Currently biodiesel is obtained from both edible and non-edible crops. In fact, no project can be considered sustainable if it is not both economically and socially sustainable. Sustainability can be framed by three inseparable dimensions: environmental, economic and social (Achten et al. 2007). If the plant like *J. curcas* is produced on lands which are not suitable for edible crop production, this will have no problem. Although even some basic agronomic characteristics of *J. curcas* are not yet fully understood, the agrotechnology is being developed rapidly. Preliminary lifecycle energy and greenhouse gas balances are positive for *J. curcas*, but the green house gas balance is expected to be much dependent on the type of land use. The other factors important for the sustainability are: cultivation intensity, the distance to markets, soil, water, vegetation structure and biodiversity. Next to biodiesel production and wasteland reclamation, *J. curcas* also hosts socio-economic development potential. The multipurpose character of the plant (Fig. 10) and the labor-intensive production chain are thought to be the main drivers for rural development in most of the developing countries.

Soybean biodiesel produce more energy than is needed to grow the crops and convert them into biodiesel. However, biodiesel cannot come close to meeting the growing demand for alternatives to petroleum products. Dedicating all current U.S. corn and soybean production to biofuels would meet only 12% of gasoline demand and 6% of diesel demand. Therefore, It may be concluded that agriculture sustainability will depend upon future technology development in coming years to make biodiesel an alternative source of energy.

9 Conclusion

Biofuels have the potential to substitute for some of that "fossil" oil. The need for substitution is driven by rapidly increasing carbon dioxide concentrations in the atmosphere, caused by burning fossil fuels, with associated global warming which is suggested to cause a 0.5 m rise in the sea level by the end of the century. Biodiesel demand is constantly increasing as the reservoir of fossil fuel are depleting. Unfortunately biodiesel produced from oil crop, waste cooking oil and animal fats are not able to replace fossil fuel. The viability of the first generation biofuels production is however questionable because of the conflict with food supply. *Jatropha* plant is projected as major player in biodisel production using marginal soils. Microalgae is other alternative for oil production in place of land crops. Microalgal biorefinery approach can be used to reduce the cost of making microalgal biodiesel. Microalgal-based carbon sequestration technologies cover the cost of carbon capture and sequestration. On the same time, quality standards for biodiesel are developing and quality certification systems have started to emerge, prompting engine manufacturers to extend their warranties. However, the economics of today's diesel prices and the prices of potential feedstock sources do not seem promising without continued government support and technological improvements. Projected increases in vegetable oil prices, especially soybean oil, will continue to squeeze margins for biodiesel producers. Even with supportive policies and infrastructure, time will be needed before second generation biofuels will be able to make an impact in any developing country, because of the research, development and demonstration requirements needed to reach the commercial implementation stage. Given the still-early point in commercial development of second-generation biofuel technologies, it is difficult to project what role developing countries are likely to take in a global biofuel economy in the long term. One possibility is that they simply become exporters of second generation feed stocks, taking advantage of their favorable natural climates and low labor costs for growing biomass. A more attractive evolution would be their becoming producers, users and exporters of finished biofuels, thereby retaining domestically more of the considerable added value involved in the conversion of the feed stocks to finished fuels.

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References

Abdelgadir HA, Johnson SD, Van Staden J (2009) Promoting branching of a biofuel crop *Jatropha curcas* L. by foliar application of plant growth regulators. Plant Growth Regul 58:287–295

Abdelgadir HA, Jager AK, Johnson SD, Van Staden J (2010) Influence of plant growth regulators on flowering, fruiting, seed oil content, and oil quality of *Jatropha curcas*. Suth Afr J Bot 76:440–446

Abdul M, Gerpen JHV (2001) The effect of biodiesel oxidation on engine performance and emissions. Biomass Bioenergy 20:317–325

Abhilash PC, Srivastava P, Jamil S, Singh N (2011) Revisited *Jatropha curcas* as an oil plant of multiple benefits: critical research needs and prospects for the future. Environ Sci Pollut Res 18:127–131

Achten WMJ, Mathijs E, Verchot L, Singh VP, Aerts R et al (2007) Jatropha biodiesel fueling sustainability? Biofuels Bioprod Biorefin 1:283–291

Achten WMJ, Verchot L, Franken YJ, Mathijs E, Singh VP, Aerts R et al (2008) Jatropha bio-diesel production and use. Biomass Bioenergy 32:1063–1084

Adebowale KO, Adedire CO (2006) Chemical composition and insecticidal properties of the underutilized *Jatropha curcas* seed oil. Afr J Biotechnol 5:901–906

Agarwal D, Kumar L, Agarwal AK (2008) Performance evaluation of a vegetable oil fuelled compression ignition engine. Renew Energy 33:1147–1156

Anonymous (2010) Impacts of the EU biofuel target on agricultural markets and land use: a Comparative modelling assessment. http://ec.europa.eu/energy/renewables/studies/doc/landuse_change/study_jrc_biofuel_target_iluc.pdf

Augustus GDPS, Jayubalan M, Seiler GJ (2002) Evaluation and bioinduction of energy components of *Jatropha curcas*. Biomass Bioenergy 23:161–164
Azam MM, Waris A, Nahar NM (2005) Prospects and potential of fatty acid methyl esters of some non-traditional seed oils for use as biodiesel in India. Biomass Bioenergy 29:293–302
Balan V, Rogers CA, Chundawat SPS, Sousa LDC, Slininger PJ, Gupta R, Dale BE (2009) Conversion of extracted oil cake fibers into bioethanol including DDGS, canola, sunflower, sesame, soy, and peanut for integrated biodiesel processing. J Am Oil Chem Soc 86:157–165
Balusamy T, Marappan R (2007) Performance evaluation of direct injection diesel engine with blends of *Thevetia peruviana* seed oil and diesel. J Sci Ind Res 66:1035–1040
Banapurmath NR, Tewari PG, Hosmath RS (2008) Performance and emission characteristics of a DI compression ignition engine operated on Honge, *Jatropha* and sesame oil methyl esters. Renew Energy 33:1982–1988
Bisen PS, Sanodiya BS, Thakur GS, Baghel RK, Prasad GBKS (2010) Biodiesel production with special emphasis on lipase-catalyzed transesterification. Biotechnol Lett 32:1019–1030
Bouaid A, Diaz Y, Martinez M, Aracil J (2005) Pilot plant studies of biodiesel production using *Brassica carinata* as raw material. Catal Today 106:193–196
Bouaid A, Martinez M, Aracil J (2009) Production of biodiesel from bioethanol and *Brassica carinata* oil: oxidation stability study. Bioresour Technol 100:2234–2239
Campbell MN (2008) Biodiesel: algae as a renewable source for liquid fuel. Guelph Eng J 1:2–7
Chapagain BP, Yehoshua Y, Wiesman Z (2009) Desert date (*Balanites aegyptiaca*) as an arid lands sustainable bioresource for biodiesel. Bioresour Technol 100:1221–1226
Christi Y (2007) Biodiesel from microalgae. Biotechnol Adv 25:294–306
Collins K (2008) The role of biofuels and other factors in increasing farm and food prices: a review of recent developments with a focus on feed grain markets and market prospects. Kraft Foods Global, Inc., Chicago
Das LM, Bora DK, Pradhan S, Naik MK, Naik SN (2009) Long-term storage stability of biodiesel produced from Karanja oil. Fuel 88:2315–2318
Dash AK, Pradhan RC, Das LM, Naik SN (2008) Some physical properties of simarouba fruit and kernel. Int Agrophys 22:111–116
Demirbas A (2006) Energy priorities and new energy strategies. Energy Educ Sci Technol 16:53–109
Deshmukh SJ, Bhuyar LB (2009) Transesterified hingan (Balanites) oil as a fuel for compression ignition engines. Biomass Bioenergy 33:108–112
Devan PK, Mahalakshmi NV (2009a) A study of the performance, emission and combustion characteristics of a compression ignition engine using methyl ester of paradise oil-eucalyptus oil blends. Appl Energy 86:675–680
Devan PK, Mahalakshmi NV (2009b) Utilization of unattended methyl ester of paradise oil as fuel in diesel engine. Fuel 88:1828–1833
Diwani GE, Attia NK, Hawash SI (2009) Development and evaluation of biodiesel fuel and by-products from Jatropha oil. Int J Environ Sci Technol 6:219–224
European Commission (2006) Communication from the Commission to the Council and the European Parliament. Renewable Energy Road Map. Renewable energies in the 21st century: building a more sustainable future. COM 848 final, Brussels
Fairless D (2007) Biofuel: the little shrub that could – maybe. Nature 449:652–655
FAOSTAT (2009) Land use classifications. Food and Agricultural Organization of the United Nations, Rome
Frohlich A, Rice B (2005) Evaluation of *Camelina sativa* oil as a feedstock for biodiesel production. Ind Crop Prod 21:25–31
Government of India (2008) The national policy on bio-fuel. Ministry of New and Renewable Energy, New Delhi
Gübitz GM, Mittelbach M, Trabi M (1999) Exploitation of the tropical oil seed plant *Jatropha curcas* L. Bioresour Technol 67:73–82
Gui MM, Lee KT, Bhatia S (2008) Feasibility of edible oil vs nonedible oil vs waste edible oil as biodiesel feedstock. Energy 33:1646–1653

Gumus M (2008) Evaluation of hazelnut kernel oil of Turkish origin as alternative fuel in diesel engines. Renew Energy 33:2448–2457
Haag AL (2007) Algae bloom again. Nature 447:520–521
Hoogwijk MA, Faaij B, de Vries EB, Turkenburg W (2005) Potential biomass energy out to 2100 for four IPCC SRES land-use scenarios. Biomass Bioenergy 29:225–257
Jachmanian I, Dobroyan M, Moltini M, Segura N, Irigaray B, Veira JP, Vieitez I, Grompone MA (2010) Continuous lipase-catalyzed alcoholysis of sunflower oil: effect of phase-equilibrium on process efficiency. J Am Oil Chem Soc 87:45–53
Jain S, Sharma MP (2010) Prospects of biodiesel from Jatropha in India: a review. Renew Sustain Energy Rev 14:763–771
Janssen M, Tramper J, Mur L, Wijffels R (2003) Enclosed outdoor photobioreactors: light regime, photosynthetic efficiency, scale-up, and future prospects. Biotechnol Bioeng 81:193–210
Kalita D (2010) Potentiality of hydrocarbon yielding plants for future energy and chemicals. In: Ramawat KG (ed) Desert plants: biology and biotechnology. Springer, Berlin/Heidelberg, pp 37–56
Kalscheuer R, Luftmann H, Steinbüchel A (2004) Synthesis of novel lipids in *Saccharomyces cerevisiae* by heterologous expression of an unspecific bacterial acyltransferase. Appl Environ Microbiol 70:7119–7125
Kalscheuer R, Stölting T, Steinbüchel A (2006) Microdiesel: *Escherichia coli* engineered for fuel production. Microbiology 152:2529–2536
Karmee SK, Chadha A (2005) Preparation of biodiesel from crude oil of *Pongamia pinnata*. Bioresour Technol 96:1425–1429
Kesari V, Rangan L (2010) Development of *Pongamia pinnata* as an alternative biofuel. Crop – current status and scope of plantations in India. J Crop Sci Biotechnol 13:127–137
Kesari V, Krishnamachari A, Rangan L (2009) Effect of auxins on adventitious rooting from stem cuttings of candidate plus tree *Pongamia pinnata* (L.), a potential biodiesel plant. Trees Struct Funct 23:597–604
Khan SA, Rashmi HMZ, Prasad S, Banerjee UC (2009) Prospects of biodiesel production from microalgae in India. Renew Sustain Energy Rev 13:2361–2372
Khemkladngoen N, Cartagena JA, Fukui K (2011) Physical wounding-assisted agrobacterium mediated transformation of juvenile cotyledons of a biodiesel-producing plant, *Jatropha curcas* L. Plant Biotechnol Rep 5:235–243
Kim SJ, Jung SM, Park YC, Park K (2007) Lipase catalyzed transesterification of soybean oil using ethyl acetate, an alternative acyl acceptor. Biotechnol Bioprocess Eng 12:441–445
Leduc S, Natarajan K, Dotzauer E, McCallum I, Obersteiner M (2009) Optimizing biodiesel production in India. Appl Energy 86:125–131
Leung DYC, Koo BCP, Guo Y (2006) Degradation of biodiesel under different storage conditions. Bioresour Technol 97:250–256
Li S, Wang Y, Dong S, Chen Y, Cao F, Chai F et al (2009) Biodiesel production from *Eruca sativa* Gars vegetable oil and motor, emissions properties. Renew Energy 34:1871–1876
Long YD, Guo F, Fang Z, Tian XF, Jiang LQ, Zhang F (2011) Production of biodiesel and lactic acid from oil using sodium silicate as catalyst. Bioresour technol 102:6884–6886
Maceiras R, Rivero JJ, Cancela MA, Urrejola S, Sanchez A (2010) Development and modeling of production of biodiesel from sunflower oil. Chem Technol Fuels Oils 46:154–159
Makkar H, Maes J, Greyt WD, Becker K (2009) Removal and degradation of phorbol esters during pre-treatment and transesterification of *Jatropha curcas* oil. J Am Oil Chem Soc 86:173–181
Mandpe S, Kadlaskar S, Degen W, Keppeler S (2005) On road testing of advanced common rail diesel vehicles with biodiesel from the *Jatropha curcas* plant. Soc Automot Eng Inc 26:356–364
Meher LC, Vidya Sagar D, Naik SN (2006) Technical aspects of biodiesel production by transesterification—a review. Renew Sustain Energy Rev 10:248–268
Miao X, Wu Q (2004) High yield bio-oil production from fast pyrolysis by metabolic controlling of *Chlorella protothecoides*. J Biotechnol 110:85–93
Mukherjee P, Varshney A, Johnson S, Jha TB (2011) *Jatropha curcas*: a review on biotechnological status and challenges. Plant Biotechnol Rep. doi:10.1007/s11816-011-0175-2

Nabi MN, Akhter MS, Shahadat MMZ (2006) Improvement of engine emissions with conventional diesel fuel and diesel–biodiesel blends. Bioresour Technol 97:372–378

Nakpong P, Wootthikanokkhan S (2010) Optimization of biodiesel production from *Jatropha curcas* L. oil via alkali-catalyzed methanolysis. J Sustain Energy Environ 1:105–109

Nieuwenhuyzen WV, Tomas MC (2008) Update on vegetable lecithin and phospholipid technologies. Eur J Lipid Sci Technol 110:472–476

NOVOD (2009) 4th R&D report on tree borne oilseeds, NOVOD Board India. http://www.novod-board.com/4th%20R&D-Report.pdf

OECD-FAO (2007) OECD-FAO Agricultural outlook 2007–2016. Organization for Economic Development and Co-operation, Paris, and the U. N. Food and Agricultural Organization of the United Nations, Rome

Oluwaniyi OO, Ibeyimi SA (2007) Efficacy of catalysts in the batch esterification of the fatty acids of *Thevetia peruviana* seed oil. J Appl Sci Environ Manage 66:1035–1040

Pal A (2007) Production of insecticides. In: Ramawat KG, Merillon JM (eds) Biotechnology secondary metabolites plants and microbes, vol 2. Science Publishers, Enfield, pp 131–149

Pegallapati AK, Nirmalakhandan N (2011) Energetic evaluation of an internally illuminated photobioreactor for algal cultivation. Biotechnol Lett 33:2161–2167

Pimentel D, Patzek TW (2005) Ethanol production using corn, switchgrass, and wood; biodiesel production using soybean and sunflower. Nat Resour Res 14:65–76

Pinto AC, Guarieiro LLN, Rezende MJC, Ribeiro NM, Torres EA, Lopes WA, Pereira PAP, Andrade JB (2005) Biodiesel: an overview. J Braz Chem Soc 16:1313–1330

Poteet MD (2006) Biodiesel crop implementation in Hawaii. Department of Agriculture, Hawaii Agriculture Research Center Aiea, HI 96701

Qin J (2005) Bio-hydrocarbons from algae. Report for the Rural Industries Research and Development Corporation, RIRDC Publication No. 05/025

Ramadhas AS, Jayaraj S, Muraleedharan C (2005) Biodiesel production from high FFA rubber seed oil. Fuel 84:335–340

Rashid U, Anwar F (2008) Production of biodiesel through base-catalyzed transesterification of safflower oil using an optimized protocol. Energy Fuel 22:1306–1312

Razon LF (2009) Alternative crops for biodiesel feedstock. CAB Rev. doi:10.1079/PAVSNNR20094056

Reddy MP, Pamidimarri DVNS (2010) Biology and biotechnology advances in *Jatropha curcas* -a biodiesel plant. In: Ramawat KG (ed) Desert plants: biology and biotechnology. Springer, Berlin/Heidelberg, pp 58–72

Reddy BVS, Ramesh S, Kumar AA, Wani SP, Ortiz R, Ceballos H, Sreedevi TK (2008) Bio-fuel crops research for energy security and rural development in developing countries. Bioenergy Res 1:248–258

Sadano Y, Toshimitsu R, Nakano JKY, Yano T (2010) Optimization of compost fermentation of glycerol by-product discharged from biodiesel fuel production process. J Mater Cycles Waste Manage 12:308–313

Sahai S (2010) http://www.genecampaign.org/Publication/Article/AG-Food-Security/Biofuel%20vs%20food%20security.pdf Accessed 15 Feb 2010

Sahoo PK, Das LM (2009) Combustion analysis of jatropha, karanja and polanga based biodiesel as fuel in a diesel engine. Fuel 88:994–999

Sahoo PK, Das LM, Babu MKG, Naik SN (2007) Biodiesel development from high acid value polanga seed oil and performance evaluation in a CI engine. Fuel 86:448–454

Sarma AK, Konwer D, Bordoloi PK (2005) A comprehensive analysis of fuel properties of biodiesel from koroch seed oil. Energy Fuel 19:656–657

Saydut A, Duz MZ, Kaya C, Kafadar AB, Hamamci C (2008) Transesterified sesame (*Sesamum indicum* L.) seed oil as a biodiesel fuel. Bioresour Technol 99:6656–6660

Scholz V, da Silva JN (2008) Prospects and risks of the use of castor oil as a fuel. Biomass Bioenergyy 32:95–100

Scott PT, Pregelj L, Chen N, Hadler JS, Djordjevic MA, Gresshoff PM (2008) *Pongamia pinnata*: an untapped resource for the biofuels industry of the future. Bioenergy Res 1:2–11

Sendzikiene E, Makareviciene V, Janulis P (2005) Oxidation stability of biodiesel fuel produced from fatty wastes. Pol J Environ Stud 14:335–339

Sharma YC, Singh B (2008) Development of biodiesel from karanja, a tree found in rural India. Fuel 87:1740–1742

Singh SP, Singh D (2010) Biodiesel production through the use of different sources and characterization of oils and their esters as the substitute of diesel: a review. Renew Sustain Energy Rev 14:200–216

Smeets EMW, Faaij APC, Lewandowski IM, Turkenburg WC (2006) A bottom-up assessment and review of global bioenergy potentials to 2050. Progress in Energy and Combustion Science 33:56–106

Tiwari AK, Kumar A, Raheman H (2007) Biodiesel production from Jatropha oil (*Jatropha curcas*) with high free fatty acids: an optimized process. Biomass Bioener 31:569–577

Tweeten L, Thompson SR (2008) Long-term global agricultural output supply–demand balance and real farm and food prices. Department of Agricultural, Environmental and Development Economics the Ohio State University

Tyagi OS, Atray N, Kumar B, Datta A (2010) Production, characterization and development of standards for biodiesel – a review. J metrol soc of India 25:197–218

Ugwu CU, Ogbonna JC, Tanaka H (2008) Photobioreactors for mass cultivation of algae. Bioresour Technol 99:4021–4028

Vasudevan PT, Briggs M (2008) Biodiesel production—current state of the art and challenges. J Ind Microbiol Biotechnol 35:421–430

Waltz E (2008) Cellulosic ethanol booms despite unproven business models. Nat Biotechnol 26:8–9

Wang ZM, Lee JS, Park JY, Wu CZ, Yuan ZH (2007) Novel biodiesel production technology from soybean soapstock. Korean J Chem Eng 24:1027–1030

Wang WG, Li R, Liu B, Li L, Wang SH, Chen F (2011) Effects of low nitrogen and drought stresses on proline synthesis of *Jatropha curcas* seedling. Acta Physiol Plant 33:1591–1595

Wawrik B, Harriman BH (2010) Rapid, colorimetric quantification of lipid from algal cultures. J Microbiol Methods 80:262–266

Xin J, Imahara H, Saka S (2009) Kinetics on the oxidation of biodiesel stabilized with antioxidant. Fuel 88:282–286

Xu YX, Hanna MA (2009) Synthesis and characterization of hazelnut oil-based biodiesel. Ind Crop Prod 29:473–479

Xu Y, Liu H, Du W, Sun Y, Ou X, Liu D (2009) Integrated production for biodiesel and 1,3-propanediol with lipase-catalyzed transesterification and fermentation. Biotechnol Lett 31:1335–1341

Yun L, Ling W, Yunjun Y (2010) Cogeneration of biodiesel and tocopherols by combining pretreatment with supercritical carbon dioxide extraction from soybean oil deodorizer distillate. Chem Technol Fuels Oils 46:79–86

Yusof B, Arif SM (2005) Vision 2020—the palm oil phenomenon. Oil Palm Ind Econ J 5:2

Zufarov O, Schmidt Š, Sekretár S (2008) Degumming of rapeseed and sunflower oils. Acta Chimica Slovaca 1:321–328

Forage Legume Intercropping in Temperate Regions: Models and Ideotypes

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Abstract This chapter shows that our models of intercropping do not increase the costs of sowing. The crude protein content in forage dry matter remains high, even with short growing seasons. The models fit easily into various cropping systems and do not require synthetic fertilisers or herbicide. Intercropping is one of the oldest agricultural practices worldwide. Intercropping is beneficial in many ways, encompassing better utilisation of soil resources such as water and nutrients, improved tolerance to abiotic and biotic stress such as low temperatures, drought, pests and diseases and environment-friendly services such as decreased demand for chemical weed control and mineral fertilisers. Despite its long tradition, intercropping offers constant challenges in examining diverse aspects of newly-designed crop associations. One of the most recent and thoroughly studied is intercropping annual temperate legumes with each other for forage production. We established four main principles for such intercropping: same time of sowing; similar growing habit; similar cutting time; and one component has good standing ability (supporting crop) and another is susceptible to lodging (supported crop). Here we review the basic agronomic performance of three main intercropping groups, namely (1) autumn- and spring-sown 'tall' cool season legumes; (2) autumn- and spring-sown 'short' cool season legumes; (3) early and late maturing warm-season annual forage legumes. Intercropping autumn-sown faba bean with autumn-sown common vetch

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had balanced total forage dry matter yield and Land Equivalent Ratio (LER) of 1.42. All combinations of autumn-sown and spring-sown intercrops of semi-leafless and normal-leafed peas resulted in LER values higher than 1. The intercrop of pigeon pea and lablab bean had LER values of 1.12 and 1.10. Majority of the intercrops are justified as economically reliable by Land Equivalent Ratio (LER) values.

Keywords Annual legumes • Environment-friendly forage production • Ideotype • Intercrop modelling • Land equivalent ratio

1 Introduction

Cropping systems based on carefully designed species mixtures have numerous potential advantages under various conditions in comparison to modern monocrop systems with clearly negative impacts on soil and water quality and on biodiversity conservation (Malézieux et al. 2009). Intercropping was one of the most ancient cropping systems, especially in Europe and before the 'fossilisation' of agriculture with synthetic fertilisers and pesticides (Hauggaard-Nielsen et al. 2011). It is generally considered beneficial in many ways, encompassing better utilisation of soil resources such as water and nutrients, improved tolerance to abiotic and biotic stress such as low temperatures, drought, pests and diseases and environment-friendly services such as decreased demand for chemical weed control and mineral fertilisers. Despite its long tradition, intercropping offers constant challenges in examining diverse aspects of newly-designed crop associations.

The term *intercropping* is commonly regarded by agronomists worldwide in an identical manner. One of the clearest definitions is that it represents simultaneous cultivation of at least two crops in the same field (Willey 1979). In a more complex context, it may be regarded as a practical application of ecological principles with respect to biodiversity, plant interactions and other natural regulation mechanisms (Vandermeer et al. 1998). The intercrop components differ in competitive ability for growth factors and therefore use resources in a complementary way (Anil et al. 1998). The reliability of an intercrop is usually evaluated by means of Land Equivalent Ratio (LER), based upon the yields of each component as sole crops and in intercrops, where an intercrop is justified if its LER value is higher than 1 (Kadžiulienė et al. 2011):

$$LER = Y_A(IC) / Y_A(SC) + Y_B(IC) / Y_B(SC),$$

where $Y_A(IC)$ is the yield of the intercrop component A in intercropping, $Y_A(SC)$ is the yield of the intercrop component A in pure stand, $Y_B(IC)$ is the yield of the intercrop component B in intercropping and $Y_B(SC)$ is the yield of the intercrop component B in pure stand. Among other parameters validating intercrops is Monetary Equivalent Ratio (MER) that measures the economic advantage of intercropping over the sole crop with the largest economic return and with a different trend than LER for the same intercrop (Adetiloye and Adekunle 1989).

Fig. 1 Intercropping of faba bean with wheat (Hauggaard-Nielsen et al. 2011)

Legumes (*Fabaceae* Lindl., syn. *Leguminosae* Juss.) are one of the largest plant families in the world, comprising annuals and perennials, herbaceous plants, shrubs and trees, and species of temperate and tropical regions, many of which are economically important (Mikić et al. 2011b). Annual legume species such as pea (*Pisum sativum* L.), chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris* Medik.), bitter vetch (*Vicia ervilia* (L.) Willd.), faba bean (*Vicia faba* L.) and grass pea (*Lathyrus sativus* L.) are considered one of the first domesticated crops, directly carrying out so-called agricultural revolution of the post-glacial Eurasia together with cereals (Mikić et al. 2011a). Lucerne (*Medicago sativa* L.) and red (*Trifolium pratense* L.) and white (*Trifolium repens* L.) clovers are among the most significant perennial forage legume crops in temperate climates. All cultivated legume species are one of the least expensive sources of quality protein for humans and animals. Due to the symbiosis with nitrogen-fixing bacteria, legumes restore and maintain soil fertility (Singh et al. 2007) and are inevitable in the remediation of wastelands and heavily degraded agroecosystems. The contribution of legumes in intercropping systems (Fig. 1) is widely acknowledged, especially by higher yields of the non-fixing intercrop components in comparison to monocultures (Corre-Hellou et al. 2006). Due to their ability of biological nitrogen fixation, legumes are largely involved in both facilitation and dynamics of nitrogen in various plant communities (Hauggaard-Nielsen and Jensen 2005; Fustec et al. 2010). Intercrops including legumes generally improve both temporal and spatial resources such as light, land, water and nutrients (Hauggaard-Nielsen et al. 2008).

2 Intercropping Legumes with Other Crops

Intercropping annual legumes such as pea or common vetch (*Vicia sativa* L.) with cereals is one of the oldest agricultural practices for both forage and grain production (Mihailović et al. 2004). The most common small grain crops intercropped with annual legumes in many European regions are common wheat (*Triticum aestivum* L.), durum wheat (*Triticum durum* Desf.), barley (*Hordeum vulgare* L.), oat (*Avena sativa* L.) and triticale (× *Triticosecale* Wittm. ex A. Camus.). As a rule, cereals profit from intercropping with annual legumes in diverse ways. Intercropping faba bean with barley produces higher yield than monoculture of each crop species (Agegnehu et al. 2006). In temperate regions, the crude protein content in grain dry matter of durum wheat may be higher up to 14% when intercropped with pea than in monoculture (Bedoussac and Justes 2010a). Also, the intercrops of barley with faba bean, pea and white lupin (*Lupinus albus* L.) produce at least the same forage dry matter yields and had significantly higher protein content in comparison to sole crop barley (Strydhorst et al. 2008). Intercrops of autumn-sown annual legumes and small grains have beneficial effects on the succeeding spring-sown forage crops (Ćupina et al. 2011a). Similarly, intercrops of annual legumes with cereals may play important role in tropical climates. Intercropping cowpea (*Vigna unguiculata* (L.) Walp.) and lablab bean (*Lablab purpureus* Sweet) with maize (*Zea mays* L.) and sorghum (*Sorghum bicolour* (L.) Moench) enhance dry season feed availability in sub-equatorial Africa (Ngongoni et al. 2007). When intercropped with rice (*Oryza sativa* L.), mung bean (*Vigna radiata* (L.) R. Wilczek) significantly improve the formation of arbuscular mycorrhizas, particularly in rice roots (Li et al. 2009). On the other hand, cereals have a higher competitive ability than legumes as a result of their below-ground competitive ability (Mariotti et al. 2009) and thus may reduce light absorption and biomass production in the legume component (Bedoussac and Justes 2010b) or decrease the yield components in comparison to its monocrop (Sobkowicz 2006).

In many temperate and humid regions, annual legumes may be intercropped with brassicas (*Brassicaceae* Juss.). Generally, brassicas such as rapeseed (*Brasica napus* L.) act as a more appropriate companion than barley in the intercrops with legumes such as pea, since the latter is too strong a competitor, resulting in more efficient nitrogen fixation (Andersen et al. 2004). The root architecture in legume-brassica intercrops, such as faba bean with rapeseed and common vetch with fodder cabbage (*Brassica oleracea* L.), differs from that in monocrops, particularly taproot, reducing the effect of competition and enhancing the nitrogen transfer from legumes to brassicas (Cortés-Mora et al. 2010). Triple intercrops, comprising various annual legumes, cereals and brassicas such as pea, wheat and rapeseed, are rather efficient in weed suppression, simultaneously producing higher yields of both dry matter and grain, confirmed by LER values higher than 1.2 (Szumigalski and Van Acker 2005). When intercropped with leafy vegetables such as lettuce (*Lactuca sativa* L.), certain tropical annual legumes with large leaves such as lablab bean provide increased yields and desirable LER values (Roy et al. 2003).

Grasses are often self-sufficient for quality silage production (Conaghan et al. 2010), either from one-species swards or mixtures of different species of the same or different genera (Lovatt et al. 2010). However, intercrops of grasses such as ryegrass (*Lolium* spp.), timothy (*Phleum pratense* L.) and fescue (*Festuca* spp.) with clovers have a long tradition in many temperate regions, especially in western, central and northern regions of Europe (Mela 2003). The leys of red clover and grasses offer numerous environment-friendly services. They leave a considerable amount of biomass and nitrogen after harvesting and thus provide substantial fertility basis for the next crop (Huss-Danell et al. 2007). Also, the swards of perennial legumes and grasses, particularly white clover and cocksfoot (*Dactylis glomerata* L.), may increase and stabilise the yield of a grassland crop and reduce invasion by unsown species by increasing its species diversity (Frankow-Lindberg et al. 2009). Generally, environmental balance of forage legumes in intercrops is positive by improving soil structure, preventing soil erosion, minimising nitrate leaching, reducing energy consumption, decreasing global warming potential of livestock systems and suppressing the invasion of pests, diseases and weeds (Peyraud et al. 2009). Advances in biotechnology and breeding of perennial forage legumes and grasses contribute to mitigating climate changes and enhancing carbon sequestration (Guiney et al. 2004; Abberton et al. 2008). In the end, numerous multiple intercrops of perennial legumes and grasses are present in many wild floras throughout the world, with little attention directed to their maintenance and assessment of their potential as crops (Muir et al. 2011).

In all the forms of intercropping legumes with non-leguminous crops, their role is notably beneficial to an intercrop as whole, resulting in better agronomic performance, a non-leguminous component, providing it with better availability of natural resources, especially nutrients, and the environment itself.

3 Mutual Intercropping of Legumes

Intercropping one legume species with another is less examined than all previously described cases, especially the intercrops of annual legumes with cereals or perennial legumes with grasses. There available literature resources recognise four basic forms of the mutual legume intercropping: (1) two or more annual forage legumes; (2) annual legume companion cropping in establishing perennial forage crops; (3) annual forage legumes drilled into an existing stand for boosting short-term yields; (4) sowing perennial legumes in between the rows of an arable crop (Koivisto 2002). The second case is of special importance, since a companion annual forage legume crop can provide an economic yield during the establishment of the perennial forage crop and thus produce an economic return already in the seeding year.

Short-season annual legumes such as field pea and vetches are suitable for intercropping with perennial forage legumes because the canopy structure is not dense enough to cause suppressive shading (Ćupina et al. 2009a). The semi-leafless field pea cultivars, with a mutation inducing the transformation of all leaflets into tendrils

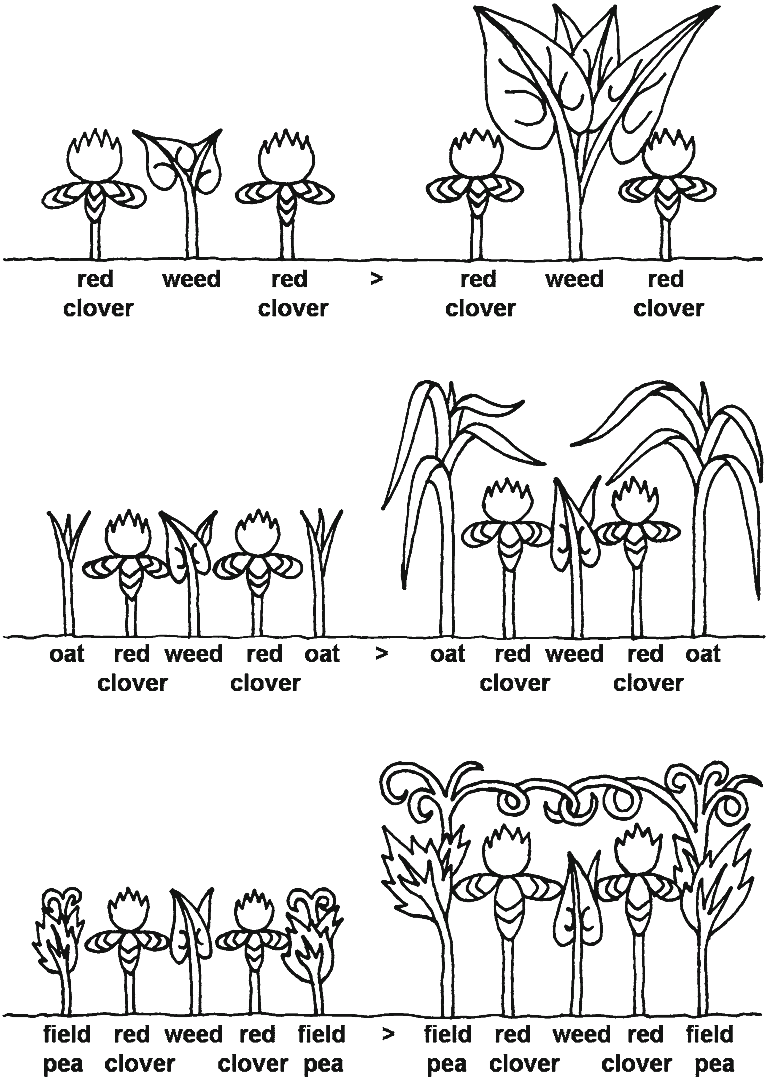

Fig. 2 Different ways of the perennial legumes establishment (Ćupina et al. 2011d): (*top row*) pure stand of red clover is easily matched by weeds; (*middle row*) oat is too aggressive to both red clover and weeds; (*bottom row*) field pea decreases weeds and enhances the growth of young red clover

and genetic structure of *afaf TLTL*, are particularly useful for this purpose since the light penetration is significantly facilitated, providing better conditions for the initial growth of the undersown crop (Ćupina et al. 2006). The establishment of a pure stand of red clover is endangered by weeds (Fig. 2, top row) and thus makes the herbicide application inevitable. A traditional way of establishing red clover accompanied with oat does prevent weeds, but also reduces the growth and the development of newly established red clover (Fig. 2, middle row). Establishing red clover together with a semi-leafless pea companion crop provides it with optimum conditions for normal growth and development, as well as with an advantage in relation to weeds (Fig. 2, bottom row). In such way, the intercropping of perennial crop with an annual legume companion crop represents a fine example of environment-friendly forage production, where the latter acts as a bioherbicide and excludes the expenses of chemical control. Also, when cut together, the total forage crude protein yield is much higher in comparison to monocrops of both components (Ćupina et al. 2010a).

The published resources on the intercrops of two or more annual legume species are even less abundant. Some intercrops are beneficial, at least for one of the components, such as in the one where lupin may use phosphorus that accompanying soybean (*Glycine max* (L.) Merr.) cannot (Braum and Helmke 1995) or where the intercropping of soybean and pigeon pea (*Cajanus cajan* (L.) Millsp.) is combined with subsoiling and may amortise the effects of droughts of unpredictable intensity (Ghosh et al. 2006a). Annual legumes with known pharmaceutical properties and prominent biochemical potential, such as fenugreek (*Trigonella phoenum-graecum* L.), effectively reduce the infection of broomrape (*Orobanche crenata* Forssk.) in faba bean (Evidente et al. 2007; Fernández-Aparicio et al. 2011). However, in the mutual intercrops of annual legumes, two components may compete for available nutrient resources resulting in the depression of growth of one of them, such as in the case of pigeon pea and soybean, where nitrogen appears as a limiting factor for growth of the former (Ghosh et al. 2006b). Also, the occurrence of the superiority of intercropping over pure stands may be rather limited, such as in the intercrop of two soybean cultivars where only the highest plant density produces the expected result (Biabani et al. 2008).

During the past decade, the Institute of Field and Vegetable Crops and the Faculty of Agriculture of the University of Novi Sad have carried out a concerted research aimed at assessing the possibility of mutual annual legume intercropping for forage production. Hundreds of the accessions of numerous annual legume species of diverse origin and status in the collection maintained in Novi Sad were screened for the potential for intercropping with each other. Among the main conclusions of this preliminary testing are the following facts: species with long and lodging stems, such as vetches, easily control weeds in pure stands but suffer from forage losses (Fig. 3, first row from above); crops with good standing ability, such as faba bean, almost regularly are heavily infested by weeds in pure stands and require chemical control (Fig. 3, second row from above); intercropping annual legumes with inadequate growth habit gives significant advantage to one component, such as common vetch, and severely affects another, such as semi-leafless pea (Fig. 3, third row from above); a fully compatible intercropping, such as the one of white lupin and common vetch, provides the best possible effects (Fig. 3, fourth row from above). The results of this long-term examination of numerous aspects of the mutual annual legume intercrops for forage production led to the establishment of four basic principles essential for a successful intercropping of two annual forage legume species (Ćupina et al. 2011d):

1. The same time of sowing;
2. The similar growing habit;

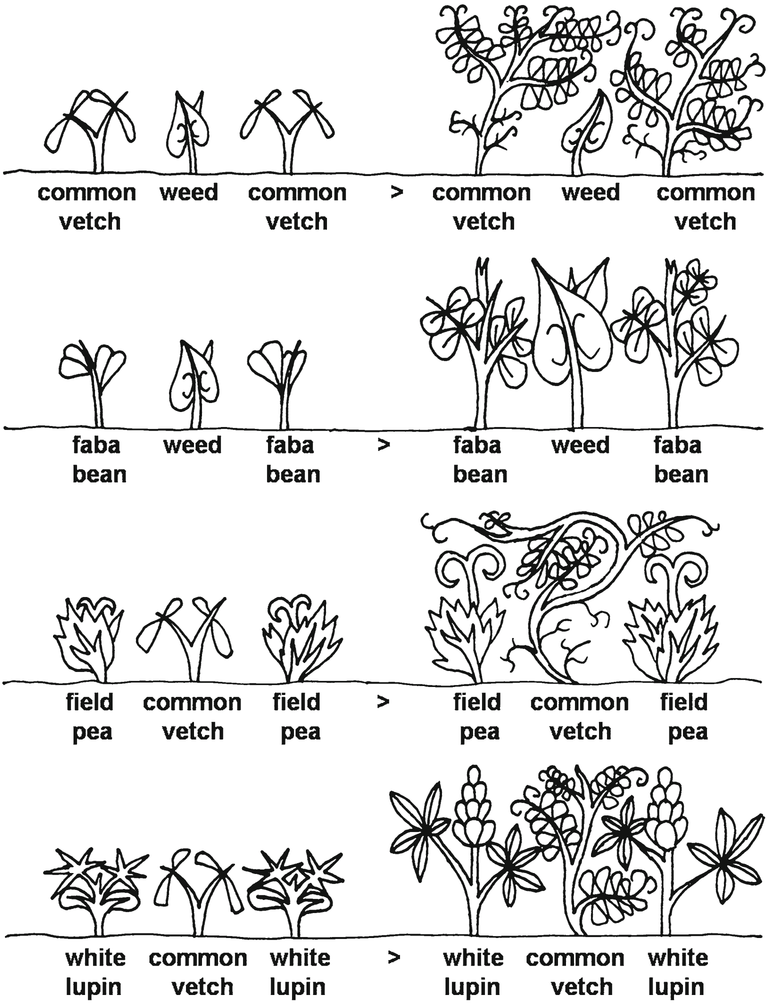

Fig. 3 Different aspects of the mutual intercropping of annual legumes (Ćupina et al. 2011d): (*first row from above*) in the pure stand of a lodging-susceptible crop such as common vetch, weeds are suppressed but lower leaves in vetch are mostly lost; (*second row from above*) in the pure stand of a lodging-resistant crop such as faba bean, weeds have favourable conditions; (*third row from above*) semi-leafless field pea and common vetch are less-beneficial choice since different growing habit; (*fourth row from above*) white lupin and common vetch are appropriate choice respecting all basic principles

3. The similar time of maturing for cutting;
4. One component has good standing ability (supporting crop) and another is susceptible to lodging (supported crop).

Following these main principles, further trials have been carried out within three main groups of annual forage legume crops:

1. Autumn- and spring-sown 'tall' cool season annual forage legumes;
2. Autumn- and spring-sown 'short' cool season annual forage legumes;
3. Early and late maturing warm-season annual forage legumes.

The proportion of both components in an intercrop was 50%:50%, with the regular sowing rate of each component reduced by half in order to avoid expensive and economically unjustified sowing. So far, all the trials were aimed at the basic above-ground aspects of intercropping such as green forage and forage dry matter yields, calculating LER values for each intercrop, namely LER for green forage yield and LER for forage dry matter yield.

The model, the basic agronomic performance related to forage production and the possible ideotypes for each of three intercrop groups are presented in details within the next three sections.

4 Intercropping 'Tall' Cool Season Annual Legumes

According to their provisional name, the annual forage legume crops in this group characterise with long stems. Within the autumn-sown sub-group (Fig. 4, left), faba bean played the role of supporting crop, while the supported crops were forage pea,

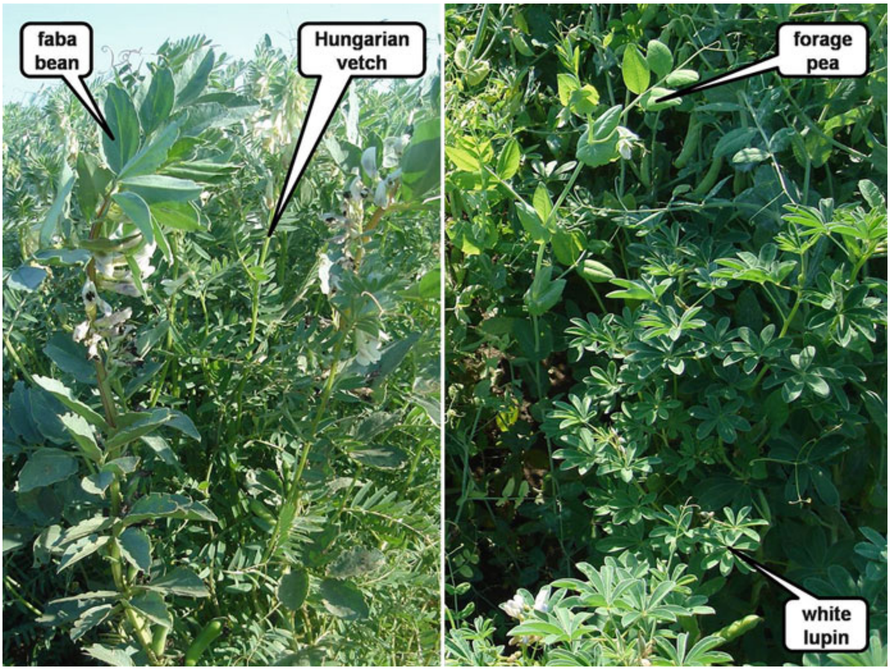

Fig. 4 Examples of intercropping 'tall' cool season legumes: autumn sowing (*left*) – faba bean with Hungarian vetch; spring sowing (*right*) – white lupin with forage pea

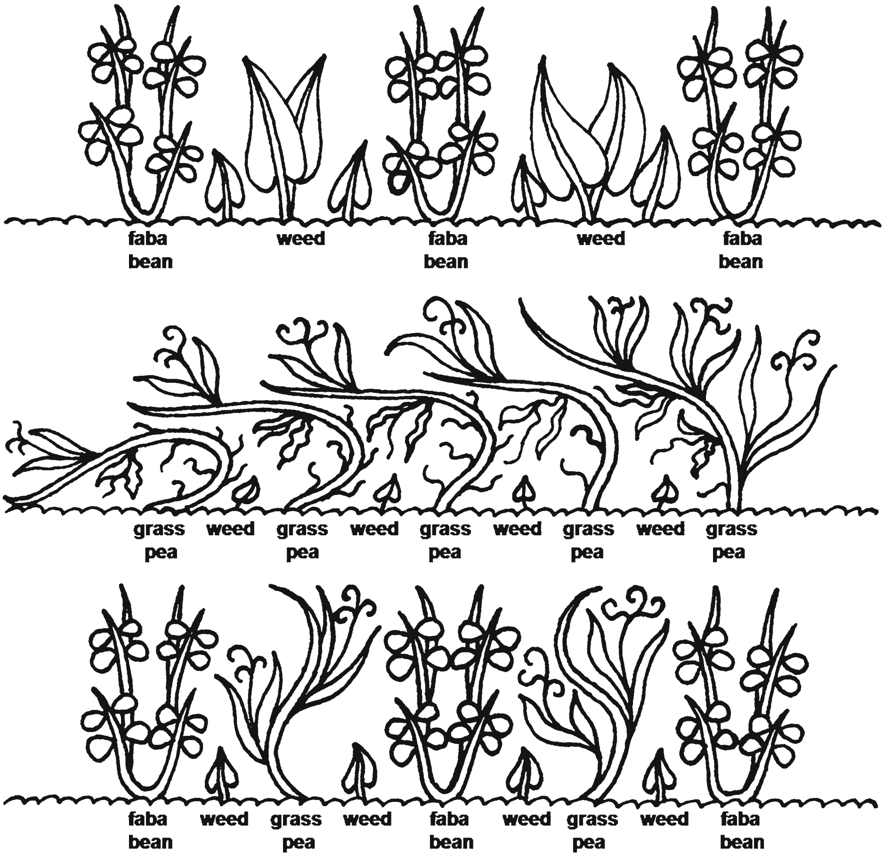

Fig. 5 Model of intercropping 'tall' cool season legumes: (*top row*) faba bean has a good standing ability, but is heavily infested by weeds; (*middle row*) grass pea easily matches weeds but suffers from forage losses; (*bottom row*) intercropping faba bean with grass pea is beneficial to both and suppress weeds

common vetch, Hungarian vetch (*Vicia pannonica* Crantz) and hairy vetch (*Vicia villosa* Roth). In the spring-sown sub-group (Fig. 4, right), faba bean and white lupin served as supporting crops, while forage pea, common vetch and grass pea were supported crops.

The trials with 'tall' cool season annual forage legumes are based upon its specific model (Fig. 5). Annual legumes with good standing ability, such as faba bean or white lupin, are usually sown in wide rows and thus provide favourable conditions for weed development (Fig. 5, top row). On the contrary, annual legumes such as forage pea, many vetches or grass pea, suppress weeds but are extremely prone to lodging, even in the optimum stages for cutting, with heavy losses or lower leaves and reduced and less quality forage yields (Fig. 5, middle row). Intercropping these two types of 'tall' cool season legumes may be beneficial for both, especially by weed reduction and preservation of photosynthetically active leaves (Fig. 5, bottom row).

Table 1 Average values of forage dry matter yield (t ha^{-1}) and land equivalent ratio for forage dry matter yield (LER_{FDMY}) in the intercrops of winter cool season annual legumes in the trial at Rimski Šančevi in 2008/2009 (Ćupina et al. 2011d)

Mixture	Forage dry matter yield (supporting crop)	Forage dry matter yield (supported crop)	LER_{FDMY}
Faba bean + forage pea	1.95	8.55	1.05
Faba bean + dual-purpose pea	3.9	3.6	1.23
Faba bean + common vetch	3.0	3.3	1.42
Faba bean + Hungarian vetch	2.7	3.3	1.06
Faba bean + hairy vetch	2.55	2.25	0.95
Least significant difference at 0.05	1.2		0.334

First results of the mutual intercrops of 'tall' cool season annual legumes for forage production generally confirm their reliability. Intercropping autumn-sown faba bean with autumn-sown common vetch proved especially efficient with both balanced contribution to the total forage dry matter yield and a high value of LER for forage dry matter yield of 1.42 (Ćupina et al. 2011d, Table 1). The trial with spring-sown 'tall' cool season annual legumes also included other cropping density ratios than 50%:50%, namely 75%:25% and 25% and 75%. Intercrops of 50% of faba bean with 50% of grass pea and 75% of white lupin and 25% of grass pea had the best performance, with values of LER for green forage yield of 1.44 and 1.21 (Ćupina et al. 2009b, Table 2). Also, the intercrops of white lupin with common vetch resulted in high values of LER for green forage yield in all three ratios, with 1.28 in average (Ćupina et al. 2011b).

Despite their preliminary character, the obtained results offer a solid basis for defining the ideotypes for 'tall' cool season annual legumes and developing their cultivars specifically for intercropping. Supporting crops, such as faba bean and white lupin, may be characterised by more prominent basal branching, especially in white lupin, as well as with slightly decreased proportion of lignin in stems enhancing the balance between standing ability and stem dry matter quality and digestibility. In addition, it may be desirable to shorten the time of flowering between the first and the second order in white lupin and thus adjust that the first- and the second-order leaves and inflorescences are in similar stages of development, mitigating too rapid maturing in the former. Supported crop genotypes, especially of hairy vetch, should be selected for determinate stem growth and smaller number of stems per plant in order to avoid a potential negative impact on the development of supporting crop.

5 Intercropping 'Short' Cool Season Annual Legumes

The annual forage legume crops within the 'short' group have, in contrast to the previous one, short stems, often with determinate growth. The autumn-sown subgroup (Fig. 6, left) comprised semi-leafless pea as supporting crop and normal-leafed pea, with a genetic structure of *AFAF TLTL*, and bitter vetch as supported crops.

Table 2 Average values of green forage yield (t ha^{-1}) and land equivalent ratio for green forage yield (LER_{GFY}) in the intercropping of grass pea with faba bean and white lupin as affected by mixture ratio (Ćupina et al. 2009b)

Supporting crop	Supported crop	Mixture ratio (%)	Green forage yield	Contribution of supporting crop	Contribution of supported crop	LER_{GFY} (total)
Faba bean	Grass pea	100 (faba bean)	25.6	1	0	1
		25:75	32.5	0.91	0.36	1.27
		50:50	39.5	0.82	0.62	1.44
		75:25	30.5	0.36	0.67	1.02
		100 (grass pea)	29.0	0	1	1
White lupin		100 (white lupin)	24.8	1	0	1
		25:75	32.0	0.88	0.27	1.14
		50:50	28.5	0.56	0.48	1.04
		75:25	32.5	0.35	0.86	1.21
		100 (grass pea)	29.0	0	1	1
Least significant difference at 0.05			5.2	0.19	0.21	0.14
Least significant difference at 0.01			7.7	0.24	0.26	0.19

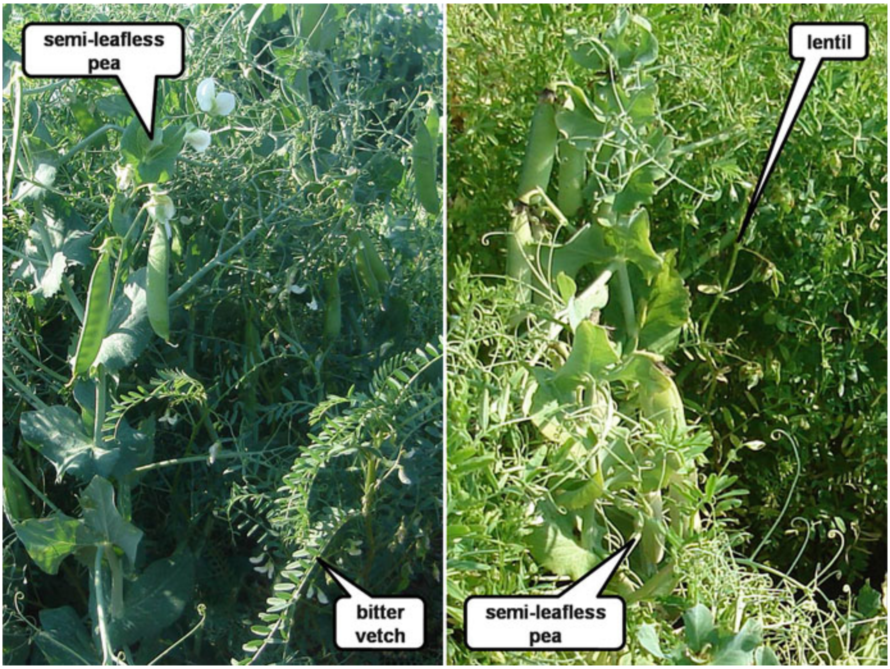

Fig. 6 Examples of intercropping 'short' cool season legumes: autumn sowing (*left*) – semi-leafless pea with bitter vetch; spring sowing (*right*) – semi-leafless pea with lentil

The spring-sown sub-group (Fig. 6, right) also had semi-leafless pea playing the role of supporting crop, while normal-leafed pea and lentil were included as supported crops.

The model of intercropping 'short' cool-season annual forage legumes respects the same basic principles (Fig. 7). Semi-leafless pea has significantly improved standing ability and profits from sunlight penetrating the whole stand, but also offers more favourable conditions for weed development (Fig. 7, top row). On the other hand, normal-leafed pea controls weeds in an easier manner, but is prone to lodging at early stages and is danger of losing lower leaves and disease infestation (Fig. 7, middle row). If intercropped with each other, semi-leafless and normal-leafed peas may profit from each other: semi-leafless pea provides improved standing ability of the intercrop, while normal-leafed pea fills the available space within the stand and contributes to better utilisation of sunlight, at the same time reducing weed growth (Fig. 7, bottom row).

A 2-year trial with autumn-sown and spring-sown intercrops of semi-leafless and normal-leafed peas resulted in an economic justification of all four possible combinations, with both LER for green forage yield and LER for forage dry matter yield values higher than 1 (Ćupina et al. 2010b, Table 3). Unpublished preliminary data on the performance of the intercrops of autumn-sown semi-leafless pea with bitter vetch and spring-sown semi-leafless pea with lentil show that the former had

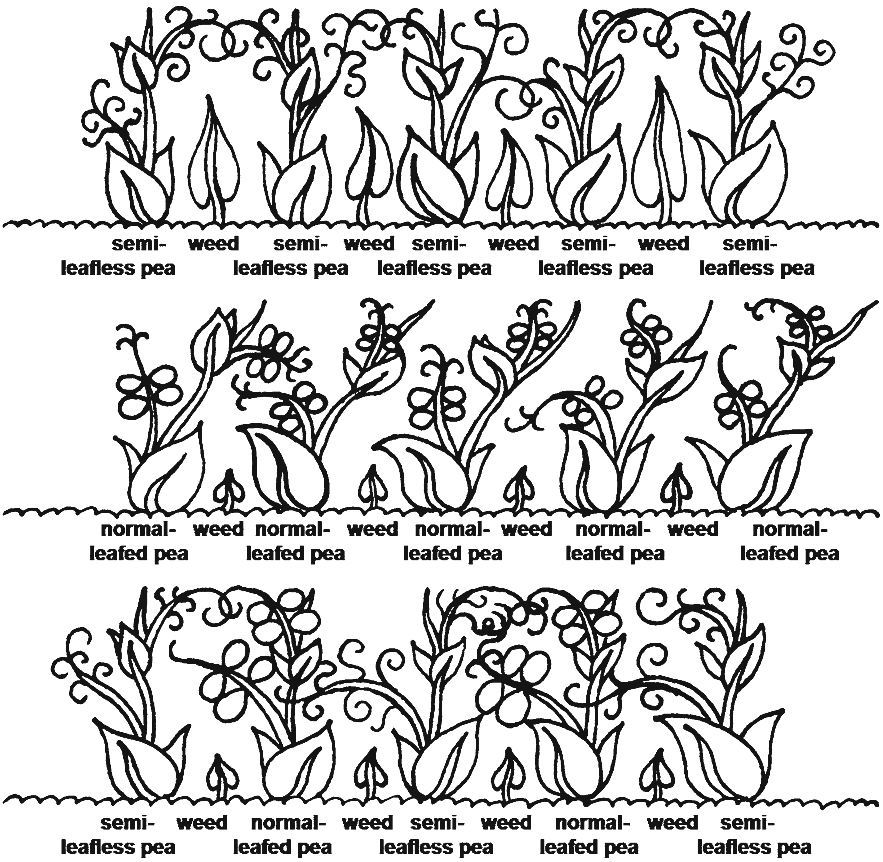

Fig. 7 Model of intercropping 'short' cool season legumes: (*top row*) semi-leafless pea has a good standing ability, but provides good conditions for weeds; (*middle row*) normal-leafed pea suppresses weeds but easily lodges; (*bottom row*) intercropping semi-leafless pea with normal-leafed pea is beneficial to both and controls weeds

somewhat lower economic return in comparison with sole crops, with a value of LER for forage dry matter yield of 0.91, while the latter surpassed the pure stands of both crops, with LER for forage dry matter yield of 1.09.

In general, ideotypes of bitter vetch and lentil cultivars specifically for intercropping are almost identical to each other, due to a rather similar morphology. In both, especially in bitter vetch, somewhat smaller number of stems could be advantageous by reducing the physical pressure on the supporting crop of semi-leafless pea. From the point of view of a breeder, the least complicated way of developing intercropping-specific cultivars is present in pea. The parental genotypes that will be used for hybridisation must have desirable characteristics for forage production. As a rule, one parent should be semi-leafless and another with normal-leaves. Due to semi-epistatic nature of this trait, all the plants in F_1 generation will have

Table 3 Average values of green forage yield (t ha^{-1}), land equivalent ratio for green forage yield (LER_{GFY}), forage dry matter yield (t ha^{-1}) and land equivalent ratio for forage dry matter yield (LER_{FDMY}) in the mutual intercrops of pea cultivars with different leaf types at Rimski Šančevi during 2008–2010 (Ćupina et al. 2010b)

Season	Treatment	Green forage yield of supporting component	Green forage yield of supported component	Total green forage yield	LER_{GFY}
Winter	Dove, pure stand	32.4	0.0	32.4	1.00
	Frijaune, pure stand	0.0	30.8	30.8	1.00
	Dove + Frijaune	23.2	11.0	34.2	1.09
Spring	Jezero, pure stand	31.3	0.0	31.3	1.00
	Javor, pure stand	0.0	30.3	30.3	1.00
	Jezero + Javor	16.4	17.5	33.8	1.11
Least significant difference at 0.05		3.7			0.08
Season	Treatment	Forage dry matter yield of supporting component	Forage dry matter yield of supported component	Total forage dry matter yield	LERFDMY
Winter	Dove, pure stand	6.8	0.0	6.8	1.00
	Frijaune, pure stand	0.0	7.8	7.8	1.00
	Dove + Frijaune	5.1	3.0	8.1	1.13
Spring	Jezero, pure stand	6.3	0.0	6.3	1.00
	Javor, pure stand	0.0	6.4	6.4	1.00
	Jezero + Javor	2.9	3.6	6.5	1.03
Least significant difference at 0.05		0.8			0.08

normal-leaves, while the segregation to both leaf types will start in F_2 generation. Since the semi-leafless plants are fully recessive may produce only the semi-leafless progenies, they may be excluded from the selection process. On the contrary, normal-leafed pea plants will keep on producing both leaf types. In the latter generations with a rather high proportion of homozigocy, such as F_5 or F_6, one semi-leafless pea line and one normal-leafed pea line may be finally selected and developed into a pair exclusively for the forage intercropping, being almost completely identical save for the leaf type. It is desirable that the semi-leafless pea cultivars have numerous nodes and large stipules in order to increase the leaf proportion in the total yield and thus improve forage quality. Most recently, fenugreek has been added as another spring-sown supporting crop.

6 Intercropping Warm Season Annual Legumes

Due to a well-known absence of winter hardiness, the warm season annual forage legumes were spring-sown only. In the early-maturing group (Fig. 8, left) soybean belonging to 00 maturity group was supporting crop, while several *Vigna* species,

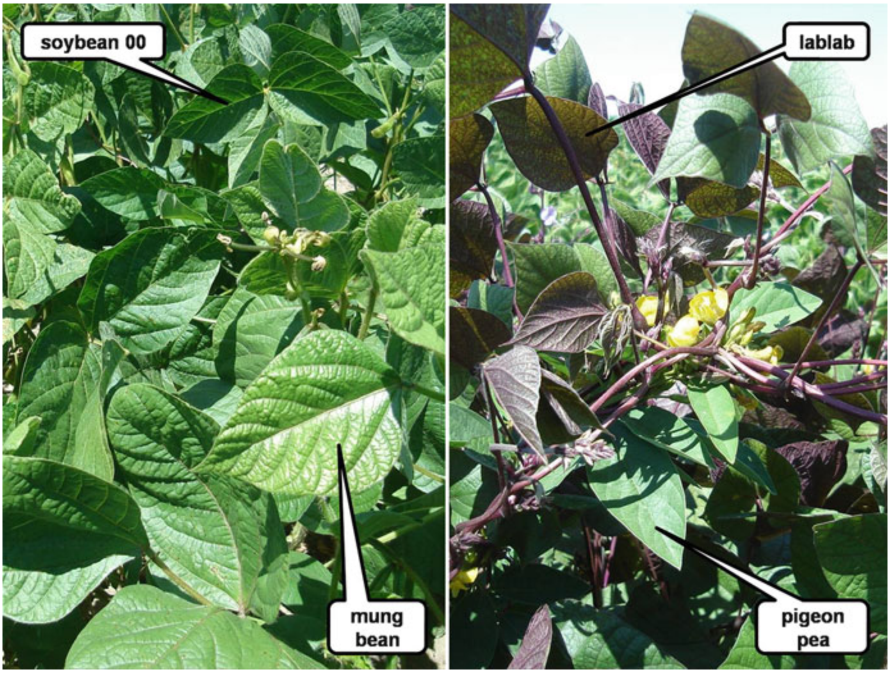

Fig. 8 Examples of intercropping warm season legumes: early-maturing (*left*) – soybean 00 with mung bean; late-maturing (*right*) – pigeon pea with lablab

Table 4 Green forage yield (t ha^{-1}), land equivalent ratio for green forage yield (LER_{GFY}), forage dry matter yield (t ha^{-1}) and land equivalent ratio for forage dry matter yield (LER_{FDMY}) in the pure stands and the intercrops of warm season annual forage legumes in average for Rimski Šančevi and Zemun Polje in 2009 (Mikić et al. 2010)

Green forage yields (t ha^{-1})							
Supported crop (Sd)	Supporting crop (Sg)	Pure stand		Intercropping			
		Sd	Sg	Sd	Sg	Sd + Sg	LER_{GFY}
Mung bean	Soybean 00	32.8	42.8	15.2	22.7	37.9	1.01
Lablab bean	Soybean I	30.8	42.0	17.8	20.5	38.3	1.05
	Pigeon pea		14.5	20.3	6.5	26.8	1.12
Least significant difference at 0.05		5.7					0.05
Least significant difference at 0.01		8.2					0.08
Forage dry matter yields (t ha^{-1})							
Supported crop (Sd)	Supporting crop (Sg)	Pure stand		Intercropping			
		Sd	Sg	Sd	Sg	Sd + Sg	LER_{FDMY}
Mung bean	Soybean 00	7.9	10.7	3.6	5.7	9.3	0.99
Lablab bean	Soybean I	8.6	12.2	5.0	5.9	10.9	1.05
	Pigeon pea		4.4	5.7	2.0	7.7	1.10
Least significant difference at 0.05		1.4					0.05
Least significant difference at 0.01		1.9					0.07

namely mung bean, adzuki bean (*Vigna angularis* (Willd.) Ohwi & H. Ohashi) and black gram (*Vigna mungo* (L.) Hepper), were used as supported crops. Within the late-maturing group (Fig. 8, right), soybean belonging to I maturity group and pigeon pea served as supporting crops, while cowpea and lablab bean played the role of supported crops.

The model of intercropping warm season annual forage legumes is essentially similar to the previous two (Fig. 9). Regardless of its maturity group, a soybean crop provides weeds with favourable conditions for rapid development and thus regularly requires intensive mechanical or chemical weed control (Fig. 9, top row). Quite opposite, *Vigna* species, most notably cowpea, as well as lablab, are exceptionally prone to lodging and by forming a mighty and creeping cover of stems and leaves are able to almost eliminate any weed species (Fig. 9, middle row), but may also lose lower leaves and cause serious difficulties during the cutting. When intercropped, soybean may bear cowpea or lablab stems and thus assist in preserving its leaves, as well as profit from significantly decreased weed infestation (Fig. 9, bottom row).

In the preliminary trials with intercrops of warm season annual forage legumes, carried out in two locations, nearly all proved as economically reliable and superior in comparison to pure stands (Mikić et al. 2010, Table 4). The intercrop of pigeon pea and lablab bean had a particularly good performance, with LER for green forage yield of 1.12 and LER for forage dry matter yield of 1.10. Additional unpublished data from the second year of the same trial reveal that the performance of the intercrops of soybean belonging to the 00 maturity

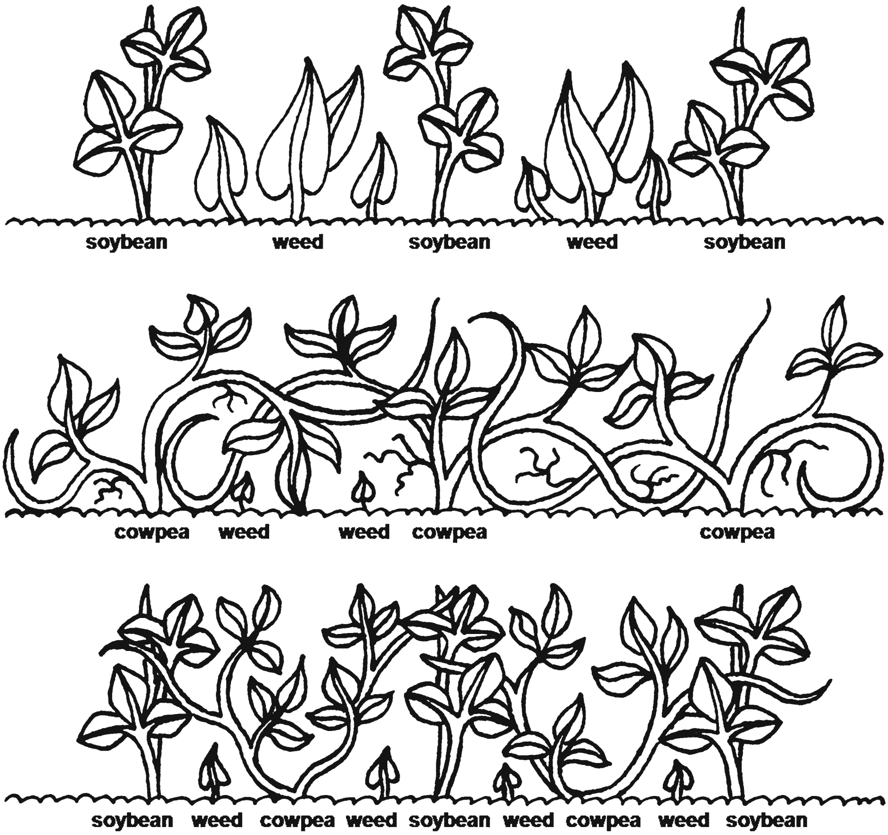

Fig. 9 Model of intercropping warm season legumes: (*top row*) soybean is lodging resistant but is in early danger from weeds; (*middle row*) cowpea almost completely eliminates weeds but extremely lodges; (*bottom row*) intercropping soybean with cowpea is beneficial to both with efficient weed control

group with adzuki bean and black gram was better than with mung bean, with LER for green forage yield of 1.08 and 1.10 and LER for forage dry matter yield of 1.07 and 1.11.

Among the solutions for the ideotype of the intercropping-specific soybean cultivars could be the introduction of larger leaflets or multifoliolate leaves with five or seven leaflets instead of usual three, leading to improved use of a crop space and resources. The soybean cultivars of later maturity groups are prone to prominent lignifications in the lower half of the stem and thus cause potential mechanical problems during the cutting, suggesting that this should be less present in the lines developed for forage intercropping. The supported crops, especially cowpea and lablab bean, should be selected for smaller number of stems per plant and determinate stem growth. In both pigeon pea and lablab bean, the genotypes with more neutral photoperiod should be targeted, ensuring seed maturing and reliability of the developed cultivars.

7 Conclusion

Although the presented results on the performance of various forms of mutual annual legume intercropping for forage production have a rather preliminary character, it may be said that the majority of the examined combinations were assessed as economically reliable and justified by appropriate LER values. It should be repeated that such intercrops do not increase the costs of sowing, since the planned reduction of sowing rates. At the same time, when both components in an intercrop are legumes, the crude protein content in forage dry matter remains high and does not decrease as in the case of intercropping with cereals. All three presented models of mutual annual forage legume intercrops are characterised by short growing seasons and thus are able to fit easily into various cropping systems. In the end, producing forage in such intercrops does not require the application of either synthetic fertiliser, since both components are legumes, or herbicides, due to an enhanced weed control, and thus confirms its value as a true environment-friendly service.

On the other hand, there are many aspects that must be thoroughly studied and integrated with the existing results into a comprehensive and solid study of such cropping system. What needs to assessed are optimum ratios for individual intercrops, impact of intercropping on forage yield components, possible correlations between total forage yields and their LER values, chemical composition of the forage dry matter in the intercrop components, and various underground aspects with a particular reference to microbiology and allelopathy. It is also necessary to provide a reliable seed production of the intercropping-specific annual forage legume cultivars in order to secure their successful use in wide production, as well as to confirm the potential of mutual annual legume intercrops for grain production in both human diets and animal feeding (Ćupina et al. 2011c).

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References

Abberton MT, Marshall AH, Humphreys MW, MacDuff JH, Collins RP, Marley CL (2008) Genetic improvement of forage species to reduce the environmental impact of temperate livestock grazing systems. Adv Agron 98:311–355

Adetiloye PO, Adekunle AA (1989) Concept of monetary equivalent ratio and its usefulness in the evaluation of intercropping advantages. Trop Agric 66:337–341

Agegnehu G, Ghizaw A, Sinebo W (2006) Yield performance and land-use efficiency of barley and faba bean mixed cropping in Ethiopian highlands. Eur J Agron 25:202–207

Andersen MK, Hauggaard-Nielsen H, Ambus P, Jensen ES (2004) Biomass production, symbiotic nitrogen fixation and inorganic N use in dual and tri-component annual intercrops. Plant Soil 266:273–287

Anil L, Park J, Phipps RH, Miller FA (1998) Temperate intercropping of cereals for forage: review of the potential for growth and utilisation with particular reference to the UK. Grass Forage Sci 53:301–317

Bedoussac L, Justes E (2010a) The efficiency of a durum wheat-winter pea intercrop to improve yield and wheat grain protein concentration depends on N availability during early growth. Plant Soil 330:19–35

Bedoussac L, Justes E (2010b) Dynamic analysis of competition and complementarity for light and N use to understand the yield and the protein content of a durum wheat–winter pea intercrop. Plant Soil 330:37–54

Biabani A, Hashemib M, Herbert SJ (2008) Agronomic performance of two intercropped soybean cultivars. Int J Plant Prod 2:215–222

Braum SM, Helmke PA (1995) White lupin utilizes soil phosphorus that is unavailable to soybean. Plant Soil 176:95–100

Ćupina B, Erić P, Mihailović V, Krstić Đ, Mikić A (2006) Perennial forage crops establishment with fodder pea as companion crop. Ratar Povrt/Field Veg Crop Res 42:41–49

Ćupina B, Mikić A, Krstić Đ (2009a) Field pea: a cover crop in establishing perennial legumes. Grain Legum 52:9

Ćupina B, Mikić A, Mihailović V, Krstić Đ, Đurić B (2009b) Intercropping of grass pea (*Lathyrus sativus*) with other grain legumes for forage production. Grain Legum 54:32

Ćupina B, Krstić Đ, Mikić A, Erić P, Vučković S, Pejić B (2010a) Field pea companion crop management on red clover establishment and productivity. Turk J Agric For 34:275–283

Ćupina B, Krstić Đ, Antanasović S, Erić P, Pejić B, Mikić A, Mihailović V (2010b) Potential of the intercrops of normal-leafed and semi-leafless pea cultivars for forage production. Pisum Genet 42:11–14

Ćupina B, Manojlović M, Krstić Đ, Čabilovski R, Mikić A, Ignjatović-Ćupina A, Erić P (2011a) Effect of winter cover crops on the dynamics of soil mineral nitrogen and yield and quality of Sudan grass [*Sorghum bicolor* (L.) Moench]. Aust J Crop Sci 5:839–845

Ćupina B, Mikić A, Krstić Đ, Antanasović S, Erić P, Đorđević V, Perić V (2011b) Intercropping white lupin with other annual legumes for forage and grain production. Book of abstracts, 13th international lupin conference, Poznań, Poland, 6–10 June 2011, p 25

Ćupina B, Mikić A, Krstić Đ, Antanasović S, Pejić B, Erić P, Ignjatović-Ćupina A (2011c) Mutual intercropping of spring annual legumes for grain production in the Balkans. Indian J Agric Sci 81:971–972

Ćupina B, Mikić A, Stoddard FL, Krstić Đ, Justes E, Bedoussac L, Fustec J, Pejić B (2011d) Mutual legume intercropping for forage production in temperate regions. In: Lichtfouse E (ed) Sustainable agriculture reviews 7: genetics, biofuels and local farming systems. Springer, Dordrecht, pp 347–365

Conaghan P, O'Kiely P, O'Mara FP (2010) Conservation characteristics of wilted perennial ryegrass silage made using biological or chemical additives. J Dairy Sci 93:628–643

Corre-Hellou G, Fustec J, Crozat Y (2006) Interspecific competition for soil N and its interaction with N_2 fixation, leaf expansion and crop growth in pea-barley intercrops. Plant Soil 282:195–208

Cortés-Mora FA, Piva G, Jamont M, Fustec J (2010) Niche separation and nitrogen transfer in *Brassica*-legume intercrops. Ratar Povrt/Field Veg Crop Res 47:581–586

Evidente A, Fernández-Aparicio M, Andolfi A, Rubiales D, Motta A (2007) Trigoxazonane, a monosubstituted trioxazonane from *Trigonella foenum-graecum* root exudate, inhibits *Orobanche crenata* seed germination. Phytochem 68:2487–2492

Fernández-Aparicio M, Emeran AA, Rubiales D (2011) Inter-cropping faba bean with berseem, fenugreek or oat can contribute to broomrape management. Grain Legum 56:31

Frankow-Lindberg BE, Brophy C, Collins RP, Conolly J (2009) Biodiversity effects on yield and unsown species invasion in a temperate forage ecosystem. Ann Bot 103:913–921

Fustec J, Lesuffleur F, Mahieu S, Cliquet JB (2010) N rhizodeposition of legumes. A review. Agron Sustain Dev 30:57–66

Ghosh PK, Mohanty M, Bandyopadhyay KK, Painuli DK, Misra AK (2006a) Growth, competition, yield advantage and economics in soybean/pigeonpea intercropping system in semi-arid tropics of India: I. Effect of subsoiling. Field Crop Res 96:80–89

Ghosh PK, Mohanty M, Bandyopadhyay KK, Painuli DK, Misra AK (2006b) Growth, competition, yields advantage and economics in soybean/pigeonpea intercropping system in semi-arid tropics of India: II. Effect of nutrient management. Field Crop Res 96:90–97

Guiney E, Milbourne D, Mullins E, Barth S, Burke JI (2004) Plant biotechnology: global perspectives and Irish priorities. Summary of papers, agricultural research forum 2004, Tullamore, Ireland, 1–2 Mar 2004, pp 116–122

Hauggaard-Nielsen H, Jensen ES (2005) Facilitative root interactions in intercrops. Plant Soil 274:237–250

Hauggaard-Nielsen H, Jørnsgaard B, Kinane J, Jensen ES (2008) Grain legume – cereal intercropping: the practical application of diversity, competition and facilitation in arable and organic cropping systems. Renew Agric Food Syst 23:3–12

Hauggaard-Nielsen H, Peoples MB, Jensen ES (2011) Faba bean in cropping systems. Grain Legum 56:32–33

Huss-Danell K, Chaia E, Carlsson G (2007) N_2 fixation and nitrogen allocation to above and below ground plant parts in red clover-grasslands. Plant Soil 299:215–226

Kadžiulienė Ž, Šarūnaitė L, Deveikytė I (2011) Effect of pea and spring cereals intercropping on grain yield and crude protein content. Ratar Povrt/Field Veg Crop Res 48:183–188

Koivisto JM (2002) Semi leafless pea: a cover crop for establishing lucerne or red clover. Ph.D. thesis, Royal Agricultural College & Coventry University, UK

Li Y, Ran W, Zhang R, Sun S, Xu G (2009) Facilitated legume nodulation, phosphate uptake and nitrogen transfer by arbuscular inoculation in an upland rice and mung bean intercropping system. Plant Soil 315:285–296

Lovatt JA, Hayes R, Sanderson R, Duller S (2010) The contribution of hybrid ryegrass (*Lolium x hybridum* Hausskn) to dry matter yield in mixtures with perennial ryegrass. In: Huyghe C (ed) Sustainable use of genetic diversity in forage and turf breeding. Springer, Dordrecht, pp 223–226

Malézieux E, Crozat Y, Dupraz C, Laurans M, Makowski D, Ozier-Lafontaine H (2009) Mixing plant species in cropping systems: concepts, tools and models. A review. Agron Sustain Dev 29:43–62

Mariotti M, Masoni A, Ercoli L, Arduini I (2009) Above- and below-ground competition between barley, wheat, lupin and vetch in a cereal and legume intercropping system. Grass Forage Sci 64:401–412

Mela T (2003) Red clover grown in a mixture with grasses: yield, persistence and dynamics of quality characteristics. Agric Food Sci Finl 12:195–212

Mihailović V, Erić P, Mikić A (2004) Growing peas and vetches for forage in Serbia and Montenegro. Grassl Sci Eur 9:457–459

Mikić A, Đorđević V, Perić V, Ćupina B, Mihailović V, Srebrić M, Krstić D (2010) Preliminary report on forage yields in mid- to late spring-sown annual legume intercrops. Biotechnol Anim Husb 26(Special issue 2):269–275

Mikić A, Medović A, Ćupina B, Mihailović V, Ignjatović-Ćupina A, Đorđević V, Kobiljski B (2011a) Paleolinguistics brings more light on the earliest history of the traditional Eurasian pulse crops. Nat Preced. doi:10.1038/npre.2011.5837.1

Mikić A, Mihailović V, Ćupina B, Kosev V, Warkentin T, McPhee K, Ambrose M, Hofer J, Ellis N (2011b) Genetic background and agronomic value of leaf types in pea (*Pisum sativum*). Ratar Povrt/Field Veg Crop Res 48:275–284

Muir JP, Pitman WD, Foster JL (2011) Sustainable, low-input, warm-season, grass–legume grassland mixtures: mission (nearly) impossible? Grass Forage Sci 66:301–315

Ngongoni NT, Mwale M, Mapiye C, Moyo MT, Hamudikuwanda H, Titterton T (2007) Evaluation of cereal-legume intercropped forages for smallholder dairy production in Zimbabwe. Livest Res Rural Dev 19:129

Peyraud JL, Le Gall A, Lüscher A (2009) Potential food production from forage legume-based-systems in Europe: an overview. Ir J Agric Food Res 48:115–135

Roy AK, Sikdar B, Rahman M, Rahman MA, Haque ME (2003) Lablab bean based intercropping system in northwest region of Bangladesh. Pak J Biol Sci 6:948–951

Singh RJ, Chung GH, Nelson RL (2007) Landmark research in legumes. Genome 50:525–537

Sobkowicz P (2006) Competition between triticale (*Triticosecale* Witt.) and field beans (*Vicia faba* var. *minor* L.) in additive intercrops. Plant Soil Environ 52(2):47–54

Strydhorst SM, King JR, Lopetinsky KJ, Harker KN (2008) Forage potential of intercropping barley with faba bean, lupin, or field pea. Agron J 100:182–190

Szumigalski A, Van Acker R (2005) Weed suppression and crop production in annual intercrops. Weed Sci 53:813–825

Vandermeer J, Van Noordwijk M, Anderson J, Ong C, Perfecto I (1998) Global change and multi-species ecosystems: concepts and issues. Agric Ecosyst Environ 67:1–22

Willey R (1979) Intercropping – its importance and research needs. 1. Competition and yield advantages. Field Crop Abstr 32:1–10

Endophytic Nitrogen-Fixing Bacteria as Biofertilizer

Garima Gupta, Jitendra Panwar, Mohd Sayeed Akhtar, and Prabhat N. Jha

Abstract Nitrogen is the most limiting nutritional factor for the growth of plants. Since plants cannot reduce atmospheric N_2, they require exogenously fixed nitrogen for growth and development. Atmospheric N_2 must be first reduced to ammonia to be used by plants. In practice, chemical N fertilizers are used to provide nitrogen nutrition to plants. However, manufacture and use of N fertilizers are associated with environmental hazards that include release of greenhouse gases at the time of manufacture, as well as contamination of underground and surface water due to leaching out of nitrates. Moreover, manufacture of chemical fertilizers requires non-renewable resources like coal and petroleum products. Excess and continuous use of chemical fertilizers to improve the yield of commercial crops has negative effect on soil fertility and reduces their agricultural sustainability. All these concerns necessitate the search for an alternative strategy that can provide nitrogen nutrition to the plants in an efficient and sustainable manner. Here biological nitrogen fixation has immense potential and can be used as an alternate to chemical fertilizers. Biological nitrogen fixation has been reported to be exclusively carried out by few members of the prokaryotic organisms. Biological nitrogen fixation is a process

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where atmospheric N_2 is reduced to NH_3. This process is catalyzed by microbial enzyme nitrogenase. Microorganisms having the capacity to fix atmospheric N_2 can be used as efficient biofertilizer.

In this chapter, we review application, properties, ecology, and advances in biology of nitrogen fixing bacteria with reference to endophytic bacteria that colonize the interior of plant without exerting any substantive harm to their host plant. Nitrogen-fixing endophytic bacteria have edge over its rhizospheric counterparts because, being sheltered inside plant tissues, they face less competition and can make available the fixed nitrogen directly to plants. Moreover, the partial pressure of oxygen inside the plant tissue is more acquiescent for efficient nitrogen fixation. Nitrogen fixing endophytic bacteria have been isolated from several plant species and found to contribute upto 47% of nitrogen derived from air, which in turn enhance plant growth. Nitrogen fixing ability of bacteria can be evaluated by total nitrogen difference method, acetylene reduction assay, analysis of nitrogen solutes in xylem and other plant parts and N-Labeling Methods. Furthermore, molecular approaches such as amplification, analysis of nitrogen-fixing genes (*nif* genes), and qualitative and quantitative estimation of their products can be used for evaluation of nitrogen fixing ability of the bacteria.

In addition to nitrogen-fixation ability, these bacteria can influence plant growth through one or more properties. These include production of phytohormones, siderophores, induced systemic tolerance through production of 1-aminocyclopropane-1-carboxylase deaminase, induced systemic resistance and antagonistic activities. The make-up of endophytic bacterial communities depends on various factors such as soil type, soil composition, soil environment, plant genotype and physiological status, bacterial colonization traits, and agricultural management regimes. Colonization and abundance of different bacterial species varies widely with host plants. Endophytic bacterial community can be analyzed employing stable isotope probing as well as various modern molecular approaches which are based on analysis of 16S ribosomal deoxyribonucleic acid (DNA), gene encoding products for nitrogen fixation and repetitive DNAs. Moreover, metagenomic approaches allow estimation and analysis of unculturable bacteria at genomic as well as functional genomic level. Colonization process of an endophytic bacterium involves various steps which include migration towards root surface, attachment and microcolony formation on plant surface, distribution along root and growth and survival of the population inside plant tissue. Ongoing progress towards in-depth analysis of genomic and whole protein profile of some of the potential endophytic bacteria such as *Azoarcus* sp., *Gluconoacetobacter diazotrophicus, Herbaspirillum seropedicae, Serratia marcesens* can help understand mechanism involved in plant-endophyte interaction which in turn will be deterministic in use of suitable formulations of endophytic bacteria to be used as biofertilizer for sustainable agriculture.

Keywords 1-aminocyclopropane-1-carboxylase deaminase • Biofertilizer • Diazotrophic • Endophytic • Nitrogen • Reverse transcription-polymerase chain reaction

Abbreviations

CO_2	Carbon dioxide
DNA	Deoxyribonucleic acid
gfp	*gfp* is a gene which encodes for green fluorescent protein
gus	*gus* is a gene which encodes for β-glucuronidase
HCN	Hydrogen cyanide
mRNA	messenger RNA which is used as template for protein synthesis.
N	Nitrogen
N_2	Atmospheric Nitrogen
NO_2	Nitric oxide
nifHDK	These are set of genes which encodes structural part of nitrogenase, an enzyme which catalyzes nitrogen fixation.
PCR	Polymerase chain reaction
PGPB	Plant growth promoting bacteria
r DNA	ribosomal DNA encodes for rRNA, a structural component of ribosome

1 Introduction

Nitrogen is an important limiting factor for plant growth in various environmental conditions. Despite abundance of atmospheric nitrogen (78%), it cannot be utilized for growth and metabolism. It must be reduced to ammonia for use by any organisms by a process called nitrogen fixation. Application of industrially manufactured nitrogen fertilizer has been one of the most popular ways to provide nitrogen nutrition to the plants to attain high crop productivity. However, excessive and continuous use of chemically synthesized fertilizer can lead to several consequences which include: (i) ground water contamination of nitrate due to leaching and denitrification which is detrimental for human and animal health, (ii) surface water contamination by eutrophication which may arise due to leaching of nitrogen in water and affects growth of aquatic organisms and (iii) production of greenhouse gases CO_2 and NO_2 during manufacture of nitrogen fertilizer using non-renewable resources like natural gas and coal, thus contributing to global warming (Bhattacharjee et al. 2008). Moreover, increase in prices of petroleum products has led to an upsurge in the cost of chemical fertilizer. Therefore, use of alternative fertilizers, which are cost effective and environmental friendly, must be sought.

Biological nitrogen fixation is considered to be the most potential way to provide fixed form of nitrogen to the plants. However, nitrogen fixation is performed solely by prokaryotes (bacteria and cyanobacteria) and archeans. The diazotrophic (N_2-fixing) bacteria are involved in the fixation process, in which these bacteria either in the free living form or in symbiosis can covert the atmospheric nitrogen into NH_3 with the help of nitrogenase enzyme. Nodulated legumes with endosymbiosis with rhizobia are among the most prominent nitrogen fixing system in agriculture.

Although most of the biologically fixed nitrogen made available to the plants is contributed by *Rhizobium* sp. and cyanobacteria, their use is restricted only to certain plant species. Applications of plant growth promoting endophytic bacteria are being considered as a potential biofertilizer in recent years (Bhattacharjee et al. 2008; Akhtar and Siddiqui 2010). This has driven intensive research towards in-depth characterization and better understanding of endophytic diazotrophic bacteria isolated from various plant species.

Any bacterium could be considered as an endophytic diazotroph if (i) it can be isolated from the surface of disinfected plant tissue or extracted inside the plants (ii) it proves to be located inside the plant, either intra- or inter-cellularly by *in-situ* identification and (iii) it fix nitrogen, as demonstrated by acetylene reduction and/or ^{15}N-enrichment. This definition includes internal colonists with apparently neutral or saprophytic behavior as well as symbionts (Hartmann et al. 2000). Endophytic bacteria are better than their rhizospheric and rhizoplanic counterparts in terms of benefiting their host through nitrogen fixation as they can provide fixed nitrogen directly to their host (Cocking 2003). As low partial oxygen pressure is necessary for the expression of the O_2 sensitive enzyme, nitrogenase, endosphere of plant root is more amenable for N_2-fixation reaction. Moreover, endophytic bacteria are less vulnerable to competition with other soil microbes for scarce resources and remain protected to various abiotic and biotic stresses (Reinhold-Hurek and Hurek 1998). In addition to diazotrophy, endophytic bacteria may enhance plant growth through one or more mechanisms which include phytohormone production, siderophore production, induced systemic tolerance and biocontrol potential. The applications of diazotrophic endophytes in various fields have been depicted in the Fig. 1. The intimate relationship of endophytic bacteria with plant can be utilized in developing efficient biofertilizer and biocontrol agents for attaining sustainable agriculture (Sevilla and Kennedy 2000). The present chapter unveils the importance of diazotrophic endophytic bacteria to exploit its properties for the development of sustainable agriculture.

2 Nitrogen Fixation by Endophytic Bacteria

In recent years, application of endophytic bacterial inoculants supplying N requirement have drawn attention for increasing plant yield in sustainable manner efficiently to the various crop plants. Percent contribution of plant nitrogen as a result of biological N_2-fixation by endophytic bacteria has been summarized in Table 1. Some of the promising endophytic biofertilizers include the members of *Azoarcus*, *Achromobacter*, *Burkholderia*, *Gluconoacetobacter*, *Herbaspirillum*, *Klebsiella* and *Serratia* (Rothballer et al. 2008; Franche et al. 2009). The efficient N supply by endophytic diazotrophic bacteria in sugarcane and kallar grass suggests the possible avenues of biological nitrogen fixation in interior niches of plants. In addition, bacteria isolated from non-leguminous plants like rice, wheat, maize, sorghum also fix the

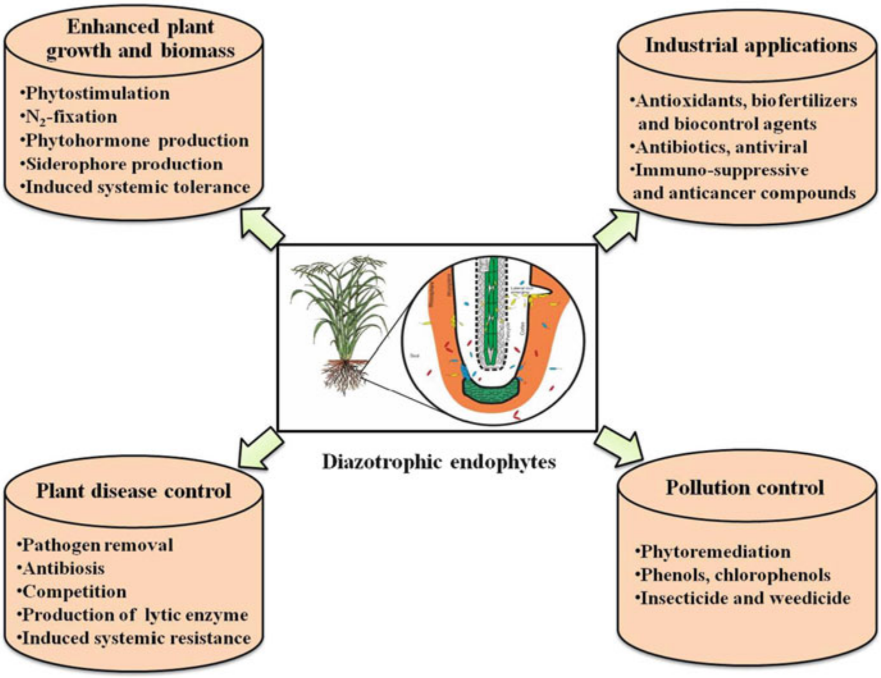

Fig. 1 Multiple applications of diazotrophic (N_2 fixing) endophytic bacteria in various fields including agricultural practices, industries and environment (Modified from Hardoim et al. 2008)

N in endophytic manner. It is evident from the reports that the *Gluconoacetobacter diazotrophicus* (*Acetobacter diazotrophicus*) is the main contributor of endophytic biological nitrogen fixation in sugarcane, and it has the ability to fix the N approximately 150 Kg N ha^{-1} $year^{-1}$ (Dobereiner et al. 1993; Muthukumarasamy et al. 2005). *Azoarcus* is recognized as another potential N_2-fixing obligate endophytic diazotroph. It dwell in the roots of kallar grass, and increased the hay yield upto 20–40 t ha^{-1} $year^{-1}$ without the addition of any N fertilizer in saline sodic, alkaline soils (Hurek and Reinhold-Hurek 2003). In addition, many energy plants (C_4 plants) like *Miscanthus sacchariflorus, Spartina pectinata* and *Penisettum purpureum* have been found to harbour bacterial population, which have the potential to support the N nutrition of the plant (Kirchhof et al. 1997). In a study, *Herbaspirillum* sp., inoculated into rice seedlings maintained in N-free Hoagland solution containing ^{15}N-labelled N, showed ^{15}N dilution amounting upto 40% increase in total N of plant (Baldani et al. 2000). Growth stimulation of wheat, corn, radish, mustard and certain varieties of rice shoots following seed inoculation with a strain of *Rhizobium leguminosarum* bv *trifolii* in pot experiment has also been reported (Hoflich et al. 1995; Webster et al. 1997). These investigations suggest that endophytic diazotrophs have a considerable potential to increase the productivity of non-legumes including important cash crop plants.

Table 1 Biological nitrogen fixation by diazotrophic endophytic bacteria

Endophytic bacteria	Associated plant	N derived from air (%)	References
Burkholderia	Rice	31	Baldani et al. (2000)
Herbaspirillum	Rice	19–47	Mirza et al. (2000)
Rhizobium leguminosarum bv. *trifolii*	Rice	19–28	Biswas et al. (2000) and Yanni et al. (2001)
K. pneumoniae 324	Rice	42	Iniguez et al. (2004)
B. vietnamiensis	Rice	40–42	Govindarajan et al. (2008)
Beijerinckia, Bacillus, Klebsiella, Enterobacter, Erwinia, Azospirillum, Herbaspirillum and *Gluconaacetobacter*	Sugarcane	18	Abeysingha and Weerarathne (2010)
Azospirillum	Rice	9.2–27.7	de Salamone et al. (2010)
Pseudomonas, Stenotrophomonas, Xanthomonas, Acinetobacter, Rhanella, Enterobacter, Pantoea, Shinella, Agrobacterium and *Achromobacter*	Sugarcane	41.2–50.3	Taule et al. (2012)
G. diazotrophicus, H. serpedicae and *H. frisingense*	Elephant grass	5.4–5.5	de Morais et al. (2012)
Microbacterium sp.	Sugarcane	5.4–6	Lin et al. (2012)
G. diazotrophicus, H. seropedicae, H. rubrisubalbicans Burkholderia sp.	Sugarcane	29–74	Urquiaga et al. (2012)

2.1 Quantification of Nitrogen Fixation

Quantification of fixed nitrogen made available to the plants by diazotrophic bacteria can be estimated by the following methods.

2.1.1 Total N Difference Method

This method measures biological nitrogen fixation on the basis of difference between the total N content of crop grown in presence of diazotrophic bacteria and their counterpart grown without bacterial inoculation. It is the oldest and simplest method which is based on an assumption that the control plant and the infected plants absorb same amount of N from soil.

2.1.2 Acetylene Reduction Assay

This technique is based on the fact that the nitrogenase enzyme involved in N_2-fixation can also reduce acetylene into ethylene as both nitrogen and acetylene are triple bonded structures. The assay is done by incubating bacterial culture with acetylene (0.03–0.1 v/v) in an air-tight vessel. Finally, the gas phase of sample is analyzed for ethylene generated as a result of reduction of acetylene by nitrogenase using a gas chromatograph. The calculated ethylene amount can either be directly used to quantify the amount of nitrogen fixed or can be converted into amount of nitrogen fixed by directly multiplying it with a factor 3. It is because in conversion of N_2 to NH_3, three pairs of electron are used while in acetylene to ethylene conversion only single pair of electrons is used. Therefore, this technique measures N_2-fixation indirectly on the basis of electron flux through nitrogenase. The acetylene reduction assay is simple, cheap and sensitive technique.

2.1.3 Analysis of N Solutes in Xylem and Other Plant Parts

In this method, the composition of nitrogenous compounds present in plant the xylem sap is analyzed. The basic idea is to differentiate between the nitrogen fixed and the soil derived nitrate N in the plant. As the uptake from soil increase, it inhibit the N_2-fixation, which results in the change of composition of nitrogenous compounds in xylem sap and by monitoring such changes, the N_2-fixation can be quantitatively analyzed.

2.1.4 N-Labeling Methods

There are three different methods for labeling viz. ^{15}N gas labeling, isotope dilution and A-value method. The principle behind this method is based on the difference of $^{15}N/^{14}N$ present in soil or plant system with that of atmosphere (0.33%). So, if the plants are incubated with ^{15}N and then, the nitrogen fixation is evaluated in plants, the ratio would differ in plants than that of present in atmosphere. And the change occurring in the ratio can be monitored and analyzed for quantifying the nitrogen fixing ability of microbes.

2.2 Molecular Analysis of Nitrogen Fixation

Bacterial communities show an immense phenotypic and genetic diversity (Ovreas and Torsvik 1998). Since, the majority of microorganisms cannot be cultured on media, estimation and analysis of natural diazotrophic bacterial communities is quite challenging (Borneman et al. 1996). However, this problem can be overcome by employing cultivation independent techniques using universal primers for amplification of gene encoding the key enzyme nitrogenase (Kirk et al. 2004).

There are three types of nitrogenases based on the presence of core metal (molybdenum (Mo), vanadium (V) and iron (Fe)) which bridge two units of this enzyme (Zehr et al. 2003; Raymond et al. 2004). Out of these three types, Mo-nitrogenase is most prevalent. There are three genes namely *nifHDK* which encodes for the structural part of nitrogenase complex. Apart from *nifHDK*, nitrogenase expression and function depends on several other genes (20 in case of *K. pneumoniae*). The genes *nifHDK* encodes for α and β fragments respectively of larger segment of nitrogenase complex called dinitrogenase ($\alpha_2\beta_2$), while *nifH* encodes smaller segment Fe protein (dinitrogenase reductase). Nitrogenase activity can be estimated by following procedures.

2.2.1 Polymerase Chain Reaction (PCR)

Amplification of *nifH*, *nifD*, and *nifK* by PCR or reverse transcriptase-PCR has been frequently employed in detection of N_2-fixing ability of bacterial and cyanobacterial isolates taken either from laboratory grown culture or directly from environmental samples (Ueda et al. 1995a, b; Chowdhury et al. 2007; Bothe et al. 2010). Sequence of *nifH* encoding Fe protein of different species has been reported to be one of the most conserved sequences, except for short species-specific sequence discrepancies, which can be used for species determination (Izquierdo and Nusslein 2006). Therefore, gene sequence of *nifH* is used for probing of nitrogenase among diazotrophic bacteria as well as analysis of diazotrophic communities growing in diverse environmental conditions (Diallo et al. 2008; Jha and Kumar 2009; Bothe et al. 2010). Based on the sequence of *nifH*, a variety of primers have been designed for analysis of both culturable and non-culturable bacteria (Zehr et al. 1998; Widmer et al. 1999; Deslippe and Egger 2006; Izquierdo and Nusslein 2006). However, some of these primers can be biased in terms of amplification efficiency (Diallo et al. 2008). In a very recent study, Islam et al. (2010) have demonstrated significant contribution of diazotrophic bacteria in paddy plants growing in natural condition, using acetylene reduction assay and *nifH* sequence analysis. Similarly, possible nitrogen contribution by *Azospirillum* sp., *Rhizobium* sp., and *P. pseudoalcaligenes* was indicated on the basis of *nifH* amplification of culturable isolates obtained from *Lasiurus sindicus*, a perennial grass growing in desert (Chowdhury et al. 2007).

In addition to the detection of *nif* gene, assessment of N_2-fixation in diazotrophic bacteria can also be studied by evaluating expression level of *nifH* gene (Terakado-Tonooka et al. 2008). Evaluation of diazotrophy by estimating the level of *nifH* expression is based on the fact that there is tight relationship between nitrogenase activity and *nifH* expression (Egener et al. 2001). Moreover, the advancement in metagenomic approaches using reverse transcription of *nifH* mRNA (messenger ribonucleic acid) has allowed identification of active diazotrophic bacteria in plants. It also facilitates the identification of bacteria which are not culturable but contributes significant nitrogen nutrition to the host plant. Based on the difference in *nifH* mRNA and deoxyribonucleic acid (DNA) profile obtained from same root extract of rice, Knauth et al. (2005) stated that presence of diazotrophs does not necessarily coincide with active diazotrophs inside the plants growing in environmental condition and reported that active diazotrophs were not related to cultured strains. Recently, metagenomic analysis of *nifH* transcript identified *R. rosttiformans* as active diazotroph of sugarcane and spruce from different locations. It was surprising as none of the known diazotrophs associated with sugarcane such as *G.diazotrophicus, H. seropedicae* or *H. rubrisubalbicans* were found to be active in sugarcane plant (Burbano et al. 2010).

2.2.2 Fluorescent In-Situ Hybridization

Detection of diazotrophic bacteria and estimation of nitrogenase activity based on expression of *nifH* mRNA employing Fluorescent in-situ hybridization is an effective approach. However, use of Fluorescent in-situ hybridization has been not used frequently due to the instability of bacterial mRNA. Use of transcript polynucleotide probes can improve the sensitivity of signal as well as reduce signal to noise ratio. Hurek et al. (1997) detected *in-planta* mRNA expression by *Azoarcus* sp. using transcript oligonucleotide probe. Further, to improve the sensitivity and reliability of the technique, Pilhofer et al. (2009) detected mRNA of *nifH* using digoxigenin-labeled transcript probe. The resultant hybrid was detected by horse-radish peroxidase marked anti-digoxigenin antibody. Subsequently the signal was amplified using catalyzed reporter deposition where tyramide molecules preconjugated with flurochrome were deposited in close proximity of horse-radish peroxidase binding site and intensifies the signal.

2.2.3 Immunoblot Analysis

Nitrogenase activity can also be assessed by detecting nitrogenase complex expressed by bacteria. Detection is based on localizing ca. 27–35 kDa of protein band of dinitrogenase either by radiolabeling or immunoblotting using antibody against Fe protein (Eckert et al. 2001; Jha and Kumar 2007). In a separate study, endophytic bacteria were localized in intercortical region of plant tissue by immunostaining of dinitrogenase reductase (Chelius and Triplett 2000).

2.2.4 Microarray or DNA-Chip

Hybridization levels can be detected by fluorescence. Microarrays with oligonucleotides of *nifH* gene can be used to determine diazotrophic communities. For example, the microarray developed from Zhang et al. (2006) compares 194 oligonucleotide probes, which covers more than 90% of all *nifH* sequences present in the *nifH* database. It is a highly reproducible and semiquantitative method of mapping.

Conclusively, the nitrogen fixing ability of bacteria can be evaluated by total nitrogen difference method, acetylene reduction assay, analysis of nitrogen solutes in xylem and other plant parts and N-Labeling Methods. Furthermore, molecular approaches such as amplification, analysis of nitrogen-fixing genes (*nif* genes), and qualitative and quantitative estimation of their products can be used for evaluation of nitrogen fixing ability of the bacteria.

3 Plant Growth Promoting Properties of Endophytic Bacteria

Apart from N_2-fixation, endophytic bacteria can benefit their host through various growth promoting effects which includes production of phytohormones (auxin and cytokinin), synthesis of siderophore, 1-aminocyclopropane-1-carboxylate-deaminase activity and antagonistic activity. Several endophytic bacteria have been reported to have ability to solubilize mineral phosphate. However, this ability may not be useful for plants as endophytes reside in the interior of plant issue where insoluble mineral phosphates are not available. The characteristics of some beneficial endophytic bacteria are discussed below.

3.1 Phytostimulatory Compounds

Plant growth promoting bacteria produce phytohormones namely auxins, cytokinins, gibberellins, certain volatiles and the co-factor pyrroquinoline quinine. Many plant associated bacteria have been shown to produce auxins chiefly indole-3-acetic acid, which enhances lateral root growth formation and thus, nutrient uptake and root exudation by plants (Spaepen et al. 2007; Ali et al. 2009; Reinhold-Hurek and Hurek 2011). Most of the beneficial bacteria synthesize indole-3-acetic acid through indole-3-pyruvate pathway. In this pathway, tryptophan to indole-3-pyruvic acid conversion occurs by an aminotransferase, which in turn gets decarboxylated by indole-3-pyruvate decarboxylase to indole-3-acetaldehyde and the oxidation of indole-3-acetaldehyde converts it to indole-3-acetic acid

(Spaepen et al. 2007). Indole-3-acetic acid synthesis occurs in stationary phase of growth and positively regulated by aromatic amino acid through a regulatory protein *Tyr*R (Ryu and Patten 2008).

Cytokinins are also involved in plant growth promotion as *Bacillus megatarium* UMCV1, a rhizospheric bacterium, can promote biomass production in *Arabidopsis thaliana* through the inhibition of primary root growth followed by increased lateral root formation and root hair length of host plant (Lopez-Bucio et al. 2007). *Methylobacterium* sp. strain NPFM-SB3, isolated from *Sesbania rostrata* was also found to produce cytokinin (Schwab et al. 2007). *Azospirillum* sp., *G. diazotrophicus*, *H. seropedicae* have also been reported to enhance plant growth by producing gibberellin (Bottini et al. 2004). Even some isolates are capable of producing more than one phytohormone. Feng et al. (2006) isolated *Pantoea agglomerans*, which produce four major plant hormones viz. abscisic acid, gibberellic acid cytokinin and indole-3-acetic acid.

Zhang et al. (2008) identified the role of bacterial volatile organic compounds by analyzing microarray results and histochemical data of *Arabidopsis* seedlings exposed with *B. subtilis* and stated that the volatile organic compounds may influence the plant growth by regulating auxin homeostasis in plants which was evident from induction of genes encoding enzymes of metabolism of indole-3-acetic acid.

3.2 Induced Systemic Tolerance

Some of the plant growth promoting bacteria (PGPB) help the associated plants to counter biotic and abiotic stresses such as drought, salt, nutrient deficiency or excess, extremes of temperature, presence of toxic metals etc. PGPB-induced physical and chemical changes in plants in response to biotic and abiotic stresses are termed as ‘induced systemic tolerance’ (Yang et al. 2009). Induced systemic tolerance results from the production of bacterial 1-aminocyclopropane-1-carboxylate deaminase activity, antioxidants, cytokinin or volatile organic compounds.

In response to the various biotic or abiotic stresses, plant produces ethylene to regulate plant homeostasis. Beyond a threshold level, production of ethylene is inhibitory as it reduces root and shoot development and hence described as “stress ethylene”. Some of the endophytes have property to synthesize 1-aminocyclopropane-1-carboxylate-deaminase, which can degrade the immediate precursor of ethylene from root exudates and convert it to α-ketobutyrate and ammonia and thus, can promote growth of plant in the vicinity (Glick et al. 2007). In addition to 1-aminocyclopropane-1-carboxylate deaminase mediated induced systemic tolerance, there are various other mechanism through which induced systemic tolerance is generated in response to stresses. It includes volatile organic compounds mediated salt tolerance (Zhang et al. 2008), affecting abscisic acid signaling of plants during stress through production of cytokinin (Figueiredo et al. 2008) and through production of antioxidant catalase (Kohler et al. 2008). The role of phytohormone produced by associative bacteria during salinity or drought stress in the promotion of plant growth has been well described (Egamberdieva 2009). Indole-3-acetic acid producing bacteria in drought condition can stimulate formation of well developed roots enough for providing sufficient water from soil (Marulanda et al. 2009).

3.3 Biocontrol Agent

The application of microorganism for the control of diseases seems to be one of the most promising ways, as it is eco-friendly and cost-effective. To become an efficient biocontrol agent, it should be stable under varying condition of pH, temperature and concentrations of different ions. Several endophytic bacteria are known to benefit host plant by reducing the growth of pathogenic organisms at laboratory, greenhouse or field level in various studies (Compant et al. 2005; Bhatia et al. 2008; Kannan and Sureendar 2009). An efficient biocontrol agent must also possess certain traits like (a) efficient colonizer of root to deliver antibiotic along the whole root system, (b) to protect itself from predators (protozoans) in the rhizosphere and (c) release antibiotic in right microniche (Lugtenberg and Kamilova 2009). Bacteria can limit pathogen directly through antagonistic property, competition for iron, detoxification or degradation of virulence factors or indirectly by inducing systemic resistance in plants against certain diseases (Lugtenberg and Kamilova 2009). Endophytic bacterial biocontrol agents can inhibit the growth of fungal or bacterial pathogens by one or more of the several mechanisms, some of which are described below.

3.3.1 Antagonism

Endophytic bacteria can exhibit biocontrol activity (antifungal and antibacterial) through production of allelochemicals or antibiotics. Gram negative biocontrol agents like *Pseudomonas* produce HCN, pyoleutorin, pyrrolnitrin, 2,4-diacetylphloroglucinol and phenazines chiefly phenazine-1-carboxylic acid and phenazine-1-carboxamide (Lugtenberg and Kamilova 2009). In *Pseudomonas fluorescens* CHA0, all these above mentioned metabolites are required for biocontrol. However, the expression of their genes may change with different plant cultivars (Rochat et al. 2010; Jousset et al. 2011). Few other compound including gluconic acid and 2-hexyl-5-propyl resorcinol produced by the antagonistic bacterial strains have also been demonstrated recently (Cazorla et al. 2006; Kaur et al. 2006). Munumbicin, an antibiotic produced by endophytic bacteria inhibits growth of phytopathogenic fungi *P. ultimum* and *F. oxysporum* (Castillo et al. 2002). Certain volatile organic compounds like 2,3-butanediol, or blends of volatiles produced by *Bacillus* sp. also act as biocontrol agents (Strobel 2006). Level of antibiotic synthesis depends upon the nutritional factors viz. type of carbon source utilized, trace elements and availability of other nutrients as well as non-nutritional factors like environmental influences (Compant et al. 2005).

Bacteria can restrict the growth of pathogens by producing hydrolytic enzymes such as chitinase, β-1,3-glucanase, protease, laminarinase etc. (Ordentlich et al. 1988). *Bacillus cepacia* has been reported to destroy *Rhizoctonia solani*, *R. rolfsii*, and *Pythium ultimum* by producing β-1,3-glucanase (Fridlender et al. 1993). Addition of endophytic bacteria *B. cereus* 65 directly to soil has been reported to protect cotton seedlings from root rot disease caused by *Rhizoctonia solani* (Pleban et al. 1997). Secretion of protease and chitinase by endophytic *Enterobacter* and *Pantoea* species isolated from cotton were found to protect the plants against fungal pathogen *Fusarium oxysporum* f. sp. *vasinfectum* (Li et al. 2010).

3.3.2 Siderophore Production

Under iron-limiting condition, some biocontrollers produce small molecular weight compound, known as siderophore, which has the capability to chelate unavailable iron and make it available to plants and cohabiting microorganism, and thus, deprive pathogen (Compant et al. 2005). An array of siderophores is produced but majority of biocontrollers are known to produce catacholate, hydroxymate and/or phenolate type (Rajkumar et al. 2010). In addition to biocontrol, siderophores are known to play multiple roles in diazotrophic bacterial species. As the diazotrophic bacteria require both iron and molybdenum for the activity of nitrogenase, the role of siderophore seems pivotal under iron deficient conditions (Kraepiel et al. 2009).

3.3.3 Induced Systemic Resistance

During their interaction with plants, endophytic bacteria results in improving the immune response of plants for future attack by pathogens, a phenomenon called as induced systemic resistance (Compant et al. 2005; van Loon 2007). In contrast to biocontrol mechanisms, extensive colonization of root system is not required for induced systemic resistance (Lugtenberg and Kamilova 2009).

The bacterial products that elicit induction of induced systemic resistance are of diverse category and show their induction in plants which possibly possess receptors for the respective ligands. These inducers may be lipopolysaccharides, flagella, siderophores, antibiotics, volatile organic compounds and quorum-sensing signals (van Loon 2007). It has been reported that both siderophore (pyochelin) and antibiotic (pyocyanin) are needed for induced systemic resistance by *P. aeruginosa* 7NSK2 (Audenaert et al. 2002). Role of volatile organic compounds such as 2,3-butanediol produced by *Bacillus* sp. in induced systemic resistance has been reported by Ryu et al. (2004). Mostly induced systemic resistance activated by plant growth promoting bacteria are jasmonate or ethylene mediated (van Loon 2007). In *Arabidopsis thaliana,* jasmonate or ethylene mediated induced systemic resistance by *Bradyrhizobium* sp. (Cartieaux et al. 2008) strain, ORS278 in *Arabidopsis thaliana* by transcriptome analysis and SA mediated by endophytic bacteria *Paenibacillus alvei* (Tjamos et al. 2005) have been reported. The level of salicylic acid production and intensity of fungal growth inhibition was found to vary with different bacterial isolates (Forchetti et al. 2010). In a recent study, *Bacillus cereus* AR156 was reported to trigger induced systemic resistance in *A. thaliana* through salicylic acid and jasmonic acid/ethylene-signaling pathways in an NPR1-dependent manner (Niu et al. 2011).

Induced systemic resistance may induce various genes to immunize the host plant mechanically or metabolically by increasing cell wall strength, alteration of host physiology or metabolic responses, enhanced synthesis of plant defense chemicals such as phenolic compounds, pathogenicity related protein (PR-1, PR-2, PR-5), chitinases, peroxidases, phenyl alanine ammonia lyase, phytoalexins, oxidase and/or chalcone synthase. These metabolic products shield the host plant from future attacks from pathogens (Duijff et al. 1997; Compant et al. 2005).

Local immune response induced by plant growth promoting bacteria has also been demonstrated in few studies. However, it is genotype specific and depends on bacterial species. *Burkholderia phytofirmans* PsJN induces local immune response by ion fluxes, salicylic acid production and defense gene activation in grapevine, while production of phenylalanine ammonia lyase, peroxidase and polyphenol oxidase activities have been observed in cucumber plant in response to *Pseudomonas s*p. (Chen et al. 2000). Moreover, production of phytoalexins such as resveratrol and viniferin in host plant have also been reported recently (Verhagen et al. 2010).

In addition to nitrogen-fixation ability, these bacteria can influence plant growth through one or more properties. These include production of phytohormones, siderophores, induced systemic tolerance through production of 1-aminocyclopropane-1-carboxylase deaminase, induced systemic resistance and antagonistic activities.

4 Ecology and Diversity of Endophytic Bacteria

The make-up of endophytic bacterial communities is very likely affected by deterministic factors as well as stochastic events (Hardoim et al. 2008). Other than soil factors, plants also offer a selective environment to microorganisms, ‘filtering out’ specific microbial groups from the diversity found at plant roots (Rosenblueth and Martinez-Romero 2006). Thus, various factors like plant genotype and physiological status, bacterial colonization traits, abiotic conditions and agricultural management regimes can affect the diversity of bacterial communities in root tissues (Hardoim et al. 2008). Out of these factors, plant genotype may play a key role in the selection of distinct bacterial communities that associate with plants (Andreote et al. 2010).

Endophytes can colonize more aggressively and displace others when inoculated with other bacteria in a competition experiment. This opinion is based on the reports where *Pantoea* sp. was found to be outcompeting *Ochrobactrum* sp. in rice (Verma et al. 2004) and with different *R. etli* strains in maize (Rosenblueth and Martinez-Romero 2006). Many endophytes have a broad host range. However, it has not been studied in a systematic and quantitative manner. Recently, *Klebsiella oxytoca* and

Achromobacter xylosoxidans originally isolated from *Typha australis* and wheat respectively were reported to colonize rice plants (Jha and Kumar 2007, 2009). However, colonization ability of different bacterial strains varies widely. Dong et al. (2003) studied the colonization pattern of *gfp*-tagged *Escherichia coli* K-12, *Salmonella enterica* serotype *Typhimurium* strain ATCC 14028, and *K. pneumoniae* 342 and concluded that significant strain specificity exists for plant entry and for most strains. They also studied the kinetics of invasion by endophytic bacteria and found a strong correlation between rhizosphere colonization and interior colonization for all strains. Despite being best colonizer in the interior, *K. pneumoniae* 342 showed the lowest correlation between rhizospheric colonization and endophytic colonization among the other six strains which showed a strong positive correlation. Based on their experiment, they deduced that single colony forming unit in the inoculum was sufficient to cause invasion of the plant interior for certain isolates while higher number of cells was required for effective colonization for other strains. They reported that endophytic colonization is an active process controlled by genetic determinants from both partners (Dong et al. 2003).

A proper understanding of interactions and resulting exchange of signals between microbial communities would facilitate the development of new strategies to promote beneficial interaction between the microorganisms and plants. The genetic diversity of bacteria can be evaluated using amplified ribosomal DNA (rDNA) restriction analysis, rep-Polymerase chain reaction genomic fingerprinting and small subunit ribosomal DNA sequencing etc. (Grange and Hungria 2004). Sequence analysis of amplified *nifH* has also been used to study the diversity of diazotrophic bacteria isolated from plants (Rosado et al. 1998; Zhang et al. 2006; Chowdhury et al. 2007). The *nifH* sequence of several known diazotroph families cluster were similar to that of 16S ribosomal RNA (rRNA) analysis. Therefore, *nifH* analysis is also used for study of diversity among diazotrophic bacteria (Ueda et al. 1995a; Zhang et al. 2006; Venieraki et al. 2011).

For studying diversity of diazotrophic bacteria, combined use of rDNA and rRNA analysis has been proposed to give more detailed understanding of bacterial community (Nogales et al. 2001). Community diversity in terms of desired metabolic activity or rRNA-based approach can also be studied comprehensively by stable isotope probing method (Manefield et al. 2002; Kiely et al. 2006). Active endophytic bacterial community has been studied recently by analyzing 16S rRNA sequences of density resolved DNA using stable isotope probing (Rasche et al. 2009). Denaturing gradient gel electrophoresis of amplified rDNA and *nifH* of culturable or unculturable rhizospheric and endophytic bacteria has also been used for studying molecular diversity (Lovell et al. 2000; Araujo et al. 2002; Abreu-Tarazi et al. 2010; Burbano et al. 2010; West et al. 2010). In addition, other methods like single strand conformation polymorphism, Terminal restriction fragment length polymorphism of rDNA can also be helpful in elucidating the prevalence of molecular diversity and studying phylogenetic relationship among bacteria. Metagenomic approach has been useful in understanding community structure of both cultivable and uncultivable bacteria. Metagenomics is the genomic analysis of uncultured microorganisms, which is of two types: a function-driven approach, in which metagenomic libraries are initially screened for an expressed trait, and a sequence-driven approach, in which libraries are initially screened for particular DNA sequences (Schloss and Handelsman 2003; Zeyaullah et al. 2009).

The make-up of endophytic bacterial communities depends on various factors such as soil type, soil composition, soil environment, plant genotype and physiological status, bacterial colonization traits, and agricultural management regimes. Colonization and abundance of different bacterial species varies widely with host plants. Endophytic bacterial community can be analyzed employing stable isotope probing as well as various modern molecular approaches which are based on analysis of 16S ribosomal deoxyribonucleic acid (DNA), gene encoding products for nitrogen fixation and repetitive DNAs. Moreover, metagenomic approaches allow estimation and analysis of unculturable bacteria at genomic as well as functional genomic level.

5 Colonization of Endophytic Bacteria

Colonization of bacteria in the plant is a complex process, which involve interplay between several bacterial traits and genes, and plant responses. The colonization is an orchestra of number of steps: (a) migration towards root surface i.e. chemotaxis, (b) attachment and microcolony formation, (c) distribution along root and, (d) growth and survival of the population. Colonization pattern of bacteria can be obtained by tagging the putative colonizing bacteria with a molecular marker such as auto-fluorescent marker such as green fluorescent protein (*gfp*) or β-glucuronidase (*gus*) followed by electron or confocal laser scanning microscopy (Singh et al. 2011). The presence of various reporter genes on the colonization of diazotrophic endophytic bacteria was summarized in Table 2. Although, molecular mechanism involved in the endophytic colonization process is not well understood, recent reports based on genomic data suggest resemblance of colonization process between pathogenic and endophytic bacteria (Krause et al. 2006; Hardoim et al. 2008).

5.1 Chemotaxis and Electrotaxis

Root colonization is the first and critical step in the establishment of plant-microbe association. Microorganisms move towards rhizosphere in response to root exudates which are rich in amino acids, organic acids, sugars, vitamins, purines/pyrimidines and other metabolic products. Thus, motility and chemotaxis play a key role in the root colonization. At the same time, in addition to providing nutritional substances, plants start cross-talk with microorganisms by secreting some signals which cause colonization by some bacteria while inhibit the others (Bais et al. 2006; Compant et al. 2010b). Like plant-rhizobia interaction, plant root exudates do influence the expression of genes in associating bacteria. Stimulation of colonization of wheat and *Brassica napus* by *A. brasilense* and *A. caulinodans* in response to flavonoids

Table 2 Effect of reporter genes on the colonization of diazotrophic endophytic bacteria

Gene	Gene product/ function	Advantages/disadvantages	References
*tfd*A	2,4-dichlorophe noxyacetate monooxygenase	Low resolution	King et al. (1991)
*pho*A	Alkaline phosphatase	Soluble end product	Reuber et al. (1991)
*xyl*E	Catechol 2,3-dioxygenase	Amplification or photographic exposure for detection	Winstanley et al. (1991)
Heavy metal resistance	Heavy metal resistance	Requires plate counting	de Lorenzo (1994)
l*ac*Z	β-galactosidase	High background in most plant and bacteria	Kovach et al. (1994)
Antibiotic resistance	Antibiotic Resistance	Requires plate counting	Kovach et al. (1995)
*cel*B	β-glucosidase	Detection after denaturation of endogenous enzymes	Voorhorst et al. (1995)
*lux*A, *luc*	Luciferase	Low resolution	Ladha and Reddy (2000)
gfp, bfp, yfp, cfp, rfp	Autoflourescent protein	High resolution. Real time application. Requires oxygen for proper folding	Godfrey et al. (2010)
*gus*A	β-glucuronidase	No background in bacteria and plants, requires substrate	Singh et al. (2011)

from host exudates indicates that flavonoid may be determinant for endophytic colonization (O'Callaghan et al. 2000). In a more recent report, naringenin, a flavonoid present in exudates of plants has been reported to modulate the expression of genes in *H. seropedicae* and this alteration in gene expression were the decisive for endophytic colonization (Tadra-Sfeir et al. 2011). In addition to chemotaxis, electrotaxis (electrogenic ion transport at the root surface) has also been considered as a possible mechanism for initiating rhizobacterial colonization (van West et al. 2002). Sloughed up root cap cells also have large impact on plant-microbe interaction (Hawes et al. 1998). Root hair regions and emergence points are preferred site for colonization (Lugtenberg and Kamilova 2009).

Colonization of root by microorganism may further induce release of exudates which can create 'biased' rhizosphere with exudation of specific metabolic products, which in turn induce flagellar motility that directs their colonization on plant surfaces. Motility of endophytic bacteria is considered as one of the most important aspect for successful colonization event. *P. fluorescence* defective in chemotaxis-driven flagellar motility was found to have reduced colonization efficiency (Lugtenberg et al. 2001). Similarly, type IV pili mediated twitching motility has been found to be instrumental in the establishment of successful endophytic colonization by *P. stutzeri, Azoarcus sp.* and *H. seropedicae* (Bohm et al. 2007; Yan et al. 2008; Pedrosa et al. 2011).

5.2 Attachment on Root Surface

Chemotaxis or electrotaxis driven migration of bacteria to roots is followed by adhesion of bacteria on root surface to get entry into the plant tissue. Adherence of these bacterial cells depends on various cell surface molecules which includes cell appendages (flagella or pili), major outer membrane proteins and secretion system of bacteria which play major role for invasion. Bacterial flagella and pili also play an important role in the colonization process by adhering on plant surfaces. Glycosylated polar flagellum is thought to act directly as a root adhesion (Croes et al. 1993). Role of flagella in colonization has evidenced recently where FliC3 (product of flagellin genes) which was considered previously as microbe associated molecular pattern, has been found to be required for endophytic colonization in the *Azoarcus*-rice interaction, most likely for spreading inside the plant (Reinhold-Hurek and Hurek 2011). Involvement of type IV pili in adherence to plant surface in *Azoarcus* has also been demonstrated (Dorr et al. 1998). Additional mechanisms can also be operative for initial plant-microbe interaction. Bilal et al. (1993) suggested that cellulose fibrils, a cell-surface protein and Ca^{2+} dependent adhesion may be implicated in the specific interaction with plants. Moreover, chemical composition of lipopolysaccharides present on the surface of bacteria might be determinative for successful colonization in host plants (Serrato et al. 2010).

Role of bacterial major outer membrane protein in early host recognition has been recognized in earlier report, where major outer membrane proteins from *Azospirillum brasilense* showed stronger adhesion to extracts of cereals than extracts of legumes and tomatoes. It suggests the involvement of major outer membrane proteins in adhesion, root adsorption and cell aggregation of bacterium (Burdman et al. 2000). Bacterial cells are equipped with various secretion systems which enable them to interact successfully with the host plant. Preston et al. (2001) identified secretion system type III (hrp) in *P. fluorescens* SBW25 by *in-vitro* expression technology, a promoter trapping technique used to identify genes expressed *in-vivo* during colonization process.

5.3 Entry and Distribution Along Root

Entry of endophytic bacteria in plant roots is known to occur through (a) wounds particularly where lateral or adventitious roots occur; (b) root hairs and (c) space between undamaged epidermal cells (Sprent and de Faria 1988). Chi et al. (2005) demonstrated that the colonization of *gfp*-tagged rhizobia in crop plants begin with surface colonization of the rhizoplane at lateral root emergence, followed by endophytic colonization within roots, and then ascending endophytic migration into the stem base, leaf sheath, and leaves where they develop high populations. *Azospirillum* may also colonize endophytically through wounds and cracks of the plant root. Spreading of *Azospirillum* from the lateral root emergence to other part of root depends on the status of the nitrogen and carbon source present in the vicinity

(Ramos et al. 2002). *G. diazotrophicus* gains its entry into micropropagated sugarcane plantlet through wounds caused by emerging lateral roots (Sevilla and Kennedy 2000). James et al. (2001) reported that *G. diazotrophicus* enters in sugarcane and other gramineous plant roots via lateral root junctions and/or root tips and subsequently colonizes the root vascular system from where it is translocated to the lower stem in the xylem. Similarly, massive colonization of *P. fluorescens* PICF7 on root hairs and site of differentiation of olive plant was noticed in a very recent report (Prieto et al. 2011). Crack entry of *A. caulinodans* ORS571 in response to release of flavonoids such as naringenin from host plant and subsequent intercellular colonization of the cortex of root systems of rice, wheat and *Arabidopsis thaliana* have earlier been observed (Gough et al. 1997; Webster et al. 1997). Compant et al. (2008) reported the chronological detection of endophytic *B. phytofirmans* PsJN on the root surfaces, in the endorhiza and inside inflorescence stalks of *Vitis vinifera* (Fig. 2).

Endophytic bacteria may colonize root tissues and spread actively in aerial parts of plants through expressing moderate amount of degradative enzymes such as pectinases and cellulases. Utilization of aforesaid enzymatic activities for colonization by *A. irakense* (Khammas and Kaiser 1991), *Azoarcus* sp. (Hurek and Reinhold-Hurek 2003) and others, has been demonstrated as one of the efficient methods to get entry into the host plant. Endoglucanase is one of the major determinants for the colonization of endorhizosphere, which was evident from the observation that *Azoarcus* strain lacking endoglucanse was not effective in colonizing the rice plant (Reinhold-Hurek et al. 2006). The endoglucanase preferably attacks oligosaccharides larger than cellobiose and releases larger oligomers from substrates such as carboxymethylcellulose, and is thus likely to loosen larger cellulose fibers, which may help bacteria to enter in the plant tissue (Hurek and Reinhold-Hurek 2003). A homologue of endoglucanase gene has also been identified in *P. stutzeri* A1501, which occasionally colonizes cortex of crop plants (Yan et al. 2008). In addition to endoglucanse, exoglucanases may also help in the colonization process. An exoglucanase having cellobiohydrolase and β-glucosidase activity on wide substrate spectrum including xylosides was identified to be key player in *Azoarcus* sp. BH72 for colonization process (Reinhold-Hurek et al. 2006). *A. irakense* isolates have been found to colonize intracellularly in rice that may be enabled by the expression of pectinolytic, cellulotyic and β-glucosidase enzymes (Somers et al. 2004). In plants like *Elaegnus* and *Mimosa* sp., the endophyte penetrates the root radial walls presumably by digesting the middle lamella and then proceeds between cells and through intercellular spaces. In *Parasponia* plants, the colonizing bacteria stimulate cell division in the outer cortex. In due course these newly formed cells rupture the epidermis resulting in an effective wound infection (Sprent and de Faria 1988). In contrast to above examples, genes encoding plant cell wall degrading enzymes has not been found in the endophytic bacteria *H. seropedicae* strain SmR1 (Pedrosa et al. 2011).

Azoarcus sp., an obligate endophyte of Kallar grass, has been critically studied by using transposon mutant expressing β-glucuronidase constitutively as a reporter gene (Hurek and Reinhold-Hurek 2003). *Azoarcus* sp. BH72 colonize apical region

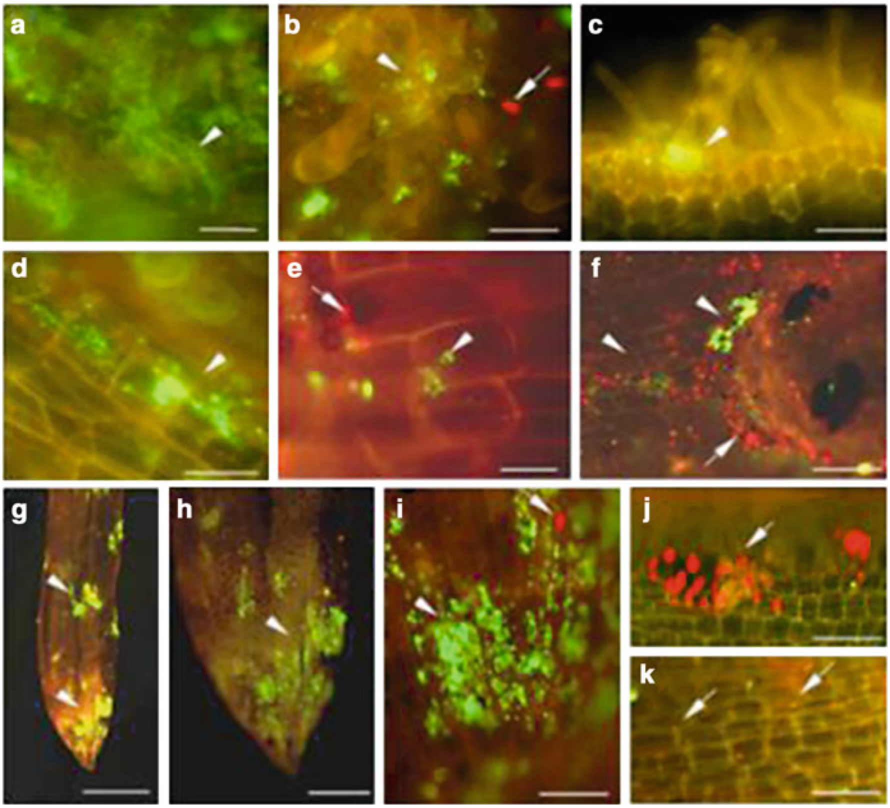

Fig. 2 Epifluorescence microscopic images of roots of grapevine fruiting cuttings inoculated with *Burkholderia phytofirmans* PsJN::*gfp*2 × 1–4 weeks after soil inoculation with 5 × 10^8 colony forming units g^{-1} of soil. Gfp-tagged bacteria (*arrows*) were visualized at the root hair zone (**a**–**c**) colonized root hairs (**a**–**c**), on other rhizodermal cells (**d** and **e**), at lateral root emergence sites (**f**) and at the root tip (**g**–**i**). A natural epiphytic microbial communities was also detected on the root surface of inoculated plants (*arrows* in **b**, **e**, **f** and **i**) as well as on the roots of control plants (*arrows* in **j** and **k**). Similar rhizoplane colonizations by strain PsJN were found from 1 to 4 weeks postinoculation. *Scale bars*: (**a**) 100 mm, (**b**) 30 mm, (**c**) 75 mm, (**d** and **e**) 30 mm, (**f**) 100 mm, (**g**) 1 mm, (**h**) 500 mm, (**i**) 250 mm and (**j** and **k**) 100 mm (Reproduced from Compant et al. (2008) with permission from Wiley)

of roots behind the meristem intensively and penetrate the rhizoplane preferentially in the zone of elongation and differentiation. It colonize in the cortex region both inter- and intra-cellularly. In older parts of the roots, it also occurs in aerenchymatic air spaces. *Azoarcus* sp. is capable of invading even the xylem vessels suggesting its systemic spreading into shoots through the transport in vessels. However, shoot colonization of Gramineae appears to be more pronounced in *Gluonoacetobacter diazotrophicus* (James and Olivares 1998) and *H. seropedicae* (Gyaneshwar et al. 2002). Intercellular colonization of endophytic bacteria in cortex as well as xylem of root has been reported in recent studies (Prieto et al. 2011; Schmidt et al. 2011).

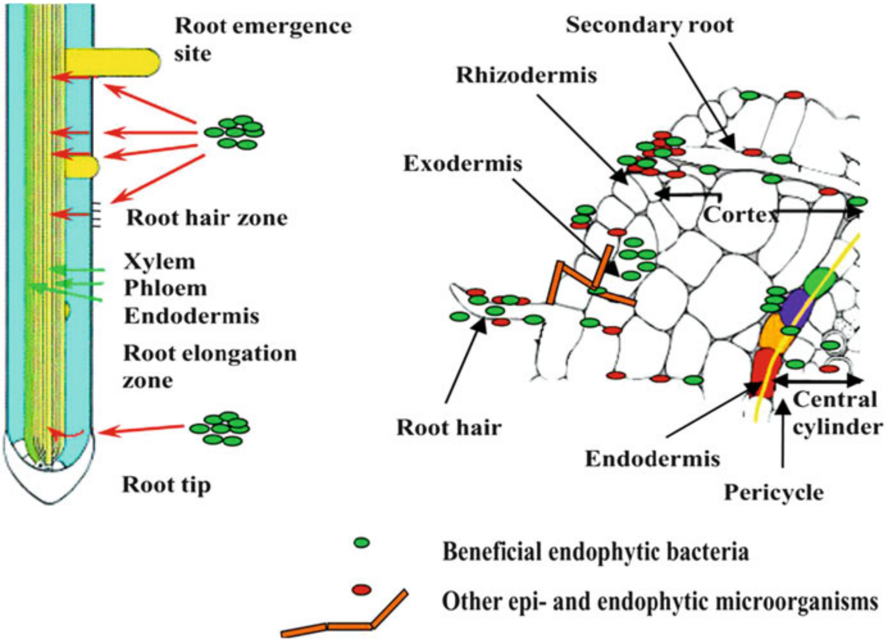

Fig. 3 Schematic presentations for the sites of plant colonization by endophytic bacteria. Figure in the *left panel* represents longitudinal section of root depicting possible sites for entry of endophytic bacteria. Figure in the *right panel* represents transverse section of root showing the distribution and colonization of endophytic bacteria (Reproduced from Compant et al. (2010a) with permission from Elsevier)

Compant et al. (2011) reported the colonization of endophytic bacteria in epidermis and xylem of even reproductive organ of grapevine. Based on the colonization pattern of *P. fluorescens* PICP2 and PICF2 in root hairs of olive plant, Prieto et al. (2011) suggested that endophytic bacteria are confined within an organelle most likely vacuole which arises by narrowing of an internal membranous structure in roots. The possible sites of colonization by diazotrophic endophytic bacteria are depicted in Fig. 3.

5.4 *Growth and Survival*

Endophytic colonization is not as specific as of *Rhizobia* but successful endophytic colonization does involve a compatible host plant (Ryan et al. 2008). However, endophytic colonization indeed depends upon the physiological changes in plants and is restricted or slowed down by the defense mechanisms (Rosenblueth and Martinez-Romero 2006). Colonization of *G. diazotrophicus* was found to be diminished in plants grown under high nitrogen fertilizer regime. This reduction in colonization was explained as a result of altered plant physiology in the presence of nitrogen fertilizer, which reduces sucrose concentration to be utilized by endophytic bacteria (Fuentes-Ramirez et al. 1999). Influence of organic amendment on endophytic population has also been demonstrated (Hallmann et al. 1997). Plant defense responses play a critical role in regulating colonization of endophytic bacteria. In dicotyledonous plants, salicylic acid and ethylene restricts the endophytic colonization. Ethylene, a signal molecule of induced systemic resistance in plants decreases endophytic colonization as observed in *Arabidopsis thaliana* inoculated with *K. pneumoniae* 342 (Iniguez et al. 2005). However, proteomic approach used to study the bacterial colonization indicated that instead of ethylene and salicylic acid, it is the jasmonic acid which contributes in restricting endophytic colonization in grasses (Miche et al. 2006). Expression of jasmonic acid induced pathogenesis related proteins (defense proteins) depends upon the compatibility of plant variety and endophytic bacteria. Inoculation of *Azoarcus* sp. to more compatible rice (*Oryza sativa*) culivar IR36 led to the expression of fewer jasmonic acid-induced pathogenesis related proteins than that of less compatible cultivar IR42. Antimicrobial peptides synthesized by some plants like rice and maize may reduce endophytic colonization (Fuentes-Ramirez et al. 1999). Understanding of molecular mechanism and conditions limiting the colonization process need to be elucidated for exploiting the beneficial endophytic or associative interaction with plants.

Colonization process of an endophytic bacterium involves various steps which include migration towards root surface, attachment and microcolony formation on plant surface, distribution along root and growth and survival of the population inside plant tissue.

6 Revamp in Genomic and Proteomic Studies of Endophytic Bacteria

This section critically summarizes the recent advances in the genomic and proteomic aspects of diazotrophic endophytic bacteria.

6.1 Genomic Studies

Prospects of potential application of endophytic bacteria led the exploration of their genomic make up so that these bacteria can be exploited for sustainable and productive agriculture advancement. In recent years, genomic sequences of several endophytic diazotrophs other than rhizobia have been summarized in tabular form (Table 3).

Recently Kaneko et al. (2010) reported the complete genome sequence of *Azospirillum* sp. B510. In this, nitrogenase genes and assembly related protein are located in three separate loci in chromosome. It is also mentioned that it possess genes of 1-aminocyclopropane-1-carboxylate deaminase activity and indole-3-acetic acid

Table 3 Some diazotrophic endophytic bacteria with their genome sizes and annonate genes

Endophytic bacteria	Genome size (bp)	No. of genes annotated	References
Azoarcus sp. EbN1	4,727,255	4,686	Rabus et al. (2005)
Azoarcus sp. BH72	5,376,040	4,073	Krause et al. (2006)
Bacillus amyloliquefaciens FZB42	3,918,589	3,693	Chen et al. (2007)
Klebsiella pneumoniae 342	5,920,257	5,881	Fouts et al. (2008)
Pseudomonas stutzeri A1501	4,567,418	4,237	Yan et al. (2008)
Gluconoacetobacter diazotrophicus Pal5	3,999,591	3,997	Bertalan et al. (2009)
Azotobacter vinelandii DJ, BAA-1303	5,365,318	5,133	Setubal et al. (2009)
Azospirillum sp. B510	3,311,395	2,893	Kaneko et al. (2010)
Enterobacter sp. 638	4,676,467	4,444	Taghavi et al. (2010)
B. subtilis BSn5	4,093,599	4,177	Deng et al. (2011)
Variovorax paradoxus S110	6,754,997	6,279	Han et al. (2011)
Azoarcus sp. strain KH32C	5,081,166	4,531	Nishizawa et al. (2012)
Herbaspirillum seropedicae	5,513,887	4,804	Pedrosa et al. (2011)
Burkholderia phytofirmans PsJN	8,214,658	7,487	Weilharter et al. (2011)
Serratia proteamaculans 568	5,448,853	4,942	Zhu et al. (2012)

production. Other than this, genes related to ion transport, Quorom sensing, secretion system (Type IV), motility genes have been described. The information provided in this study can be exploited to study the role of various genes in the colonization process described above as well as augmentation of genes related to plant growth promoting bacteria activities can provide a potential genetically modified biofertilizer candidate.

The strain *Azoarcus* sp. BH72 consists of gene cluster that encodes cell surface components (Type IV pili) which seems to be important factor for host-microbe interaction as this cluster is absent in related non-endophytic soil bacteria *Azoarcus* sp. EbN1 (Krause et al. 2006). Genes encoding products for exopolysaccharide polymerization, translocation (*gum* operon of *Xanthomonas compestris* and rhizobial *pss* gene cluster) and products required for plant-microbe interaction resemble to that of pathogenic bacteria and rhizobium. It differs from that of pathogenic bacteria in not secreting hydrolases. However, genes for producing low amount of macerating enzyme endoglucanase (*EglA*) is synthesized which is probably required for endophytic colonization (Krause et al. 2006).

Analysis of the *G. diazotrophicus* Pal5 complete genome sequence provides important insights into the endophytic relationship, and suggests many interesting candidate genes for post-genomic experiments (Bertalan et al. 2009). The genome sequence results showed unexpectedly high number of mobile elements for an endophytic bacterium; thus suggest a high number of horizontal gene transfer events. To change niche from rhizosphere to endophytic, the bacteria should penetrate the plant. The putative *gum*-like cluster containing an endoglucanase could be important in this regard. The genome shows various properties such as biological nitrogen fixation, phytohormones and biocontrol genes. Several features for an endophytic lifestyle are found in genome islands, including type IV secretion systems, flagella, pili, chemotaxis, biofilm, capsular polysaccharide and some transport proteins.

Comparison of *G. diazotrophicus* and *Azoarcus sp*. BH72 genome sequences showed that these endophytic diazotrophic bacteria adopted very different strategies to colonize plants. Features like, large number of TonB receptors, *gum*-like and *nif* clusters, and osmotolerance mechanisms are common to both endophytic diazotrophic bacteria. Presence of TonB receptors has been found to be common in almost all endophytic bacteria (Reinhold-Hurek and Hurek 2011). Apart from this, *G. diazotrophicus* has a larger number of transport systems, and it is capable of growing on a wide variety of carbon sources, while *Azoarcus* sp. BH72 has rather complex signaling mechanisms to communicate with its plant host (Bertalan et al. 2009).

Herbaspirillum seropedicae is well characterized diazotrophic endophytic bacteria, which is known to interact with several plant species belonging to family Graminiae. Presence of few mobile elements in most of the endophytic bacteria with exception of *G. diazotrophicus* Pal5 indicates low recombination or rearrangement which might be a reason for their adaptation to endophytic lifestyle (Krause et al. 2006; Pedrosa et al. 2011). It is well equipped with *nif* gene cluster with 46 open reading frames which includes structural and regulatory genes for nitrogenase synthesis and activity, molybdenum uptake, electron transport, metal cluster analysis and other related functions. In addition to diazotrophy, *H. seropedicae* possesses complete machinery for auxin, siderophore and 1-aminocyclopropane-1-carboxylate deaminase synthesis which make it suitable candidate for plant growth promotion. In order to colonize plant, *H. seropedicae* expresses a variety of secretion system including secretion system type II, III, V and type IV pili. Genes for type IV pili encode for proteins which play role in attachment to surfaces, twitching motility, biofilm formation, virulence and protein secretion. Expression of lytic transglycolase help in plant-microbe interaction through its ability to degrade peptidoglycan partially which allow efficient assembly and anchoring of transport complexes (secretions system type II and III) and type IV pili to the cell envelope. However, expression of type III secretion system has not been observed in proteomic analysis which suggest for availability of suitable physiological condition for their synthesis and function. Since, invasion of bacteria into the plant is kind of stress for both microbe and host, genes encoding function to combat osmotic stress, salinity desiccation, nitrogen starvation, ultraviolet radiation, pH and other stresses are present in *H. seropedicae.* These include, synthesis of amylopectin like polysaccharides, trehalose and $Na^+(K^+)/H^+$ antiporter which contribute to defense against osmotic stress. Genes for hemolysin and hemagglutins have also been found which may be presumably required for surface attachment and biofilm formation during plant tissue colonization. Moreover, availability for metabolic pathways for the degradation of several aromatic compounds indicate that these pathways may be instrumental for given bacteria to thrive on plant tissue owing to their metabolic flexibility and defense against toxic phytochemicals (Pedrosa et al. 2011).

Comparative genomic analyses of diazotrophic *K. pneumoniae* 342 with the presumed human pathogen *K. pneumoniae* MGH78578 suggested that the later apparently cannot fix nitrogen, and the distribution of genes essential to surface attachment, secretion, transport, and regulation and signaling varied between both genome, which revealed a critical divergences between the strains that influence their preferred host ranges and lifestyles. Little genome information is available concerning endophytic bacteria. The *K. pneumoniae* 342 genome unveil bacterial-plant host relationships, which could ultimately enhance growth and nutrition of important agricultural crops and development of plant-derived products and biofuels (Fouts et al. 2008).

In addition to above mentioned bacterial species, genome of other endophytic bacteria namely *B. phytofirmans* (strain DSM 17436/PsJN), *Enterobacter* sp. (strain 638), *Methylobacterium populi* (strain ATCC BAA-705/NCIMB 13946/BJ001), *P. putida* (strain W619), *Serratia proteamaculans* (strain 568), *Stenotrophomonas maltophilia* (strain R551-3) have also been sequenced (Table 3). Although, genome sequences of many endophytic bacteria are being compiled, it still needs thoughtful analysis and strategy to develop a potential biofertilizer strain which can be manipulated in different environmental conditions.

6.2 Proteomics Studies

In the last decade, proteomics has been applied for the identification of proteins that are important in plant responses to microorganisms. Several grasses have interaction with plant growth-promoting N_2-fixing bacteria which do not form a specific root structure like a nodule. A large number of proteomic techniques are available for the analysis of various aspects of proteins, including their post-translational modification, expression profile, and interaction network (Pandey and Mann 2000). The two most famous proteomic methods are two-dimensional gel electrophoresis and mass spectrometry. Differential display tool difference gel electrophoresis is an extension of two-dimensional gel electrophoresis technique to compare multiple samples simultaneously. There are numerous other gel-based or gel-free and quantitative or qualitative proteomic methods, which includes isotope-coded affinity tag, isobaric tag for relative and absolute quantification, stable isotope labeling with amino acids in cell culture, label-free comparative Liquid chromatography-Mass Spectrometry, protein phosphorylation identification, and protein microarrays, that can be used to elucidate plant bacterial interactions (Xing et al. 2004; Roe and Griffin 2006; America and Cordewener 2008; Gong et al. 2008).

Proteome analysis of rice roots infected with *Azoarcus* sp. was carried out to characterize the plant responses to these endophytes. Out of 1,000 displayed proteins, 47 responded to inoculation, including salt-stress and pathogenesis-related proteins and a putative receptor kinase. These proteins are involved in the endophyte infection process as they were also induced by jasmonic acid, which inhibits the infection process (Miche et al. 2006)

In *G. diazotrophicus,* 583 proteins were identified by two-dimensional gel electrophoresis /Matrix associated laser desorption/ionization for the establishment of a proteome reference map (Lery et al. 2008). Proteins of various pathways related to nucleotides, amino acids, carbohydrates, lipids, cofactors and energy metabolism have been described for comparative studies with other bacterial species. Various proteins related to Nitrogen-fixation, ion transporting, adaptation and protection related, regulatory and metabolic pathway proteins. When *G. diazotrophicus* colonizes sugarcane present in the cocultivation medium, changes occurs in various metabolic pathways, membrane-associated structure, redox reactions, transcript and translational regulation, and energy metabolism in comparison to control. So, the differentially expressed proteins showing modifications in bacterial metabolism and physiology in *G. diazotrophicus* during cocultivation with sugarcane provided information about the proteins involved in plant-bacterial interaction. The knowledge of metabolic fundamentals and coordination of these pathways are important for studying plant-endophyte interaction for attaining sustainable agriculture (Lery et al. 2008).

Cheng et al. (2009) observed that among the 275 identified proteins, in *P. putida* UW4, 1-aminocyclopropane-1-carboxylate deaminase is noteworthy because of its substantial role in plant growth-promoting activity. Furthermore, majority of the identified proteins were in bacterial cytosol. The identification of periplasmic, membrane-spanning and extracellular proteins indicated that the methodology is capable of providing a degree of representation from all cellular compartments including less soluble membrane fractions. One additional protein was identified as homologous to a *Bradyrhizobium japonicum* protein, which was later confirmed by several independent mass spectrometry analyses. It suggested a possibility that this protein was acquired from *Bradyrhizobium* species via lateral transfer, but it needs further confirmation. The functional diversity of the identified proteins can be used to investigate the responses of *P. putida* UW4 in response to various environmental signals. This data set will be helpful to unveil plant growth-promoting mechanisms present in this and similar bacteria, and in future to characterize bacterial interactions in the environment (Cheng et al. 2009).

Chaves et al. (2007) reported the proteome reference map of *H. seropedicae*. Out of 205 proteins identified during their study, 17 were hypothetical or conserved hypothetical proteins. The annotated proteins were classified in 19 clusters of orthologous groups categories, except proteins involved in defense mechanisms which all were identified. The clusters of orthologous groups categories were grouped in four classes: proteins involved in metabolism, information storage and processing, cellular processes and poorly characterized class (Chaves et al. 2007). Analysis of samples for meta-proteogenomics can be performed following the major steps mentioned as shown in Fig. 4.

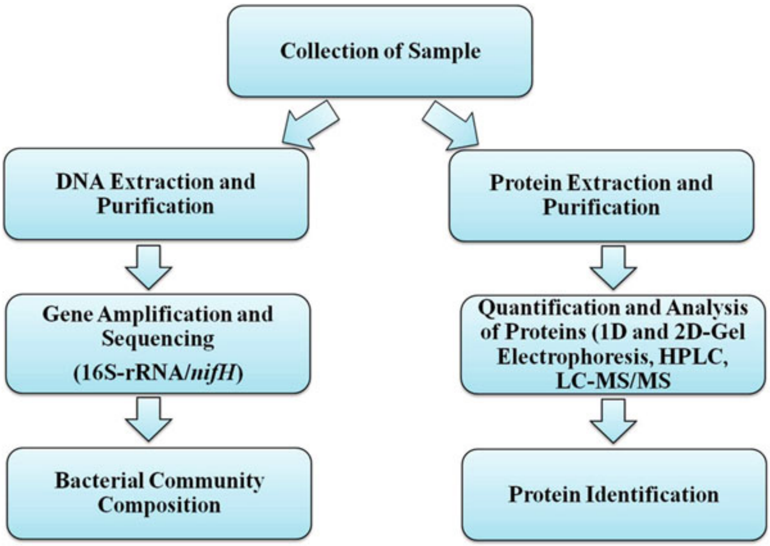

Fig. 4 Diagrammatic representation of meta-proteogenomics sample analysis methods

Ongoing progress towards in-depth analysis of genomic and whole protein profile of some of the potential endophytic bacteria such as *Azoarcus* sp., *Gluconoacetobacter diazotrophicus, Herbaspirillum seropedicae, Serratia marcesens* can help understand mechanism involved in plant-endophyte interaction which in turn will be deterministic in use of suitable formulations of endophytic bacteria to be used as biofertilizer for sustainable agriculture.

7 Conclusion

Exploration of endophytic bacteria and their abilities to enhance plant growth and productivity indeed indicates the existence of their natural associations and beneficial impact which can be exploited to feed burgeoning population of the world. Despite the fact that a large number of associative and endophytic bacteria have shown the potential in laboratory and green house condition but their consistent performance was failed under natural condition (Lucy et al. 2004). The reduction in the efficiency was noted when plants inoculated with endophytic bacteria were shifted from pot to field conditions (Gyaneshwar et al. 2002). These factors that affect colonization and the bacteria derived benefit to plants may be soil type, nutritional status of soil, host plant genotype and age as well as climatic conditions (Muthukumarasamy et al. 2005). It has been also evident from the earlier reports that plant growth and yield can be increased by the combined use of fertilizers and endophytic bacteria. This practice reduced the inputs of chemical fertilizers in the soil (Yanni et al. 1997; Saleh and Glick 2001). However, high amount of available utilizable N results in reduced colonization of endophytic bacteria and also reduce the process of

N_2-fixation due to regulatory mechanism acting in the diazotrophs. As observed in some studies, high nitrogen fertilized soil reduced the colonization of sugarcane by *G. diazotrophicus* and *H. seropedicae* (Fuentes-Ramirez et al. 1999; dos Reis et al. 2000). Therefore, a challenge is posed for systematic optimization for the application of suitable diazotrophs isolates and the amount of fertilizer to be added to obtain maximum output. Use of compost may be useful at some extent which provides utilizable N to support the growth of microorganism and make the plant evade from negative effects of diazotrophs colonization (Muthukumarasamy et al. 2007).

One of the major challenges includes selection of plant genotype and age, and compatible associative bacteria. Understanding of this compatibility would be helpful in enhancing the productivity using specific bacterial strain. Since, the colonization of associative bacteria depends upon seasonal changes and soil hydric stress, multiples field trials are required to optimize the parameters for obtaining the maximum output. Another factor which might be plays a crucial role is the plant defense response because it may limit or reduce the colonization of associative bacteria. In addition, the colonization mechanism is still not well understood. Intelligent analysis of genomic and functional genomic studies can help to manipulate the conditions in order to enhance the bacterial colonization process and increased plant growth attributes and also provides a better way to understand the ecology and behaviours of the endophytic diazotrophs.

References

Abeysingha NS, Weerarathne CS (2010) A preliminary study on quantification of biological nitrogen fixation in sugarcane grown in Sevanagala in Sri Lanka. J Nat Sci Found Sri Lanka 38:207–210. doi:10.4038/jnsfsr.v38i3.2311

Abreu-Tarazi MF, Navarrete AA, Andreote FD, Almeida CV, Tsai SM, Almeida M (2010) Endophytic bacteria in long-term in vitro cultivated “axenic” pineapple microplants revealed by PCR-DGGE. World J Microbiol Biotechnol 26:555–560. doi:10.1007/s11274-009-0191-3

Akhtar MS, Siddiqui ZA (2010) Role of Plant growth promoting rhizobacteria in biocontrol of plant diseases and sustainable agriculture. In: Maheshwari DK (ed) Plant growth and health promoting bacteria, vol 18, Microbiology monographs. Springer, Berlin/Heidelberg, pp 157–196. doi:10.1007/978-3-642-13612-2_7

Ali SKZ, Sandhya V, Grover M, Kishore N, Rao LV, Venkateswarlu B (2009) *Pseudomonas* sp. strain AKM-P6 enhances tolerance of sorghum seedlings to elevated temperatures. Biol Fertil Soils 46:45–55. doi:10.1007/s00374-009-0404-9

America AHP, Cordewener JHG (2008) Comparative LC-MS: a landscape of peaks and valleys. Proteomics 8:731–749. doi:10.1002/pmic.200700694

Andreote FD, Rocha UN, Araujo WL, Azevedo JL, van Overbeek LS (2010) Effect of bacterial inoculation, plant genotype and developmental stage on root-associated and endophytic bacterial communities in potato (*Solanum tuberosum*). Antonie van Leeuwen 97:389–399. doi:10.1007/s10482-010-9421-9

Araujo WL, Marcon J, Maccheroni WJ, van Elsas JD, van Vuurde JWL, Azevedo JL (2002) Diversity of endophytic bacterial populations and their interaction with *Xylella fastidiosa* in citrus plants. Appl Environ Microbiol 68:4906–4914. doi:10.1128/AEM.68.10.4906-4914.2002

Audenaert K, Pattery T, Comelis P, Hofte M (2002) Induction of systemic resistance to *Botrytis cinerea* in tomato by *Pseudomonas aeruginosa* 7NSK2: role of salicylic acid, pyochelin, and pyocyanin. Mol Plant Microbe Interact 15:1147–1156. doi:10.1094/MPMI.2002.15.11.1147

Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. Annu Rev Plant Biol 57:233–266. doi:10.1146/annurev.arplant.57.032905.105159

Baldani VLD, Baldani JI, Dobereiner J (2000) Inoculation of rice plants with the endophytic diazotrophs *Herbaspirillum seropedicae* and *Burkholderia* spp. Biol Fertil Soils 30:485–491. doi:10.1007/s003740050027

Bertalan M, Albano R, de Padua V, Rouws L, Rojas C, Hemerly A, Teixeira K, Schwab S, Araujo J, Oliveira A, França L, Magalhaes V, Alqueres S, Cardoso A, Almeida W, Loureiro MM, Nogueira E, Cidade D, Oliveira D, Simao T, Macedo J, Valadao A, Dreschsel M, Freitas F, Vidal M, Guedes H, Rodrigues E, Meneses C, Brioso P, Pozzer L, Figueiredo D, Montano H, Junior J, de Souza FG, Flores VMQ, Ferreira B, Branco A, Gonzalez P, Guillobel H, Lemos M, Seibel L, Macedo J, Alves-Ferreira M, Sachetto-Martins G, Coelho A, Santos E, Amaral G, Neves A, Pacheco AB, Carvalho D, Lery L, Bisch P, Rossle SC, Urmenyi T, Pereira AR, Silva R, Rondinelli E, von Kruger W, Martins O, Baldani JI, Ferreira PC (2009) Complete genome sequence of the sugarcane nitrogen-fixing endophyte *Gluconacetobacter diazotrophicus* Pal5. BMC Genomics 10:450. doi:10.1186/1471-2164-10-450

Bhatia S, Maheshwari DK, Dubey RC, Arora DS, Bajpai VK, Kang SC (2008) Beneficial effects of fluorescent Pseudomonads on seed germination, growth promotion, and suppression of charcoal rot in groundnut (*Arachis hypogea* L.). J Microbiol Biotechnol 18:1578–1583

Bhattacharjee RB, Singh A, Mukhopadhyay SN (2008) Use of nitrogen-fixing bacteria as biofertiliser for non-legumes: prospects and challenges. Appl Microbiol Biotechnol 80:199–209. doi:10.1007/s00253-008-1567-2

Bilal R, Rasul G, Arshad M, Malik KA (1993) Attachment, colonization and proliferation of *Azospirillum brasilense* and *Enterobacter* spp. on root surface of grasses. World J Microbiol Biotechnol 9:63–69. doi:10.1007/BF00656519

Biswas JC, Ladha JK, Dazzo FB, Yanni YG, Rolfe BG (2000) Rhizobial inoculation influences seedling vigor and yield of rice. Agron J 92:880–886

Bohm M, Hurek T, Reinhold-Hurek B (2007) Twitching motility is essential for endophytic rice colonization by the N_2-fixing endophyte *Azoarcus* sp. strain BH72. Mol Plant Microbe Interact 20:526–533. doi:10.1094/MPMI-20-5-0526

Borneman J, Skroch PW, O'Sullivan KM, Palus JM, Rumjanek NG, Jansen J, Nienhuis J, Triplett EW (1996) Molecular microbial diversity of an agricultural soil in Wisconsin. Appl Environ Microbiol 62:1935–1943

Bothe H, Schmitz O, Yates MG, Newton WE (2010) Nitrogen fixation and hydrogen metabolism in cyanobacteria. Microbiol Mol Boil Rev 74:529–551. doi:10.1128/MMBR.00033-10

Bottini R, Cassan F, Piccoli P (2004) Gibberellin production by bacteria and its involvement in plant growth promotion and yield increase. Appl Microbiol Biotechnol 65:497–503. doi:10.1007/s00253-004-1696-1

Burbano CS, Reinhold-Hurek B, Hurek T (2010) LNA-substituted degenerate primers improve detection of nitrogenase gene transcription in environmental samples. Environ Microbiol Rep 2:251–257. doi:10.1111/j.1758-2229.2009.00107.x

Burdman S, Jurkevitch E, Okon Y (2000) Recent advances in the use of plant growth promoting rhizobacteria (PGPR) in agriculture. In: Rao NS, Dommergues YR (eds) Microbial interactions in agriculture and forestry. Science Publishers, Plymouth, pp 29–250

Cartieaux F, Contesto C, Gallou A, Desbrosses G, Kopka J, Taconnat L, Renou JP, Touraine B (2008) Simultaneous interaction of *Arabidopsis thaliana* with *Bradyrhizobium* sp. Strain ORS278 and *Pseudomonas syringae* pv. tomato DC3000 leads to complex transcriptome changes. Mol Plant Microbe Interact 21:244–259. doi:10.1094/MPMI-21-2-0244

Castillo UF, Strobel GA, Ford EJ, Hess WM, Porter H, Jensen JB, Albert H, Robison R, Condron MAM, Teplow DB (2002) Munumbicins, wide-spectrum antibiotics produced by *Streptomyces* NRRL 30562, endophytic on *Kennedia nigriscans*. Microbiology 148:2675–2685

Cazorla FM, Duckett SB, Bergstrom ET (2006) Biocontrol of *Avocado dematophora* root rot by the antagonistic *Pseudomonas fluorescens* PCL1606 correlates with the production of 2-hexyl 5-propyl resorcinol. Mol Plant Microbe Interact 19:418–428. doi:10.1094/MPMI-19-0418

Chaves DFS, Ferrer PP, de Souza EM, Gruz LM, Monteriro RA, de Oliveira PF (2007) A two-dimensional proteome reference map of *Herbaspirillum seropedicae* proteins. Proteomics 7:3759–3763. doi:10.1002/pmic.200600859

Chelius MK, Triplett EW (2000) Immunolocalization of dinitrogenase reductase produced by *Klebsiella pneumoniae* in association with *Zea mays* L. Appl Environ Microbiol 66:783–787. doi:10.1128/AEM.66.2.783-787.2000

Chen C, Belanger R, Benhamou N, Paulitz TC (2000) Defence enzymes induced in cucumber roots by treatment with plant growth-promoting rhizobacteria (PGPR) and *Pythium aphanidermatum*. Physiol Mol Plant Pathol 56:13–23. doi:10.1006/pmpp. 1999.0243

Chen XH, Koumoutsi A, Scholz R, Eisenreich A, Schneider K, Heinemeyer I, Morgenstern B, Voss B, Hess WR, Reva O, Junge H, Voigt B, Jungblut PR, Vater J, Sussmuth R, Liesegang H, Strittmatter A, Gottschalk G, Borriss R (2007) Comparative analysis of the complete genome sequence of the plant growth-promoting bacterium Bacillus *amyloliquefaciens* FZB42. Nat Biotechnol 25:1007–1014. doi:10.1038/nbt1325

Cheng Z, Woody OZ, Song J, Glick BR, McConkey BJ (2009) Proteome reference map for the plant growth-promoting bacterium *Pseudomonas putida* UW4. Proteomics 9:4271–4274. doi:10.1002/pmic.200900142

Chi F, Shen SH, Cheng HP, Jing YX, Yanni YG, Dazzo FB (2005) Ascending migration of endophytic rhizobia, from roots to leaves, inside rice plants and assessment of benefits to rice growth physiology. Appl Environ Microbiol 71:7271–7278. doi:10.1128/AEM.71.11.7271-7278.2005

Chowdhury SP, Schmid M, Hartmann A, Tripathi AK (2007) Identification of diazotrophs in the culturable bacterial community associated with roots of *Lasiurus sindicus*, a perennial grass of Thar desert, India. Microb Ecol 54:82–90. doi:10.1007/s00248-006-9174-1

Cocking EC (2003) Endophytic colonization of plant roots by nitrogen-fixing bacteria. Plant Soil 252:169–175. doi:10.1023/A:1024106605806

Compant S, Duffy B, Nowak J, Clement C, Barka EA (2005) Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl Environ Microbiol 71:4951–4959. doi:10.1128/AEM.71.9.4951-4959.2005

Compant S, Kaplan H, Sessitsch A, Nowak J, Barka EA, Clement C (2008) Endophytic colonization of *Vitis vinifera* L. by *Burkholderia phytofirmans* strain PsJN: from the rhizosphere to inflorescence tissues. FEMS Microbiol Ecol 63:84–93. doi:10.1111/j.1574-6941.2007.00410.x

Compant S, Clement C, Sessitsch A (2010a) Plant growth-promoting bacteria in the rhizo- and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. Soil Biol Biochem 42:669–678. doi:10.1016/j.soilbio.2009.11.024

Compant S, van der Heijden MGA, Sessitsch A (2010b) Climate change effects on beneficial plant microorganism interactions. FEMS Microbiol Ecol 73:197–214. doi:10.1111/j.1574-6941.2010.00900.x

Compant S, Mitter B, Colli-Mull JG, Gangl H, Sessitsch A (2011) Endophytes of grapevine flowers, berries, and seeds: identification of cultivable bacteria, comparison with other plant parts, and visualization of niches of colonization. Microb Ecol 62:188–197. doi:10.1007/s00248-011-9883-y

Croes CL, Moens S, van Bastelaere E, Vanderleyden J, Michiels KW (1993) The polar flagellum mediates *Azospirillum brasilense* adsorption to wheat roots. J Gen Microbiol 139:960–967. doi:10.1099/00221287-139-9-2261

de Lorenzo V (1994) Designing microbial system for gene expression in the field. Trends Biotechnol 12:365–371. doi:10.1016/0167-7799(94)90037-X

de Morais RF, Quesada DM, Reis VM, Urquiaga S, Alves BJR, Boddey RM (2012) Contribution of biological nitrogen fixation to elephant grass (*Pennisetum purpureum* Schum.). Plant Soil 356:23–34. doi:10.1007/s11104-011-0944-2

de Salamone IEG, Salvo LPD, Ortega JSE, Sorte PMFB, Urquiaga S, Teixeira KRS (2010) Field response of rice paddy crop to *Azospirillum* inoculation: physiology of rhizosphere bacterial communities and the genetic diversity of endophytic bacteria in different parts of the plants. Plant Soil 336:351–362. doi:10.1007/s11104-010-0487-y

Deng Y, Zhu Y, Wang P, Zhu L, Zheng J, Li R, Ruan L, Peng D, Sun M (2011) Complete Genome Sequence of *Bacillus subtilis* BSn5, an endophytic bacterium of *Amorphophallus konjac* with

antimicrobial activity to plant pathogen *Erwinia carotovora* subsp. *carotovora*. J Bacteriol 193:2070–2071. doi:10.1128/JB.00129-11
Deslippe JR, Egger KN (2006) Molecular diversity of *nifH* genes from bacteria associated with high arctic dwarf shrubs. Microb Ecol 51:516–525. doi:10.1007/s00248-006-9070-8
Diallo MD, Reinhold-Hurek B, Hurek T (2008) Evaluation of PCR primers for universal *nifH* gene targeting and for assessment of transcribed *nifH* pools in roots of *Oryza longistaminata* with and without low nitrogen input. FEMS Microbiol Ecol 65:220–228. doi:10.1111/j.1574-6941.2008.00545.x
Dobereiner J, Reis VM, Paula MA, Olivares F (1993) Endophytic diazotrophs in sugarcane cereals and tuber crops. In: Palacios R, Moor J, Newton WE (eds) New horizons in nitrogen fixation. Kluwer, Dordrecht, pp 671–674
Dong Y, Iniguez AL, Ahmer BMM, Triplett EW (2003) Kinetics and strain specificity of rhizosphere and endophytic colonization by enteric bacteria on seedlings of *Medicago sativa* and *Medicago truncatula*. Appl Environ Microbiol 69:1783–1790. doi:10.1128/AEM.69.3.1783-1790.2003
Dorr J, Hurek T, Reinhold-Hurek B (1998) Type IV pili are involved in plant-microbe and fungus-microbe interactions. Mol Microbiol 30:7–17. doi:10.1046/j.1365-2958.1998.01010.x
dos Reis FB Jr, Reis VM, Urquiaga S, Dobereiner J (2000) Influence of fertilization on the population of diazotrophic bacteria *Herbaspirillum* spp. and *Acetobacter diazotrophicus* in sugar cane (*Saccharum* spp.). Plant Soil 219:153–159. doi:10.1023/A:1004732500983
Duijff BJ, Gianinazzi-Pearson V, Lemanceau P (1997) Involvement of the outer membrane lipopolysaccharides in the endophytic colonization of tomato roots by biocontrol *Pseudomonas fluorescens* strain WCS417r. New Phytol 135:325–334. doi:10.1046/j.1469-8137.1997.00646.x
Eckert B, Weber OB, Kirchhof G, Halbritter A, Stoffels M, Hartmann A (2001) *Azospirillum doebereinerae* sp. nov., a nitrogen-fixing bacterium associated with the C_4-grass *Miscanthus*. Int J Syst Evol Microbiol 51:17–26
Egamberdieva D (2009) Alleviation of salt stress by plant growth regulators and IAA producing bacteria in wheat. Acta Physiol Plant 31:861–864. doi:10.1007/s11738-009-0297-0
Egener T, Martin DE, Sarkar A, Reinhold-Hurek B (2001) Role of a ferredoxin gene cotranscribed with the *nifHDK* operon in N_2 fixation and nitrogenase "switch-off" of *Azoarcus* sp. strain BH72. J Bacteriol 183:3752–3760. doi:10.1128/JB.183.12.3752-3760.2001
Feng Y, Shen D, Song W (2006) Rice endophyte *Pantoea agglomerans* YS19 promotes host plant growth and affects allocations of host photosynthates. J Appl Microbiol 100:938–945. doi:10.1111/j.1365-2672.2006.02843.x
Figueiredo MVB, Martinez CR, Burity HA, Chanway CP (2008) Plant growth-promoting rhizobacteria for improving nodulation and nitrogen fixation in the common bean (*Phaseolus vulgaris* L.). World J Microbiol Biotechnol 24:1187–1193. doi:10.1007/s11274-007-9591-4
Forchetti G, Masciarelli O, Izaguirre MJ, Alemano S, Alvarez D, Abdala G (2010) Endophytic bacteria improve seedling growth of sunflower under water stress, produce salicylic acid, and inhibit growth of pathogenic fungi. Curr Microbiol 61:485–493. doi:10.1007/s00284-010-9642-1
Fouts DE, Tyler HL, DeBoy RT, Daugherty S, Ren Q, Badger JH, Durkin AS, Huot H, Srivastava S, Kothari S, Dodson RJ, Mohamoud Y, Khouri H, Roesch LFW, Krogfelt KA, Struve C, Triplett EW, Mathe BA (2008) Complete Genome sequence of the N_2-fixing broad host range endophyte *Klebsiella pneumoniae* 342 and virulence predictions verified in mice. PLoS Genet 4:e1000141. doi:10.1371/journal.pgen.1000141
Franche C, Lindstrom K, Elmerich C (2009) Nitrogen-fixing bacteria associated with leguminous and non-leguminous plants. Plant Soil 321:35–59. doi:10.1007/s11104-008-9833-8
Fridlender M, Inbar J, Chet I (1993) Biological control of soilborne plant pathogens by a β-1,3-glucanase-producing *Pseudomonas cepacia*. Soil Biol Biochem 25:1211–1221. doi:10.1016/0038-0717(93)90217-Y
Fuentes-Ramirez LE, Caballero-Mellado J, Sepulveda J, Martinez-Romero E (1999) Colonization of sugarcane by *Acetobacter diazotrophicus* is inhibited by high N-fertilization. FEMS Microbiol Lett 29:117–128. doi:10.1111/j.1574-6941.1999.tb 00603.x
Glick BR, Cheng Z, Czarny J, Duan J (2007) Promotion of plant growth by ACC deaminase-producing soil bacteria. Eur J Plant Pathol 119:329–339. doi:10.1007/s10658-007-9162-4

Godfrey SAC, Mansfield JW, Corry DS, Lovell HC, Jackson RW, Arnold DL (2010) Confocal imaging of *Pseudomonas syringae* pv. *phaseolicola* colony development in bean reveals reduced multiplication of strains containing the genomic island PPHGI-1. Mol Plant Microbe Interact 23:1294–1302. doi:10.1094/MPMI-05-10-0114

Gong W, He K, Covington M, Dinesh-Kumar SP, Snyder M, Harmer SL, Zhu YX, Deng XW (2008) The development of protein microarrays and their applications in DNA-protein and protein-protein interaction analyses of *Arabidopsis* transcription factors. Mol Plant 1:27–41. doi:10.1093/mp/ssm009

Gough C, Galera C, Vasse J, Webster G, Cocking EC, Denarie J (1997) Specific flavonoids promote intercellular root colonization of *Arabidopsis thaliana* by *Azorhizobium caulinodans* ORS571. Mol Plant Microbe Interact 10:560–570. doi:10.1094/MPMI.1997.10.5.560

Govindarajan M, Balandreau J, Kwon SW, Weon HY, Lakshminarasimhan C (2008) Effects of the inoculation of *Burkholderia vietnamiensis* and related endophytic diazotrophic bacteria on grain yield of rice. Microb Ecol 55:21–37. doi:10.1007/s00248-007-9247-9

Grange L, Hungria M (2004) Genetic diversity of indigenous common bean (*Phaseolus vulgaris*) rhizobia on two Brazilian ecosystem. Soil Biol Biochem 36:1389–1398. doi:10.1016/j.soilbio.2004.03.005

Gyaneshwar P, James EK, Reddy PM, Ladha JK (2002) *Herbaspirillum* colonization increases growth and nitrogen accumulation in aluminium-tolerant rice varieties. New Phytol 154:131–145. doi:10.1046/j.1469-8137.2002.00371.x

Hallmann J, Quadt-Hallmann A, Mahaffee WF, Kloepper JW (1997) Bacterial endophytes in agricultural crops. Can J Microbiol 43:895–914. doi:10.1139/m97-131

Han J, Choi HK, Lee SW, Orwin PM, Kim J, LaRoe SL, Kim TG, O'Neil J, Leadbetter JR, Lee SY, Hur CG, Spain JC, Ovchinnikova G, Goodwin L, Han C (2011) Complete genome sequence of the metabolically versatile plant growth-promoting endophyte *Variovorax paradoxus* S110. J Bacteriol 193:1183–1190. doi:10.1128/JB.00925-10

Hardoim PR, van Overbeek LS, Elsas JD (2008) Properties of bacterial endophytes and their proposed role in plant growth. Trends Microbiol 16:463–471. doi:10.1016/j.tim.2008.07.008

Hartmann A, Stoffels M, Eckert B, Kirchhof G, Schloter M (2000) Analysis of the presence and diversity of diazotrophic endophytes. In: Triplett EW (ed) Prokaryotic nitrogen fixation: a model system for the analysis of a biological process. Horizon Scientific Press, Wymondham, UK, pp 727–736

Hawes MC, Brigham LA, Wen F, Woo HH, Zhu Y (1998) Function of root border cells in plant health: pioneers in the rhizosphere. Annu Rev Phytopathol 36:311–327. doi:10.1146/annurev.phyto.36.1.311

Hoflich G, Wiehe W, Hecht-Buchholz C (1995) Rhizosphere colonization of different crops with growth promoting *Pseudomonas* and *Rhizobium* bacteria. Microbiol Res 150:139–147. doi:10.1016/S0944-5013(11)80048-0

Hurek T, Reinhold-Hurek B (2003) *Azoarcus* sp. strain BH72 as a model for nitrogen-fixing grass endophytes. J Biotechnol 106:169–178. doi:10.1016/j.jbiotec.2003.07.010

Hurek T, Wagner B, Reinhold-Hurek B (1997) Identification of N_2-fixing plant and fungus associated *Azoarcus* species by PCR-based genomic fingerprints. Appl Environ Microbiol 63:4331–4339

Iniguez AL, Dong Y, Triplett EW (2004) Nitrogen fixation in wheat provided by *Klebsiella pneumoniae* 342. Mol Plant Microbe Interact 17:1078–1085. doi:10.1094/MPMI.2004.17.10.1078

Iniguez AL, Dong Y, Carter HD, Ahmer BMM, Stone JM, Triplett EW (2005) Regulation of enteric endophytic bacterial colonization by plant defenses. Mol Plant Microbe Interact 18:169–178. doi:10.1094/MPMI-18-0169

Islam R, Trivedi P, Madhaiyan M, Seshadri S, Lee G, Yang J, Kim Y, Kim M, Han G, Chauhan PS, Sa T (2010) Isolation, enumeration, and characterization of diazotrophic bacteria from paddy soil sample under long-term fertilizer management experiment. Biol Fertil Soils 46:261–269. doi:10.1007/s00374-009-0425-4

Izquierdo JA, Nusslein K (2006) Distribution of extensive *nifH* gene diversity across physical soil microenvironments. Microb Ecol 51:441–452. doi:10.1007/s00248-006-9044-x

James EK, Olivares FL (1998) Infection and colonization of sugarcane and other gramineous plants by endophytic diazotrophs. Crit Rev Plant Sci 17:77–119. doi:10.1080/07352689891304195

James EK, Olivares FL, de Oliveira ALM, dos Reis Jr FB, da Silva LG, Reis M (2001) Further observations on the interaction between sugarcane and *Gluconoacetobacter diazotrophicus* under laboratory and greenhouse conditions. J Exp Bot 52:747–760. doi:10.1093/jexbot/52.357.747

Jha PN, Kumar A (2007) Endophytic colonization of *Typha australis* by a plant growth-promoting bacterium *Klebsiella oxytoca* strain GR-3. J Appl Microbiol 103:1311–1320. doi:10.1111/j.1365-2672.2007.03383.x

Jha P, Kumar A (2009) Characterization of novel plant growth promoting endophytic bacterium *Achromobacter xylosoxidans* from wheat plant. Microb Ecol 58:179–188. doi:10.1007/s00248-009-9485-0

Jousset A, Rochat L, Lanoue A, Bonkowski M, Keel C, Scheu S (2011) Plants respond to pathogen infection by enhancing the antifungal gene expression of root-associated bacteria. Mol Plant Microbe Interact 24:352–358. doi:10.1094/MPMI-09-10-0208

Kaneko T, Minamisawa K, Isawa T, Nakatsukasa H, Mitsui H, Kawaharada Y, Nakamura Y, Watanabe A, Kawashima K, Ono A, Shimizu Y, Takahashi C, Minami C, Fujishiro T, Kohara M, Katoh M, Nakazaki N, Nakayama S, Yamada M, Tabata S, Sato S (2010) Complete genomic structure of the cultivated rice endophyte *Azospirillum* sp. B510. DNA Res 17:37–50. doi:10.1093/dnares/dsp026

Kannan V, Sureendar R (2009) Synergistic effect of beneficial rhizosphere microflora in biocontrol and plant growth promotion. J Basic Microbiol 49:158–164. doi:10.1002/jobm.200800011

Kaur R, Macleod J, Foley W, Nayudu M (2006) Gluconic acid: an antifungal agent produced by *Pseudomonas* species in biological control of take-all. Phytochemistry 67:595–604. doi:10.1016/j.phytochem.2005.12.011

Khammas KM, Kaiser P (1991) Characterization of a pectinolytic activity in *Azospirillum irakense*. Plant Soil 137:75–79. doi:10.1007/BF02187435

Kiely PD, Haynes JM, Higgins CH, Franks A, Mark GL, Morrissey JP, O'Gara F (2006) Exploiting new systems-based strategies to elucidate plant-bacterial interactions in the rhizosphere. Microb Ecol 51:257–266. doi:10.1007/s00248-006-9019-y

King RJ, Short KA, Seidler RJ (1991) Assay for detection and enumeration of genetically engineered microorganisms which is based on the activity of a deregulated 2,4-dichlorophenoxyacetate monooxygenase. Appl Environ Microbiol 57:1790–1792

Kirchhof G, Reis VM, Baldani JI, Eckert B, Dobereiner J, Hartmann A (1997) Occurrence, physiological and molecular analysis of endophytic diazotrophic bacteria in gramineous energy plants. Plant Soil 194:45–55. doi:10.1023/A:1004217904546

Kirk JL, Beaudette LA, Hart M, Moutoglis P, Klironomos JN, Lee H, Trevors JT (2004) Methods of studying soil microbial diversity. J Microbiol Methods 58:169–188. doi:10.1016/j.mimet.2004.04.006

Knauth S, Hurek T, Brar D, Reinhold-Hurek B (2005) Influence of different *Oryza* cultivars on expression of *nifH* gene pools in roots of rice. Environ Microbiol 7:1725–1733. doi:10.1111/j.1462-2920.2005.00841.x

Kohler J, Hernandez JA, Caravaca F, Roldan A (2008) Plant-growth-promoting rhizobacteria and arbuscular mycorrhizal fungi modify alleviation biochemical mechanisms in water-stressed plants. Funct Plant Biol 35:141–151. doi:10.1071/FP07218

Kovach ME, Phillips RW, Elzer PH, Roop RM, Peterson KM (1994) pBBR1MCS: a broad-host-range cloning vector. Biotechniques 16:800–802

Kovach ME, Elzer PH, Hill DS, Robertson GT, Farris MA, Roop RM, Peterson KM (1995) Four new derivatives of the broad-host range cloning vector pBBR1MCS, carrying antibiotic-resistance cassettes. Gene 166:175–176. doi:10.1016/0378-1119(95)00584-1

Kraepiel AML, Bellenge JP, Wichard T, Morel FMM (2009) Multiple roles of siderophores in free-living nitrogen-fixing bacteria. Biometals 22:573–581. doi:10.1007/s10534-009-9222-7

Krause A, Ramakumar A, Bartels D, Battistoni F, Bekel T, Bosh J, Bohm M, Friedrich F, Hurek T, Krause L, Linke B, McHardy AC, Sarkar A, Schneiker S, Syed AA, Thauer R, Vorholter FJ, Weinder S, Puhler A, Reinhold-Hurek B, Kaiser O, Goesmann A (2006) Complete genome of the mutualistic, N_2-fixing grass endophyte *Azoarcus* sp. strain BH72. Nat Biotechnol 24:1385–1391. doi:10.1038/nbt1243

Ladha JK, Reddy PM (2000) The quest for nitrogen fixation in rice. In: Proceeding of the third working group meeting on assessing opportunities for nitrogen fixation in rice, 9–12 Aug 1999. International Rice Research Institute, Makati City, pp 354

Lery LMS, Coelho A, von Kruger WMA, Goncalves MSM, Santos MF, Valente RH, Santos EO, Rocha SLG, Perales J, Domont GB, Teixeira KRS, Bisch PM (2008) Protein expression profile of *Gluconacetobacter diazotrophicus* PAL5, a sugarcane endophytic plant growth-promoting bacterium. Proteomics 8:1631–1644. doi:10.1002/pmic.200700912

Li CH, Zhao MW, Tang CM, Li SP (2010) Population dynamics and identification of endophytic bacteria antagonistic toward plant-pathogenic fungi in cotton root. Microb Ecol 59:344–356. doi:10.1007/s00248-009-9570-4

Lin L, Guo W, Xing Y, Zhang X, Li Z, Hu C, Li S, Li Y, An Q (2012) The actinobacterium *Microbacterium* sp. 16SH accepts pBBR1-based pPROBE vectors, forms biofilms, invades roots, and fixes N_2 associated with micropropagated sugarcane plants. Appl Microbiol Biotechnol 93:1185–1195. doi:10.1007/s00253-011-3618-3

Lopez-Bucio J, Campos-Cuevas JC, Hernandez-Calderon E, Velasquez-Becerra C, Farias-Rodriguez R, Macias-Rodriguez LI, Valencia-Cantero E (2007) *Bacillus megaterium* rhizobacteria promote growth and alter root-system architecture through an auxin- and ethylene-independent signaling mechanism in *Arabidopis thaliana*. Mol Plant Microbe Interact 20:207–217. doi:10.1094/MPMI-20-2-0207

Lovell CR, Piceno YM, Quattro JM, Bagwell CE (2000) Molecular analysis of diazotroph diversity in the rhizosphere of the smooth Cordgrass, *Spartina alterniflora*. Appl Environ Microbiol 66:3814–3822. doi:10.1128/AEM.66.9.3814-3822.2000

Lucy M, Reed E, Glick BR (2004) Applications of free living plant growth promoting rhizobacteria. Antonie Van Leeuwenhoek 86:1–25. doi:10.1023/B:ANTO. 0000024903.10757.6e

Lugtenberg B, Kamilova F (2009) Plant-growth-promoting Rhizobacteria. Annu Rev Microbiol 63:541–556. doi:10.1146/annurev.micro.62.081307.162918

Lugtenberg BJJ, Dekkers LC, Bloemberg GV (2001) Molecular determinants of rhizosphere colonization by *Pseudomonas*. Annu Rev Phytopathol 39:461–490. doi:10.1146/annurev.phyto.39.1.461

Manefield M, Whiteley AS, Griffiths RI, Bailey MJ (2002) RNA stable isotope probing, a novel means of linking microbial community function to phylogeny. Appl Environ Microbiol 68:5367–5373. doi:10.1128/AEM.68.11.5367-5373.2002

Marulanda A, Barea JM, Azcon R (2009) Stimulation of plant growth and drought tolerance by native microorganisms (AM fungi and bacteria) from dry environments: mechanisms related to bacterial effectiveness. J Plant Growth Regul 28:115–124. doi:10.1007/s00344-009-9079-6

Miche L, Battistoni F, Gernmer S, Belghazi M, Reinhold-Hurek B (2006) Upregulation of jasmonate-inducible defense proteins and differential colonization of roots of *Oryza sativa* cultivars with the endophyte *Azoarcus* sp. Mol Plant Microbe Interact 19:502–511. doi:10.1094/MPMI-19-0502

Mirza MS, Rasul G, Mehnaz S, Ladha JK, So RB, Ali S, Malik KA (2000) Beneficial effects of inoculated nitrogen-fixing bacteria on rice. In: Ladha JK, Reddy PM (eds) The quest for nitrogen fixation in rice. International Rice Research Institute, Manila, pp 191–204

Muthukumarasamy R, Cleenwerck I, Revathi G, Vadivelu M, Janssens D, Hoste B, Gum KU, Park K, Son CY, Sa T, Caballero-Mellado J (2005) Natural association of *Gluconoacetobacter diazotrophicus* and diazotrophic *Acetobacter peroxydans* with wetland rice. Syst Appl Microbiol 28:277–286. doi:10.1016/j.syapm.2005.01.006

Muthukumarasamy R, Kang UG, Park KD, Jeon WT, Park CY, Cho YS, Kwon SW, Song J, Roh DH, Revathi G (2007) Enumeration, isolation and identification of diazotrophs from Korean wetland rice varieties grown with long-term application of N and compost and their short-term inoculation effect on rice plants. J Appl Microbiol 102:981–991. doi:10.1111/j.1365-2672.2006.03157.x

Nishizawa T, Tago K, Oshima S, Hattori M, Ishii S, Otsuka S, Senoo K (2012) Complete genome sequence denitrifying and N_2O-reducing bacterium *Azoarcus* sp. strain KH32C. J Bacteriol 194:1255. doi:10.1128/JB.06618-11

Niu DD, Liu HX, Jiang CH, Wang YP, Jin HL, Guo JH (2011) The plant growth-promoting rhizobacterium *Bacillus cereus* AR156 induces systemic resistance in *Arabidopsis thaliana,* by simultaneously activating salicylate- and jasmonate/ethylene-dependent signaling pathways. Mol Plant Microbe Interact 24:533–542. doi:10.1094/MPMI-09-10-0213

Nogales B, Moore ERB, Llobet-Brossa E, Rossello-Mora R, Amann R, Timmis KN (2001) Combined use of 16S ribosomal DNA and 16S rRNA to study the bacterial community of polychlorinated biphenyl-polluted soil. Appl Environ Microbiol 67:1874–1884. doi:10.1128/AEM.67.4.1874-1884.2001

O'Callaghan KJ, Stone PJ, Hu X, Griffiths DW, Davey MR, Cocking EC (2000) Effects of glucosinolates and flavonoids on colonization of the roots of *Brassica napus* by *Azorhizobium caulinodans* ORS571. Appl Environ Microbiol 66:2185–2191. doi:10.1128/AEM.66.5.2185-2191.2000

Ordentlich A, Elad Y, Chet I (1988) The role of chitinase of *Serratia marcescens* in biocontrol of *Sclerotium rolfsii*. Phytopathology 78:84–88. doi:10.1094/Phyto-78-84

Ovreas L, Torsvik VV (1998) Microbial diversity and community structure in two different agricultural soil communities. Microb Ecol 36:303–315. doi:10.1007/s002489900117

Pandey A, Mann M (2000) Proteomics to study genes and genomes. Nature 405:837–846. doi:10.1038/35015709

Pedrosa FO, Monteiro RA, Wassem R, Cruz LM, Ayub RA, Colauto NB, Fernandez MA, Fungaro MHP, Grisard EC, Hungria M, Madeira HMF, Nodari RO, Osaku CA, Petzl-Erler ML, Terenzi H, Vieira LGE, Steffens MBR, Weiss VA, Pereira LFP, Almeida MIM, Alves LR, Marin A, Araujo LM, Balsanelli E, Baura VA, Chubatsu LS, Faoro H, Favetti A, Friedermann G, Glienke C, Karp S, Kava-Cordeiro V, Raitzz RT, Ramos HJO, Ribeiro EMSF, Rigo LU, Rocha SN, Schwab S, Silva AG, Souza EM, Tadra-Sfeir MZ, Torres RA, Dabul ANG, Soares MAM, Gasques LS, Gimenes CCT, Valle JS, Ciferri RR, Correa LC, Murace NK, Pamphile JA, Patussi EV, Prioli AJ, Prioli SMA, Rocha CLMSC, Arantes OMN, Furlaneto MC, Godoy LP, Oliveira CEC, Satori D, Vilas-Boas LA, Watanabe MAE, Dambros BP, Guerra MP, Mathioni SM, Santos KL, Steindel M, Vernal J, Barbellos FG, Campo RJ, Chueira LMO, Nicholas MF, Pereira-Ferrari L, da Conceicao Silva JL, Gioppa NMR, Margarido VP, Menck-Soares MA, Pinto FGS, Simao RDCG, Takahashi EK, Yates MG, Souza EM (2011) Genome of *Herbaspirillum seropedicae* strain SmR1, a specialized diazotrophic endophyte of tropical grasses. PLoS Genet 7:e1002064. doi:10.1371/journal.pgen.1002064

Pilhofer M, Pavlekovic M, Lee NM, Ludwig W, Schleifer KH (2009) Fluorescence *in situ* hybridization for intracellular localization of *nifH* mRNA. Syst Appl Microbiol 32:186–192. doi:10.1016/j.syapm.2008.12.007

Pleban S, Chernin L, Chet I (1997) Chitinolytic activity of an endophytic strain of *Bacillus cereus*. Lett Appl Microbiol 25:284–288. doi:10.1046/j.1472-765X.1997.00224.x

Preston GM, Bertrand N, Rainey PB (2001) Type III secretion in plant growth-promoting *Pseudomonas fluorescens* SBW25. Mol Microbiol 41:999–1014. doi:10.1046/j.1365-2958.2001.02560.x

Prieto P, Schiliro E, Maldonado-Gonzalez MM, Valderrama R, Barroso-Albarracin JB, Mercado-Blanco J (2011) Root hairs play a key role in the endophytic colonization of olive roots by *Pseudomonas* spp. with biocontrol activity. Microb Ecol 62:435–445. doi:10.1007/s00248-011-9827-6

Rabus R, Kube M, Heider J, Beck A, Heitmann K, Widdel F, Reinhardt R (2005) The genome sequence of an anaerobic aromatic-degrading denitrifying bacterium strain EbN1. Arch Microbiol 183:27–36. doi:10.1007/s00203-004-0742-9

Rajkumar M, Ae N, Prasad MNV, Freitas H (2010) Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. Trends Biotechnol 28:142–149. doi:10.1016/j.tibtech.2009.12.002

Ramos HJO, Roncato-Maccari LDB, Souza EM, Soares-Ramos JR, Hungria M, Pedrosa FO (2002) Monitoring *Azospirillum*-wheat interactions using the *gfp* and *gus*A genes constitutively expressed from a new broad-host range vector. J Biotechnol 97:243–252. doi:10.1016/S0168-1656(02)00108-6

Rasche F, Lueders T, Schloter M, Schaefer S, Buegger F, Gattinger A, Hood-Nowotny RC, Sessitsch A (2009) DNA-based stable isotope probing enables the identification of active bacterial endophytes in potatoes. New Phytol 181:802–807. doi:10.1111/j.1469-8137.2008.02744.x

Raymond J, Siefert JL, Staples CR, Blankenship RE (2004) The natural history of nitrogen fixation. Mol Biol Evol 21:541–554. doi:10.1093/molbev/msh047

Reinhold-Hurek B, Hurek T (1998) Interactions of gramineous plants with *Azoarcus* spp. and other diazotrophs: identification, localization and perspectives to study their function. Crit Rev Plant Sci 17:29–54. doi:10.1080/07352689891304186

Reinhold-Hurek B, Hurek T (2011) Living inside plants: bacterial endophytes. Curr Opin Plant Biol 14:435–443. doi:10.1016/j.pbi.2011.04.004

Reinhold-Hurek B, Maes T, Gemmer S, Montagu MV, Hurek T (2006) An endoglucanase is involved in infection of rice roots by the not-cellulose-metabolizing endophyte *Azoarcus* Sp. strain BH72. Mol Plant Microbe Interact 19:181–188. doi:10.1094/MPMI-19-01810

Reuber TL, Long S, Walker GC (1991) Regulation of *Rhizobium meliloti* exo genes in free-living cells and in planta examined by using TnphoA fusions. J Bacteriol 173:426–434

Rochat L, Pechy-Tarr M, Baehler E, Maurhofer M, Keel C (2010) Combination of fluorescent reporters for simultaneous monitoring of root colonization and antifungal gene expression by a biocontrol Pseudomonad on cereals with Flow cytometry. Mol Plant Microbe Interact 23:949–961. doi:10.1094/MPMI-23-7-0949

Roe MR, Griffin TJ (2006) Gel-free mass spectrometry-based high throughput proteomics: tools for studying biological response of proteins and proteomes. Proteomics 6:4678–4687. doi:10.1002/pmic.200500876

Rosado AS, Duarte GF, Seldin L, Elsas JDV (1998) Genetic Diversity of *nifH* gene sequences in *Paenibacillus azotofixans* strains and soil samples analyzed by Denaturing gradient gel electrophoresis of PCR-amplified gene fragments. Appl Environ Microbiol 64:2770–2779

Rosenblueth M, Martinez-Romero E (2006) Bacterial endophytes and their interactions with hosts. Mol Plant Microbe Interact 19:827–837. doi:10.1094/MPMI-19-0827

Rothballer M, Eckert B, Schmid M, Fekete A, Schloter M, Lehner A, Pollmann S, Hartmann A (2008) Endophytic root colonization of gramineous plants by *Herbaspirillum frisingense*. FEMS Microbiol Ecol 66:85–95. doi:10.1111/j.1574-6941.2008.00582.x

Ryan RP, Germaine K, Franks A, Ryan DJ, Dowling DN (2008) Bacterial endophytes: recent developments and applications. FEMS Microbiol Lett 278:1–9. doi:10.1111/j.1574-6968.2007.00918.x

Ryu RJ, Patten CL (2008) Aromatic amino acid-dependent expression of indole-3-pyruvate decarboxylase is regulated by *TyrR* in *Enterobacter cloacae* UW5. J Bacteriol 190:7200–7208. doi:10.1128/JB.00804-08

Ryu CM, Farag MA, Hu CH, Reddy MS, Kloepper JW, Pare PW (2004) Bacterial volatiles induce systemic resistance in *Arabidopsis*. Plant Physiol 134:1017–1026. doi:10.1104/pp.103.026583

Saleh SS, Glick BR (2001) Involvement of *gacS* and *rpoS* in enhancement of the plant growth-promoting capabilities of *Enterobacter cloacae* CAL2 and UW4. Can J Microbiol 47:698–705. doi:10.1139/cjm-47-8-698

Schloss PD, Handelsman J (2003) Biotechnological prospects from metagenomics. Curr Opin Biotechnol 14:303–310. doi:10.1016/S0958-1669(03)00067-3

Schmidt MA, Souza EM, Baura V, Wassem R, Yates MG, Pedrosa FO, Monteiro RA (2011) Evidence for the endophytic colonization of *Phaseolus vulgaris* (common bean) roots by the diazotroph *Herbaspirillum seropedicae*. Braz J Med Biol Res 44:182–185. doi:10.1590/S0100-879X2011007500004

Schwab S, Ramos HJ, Souza EM, Pedrosa FO, Yates MG, Chubatsu LS, Rigo LU (2007) Identification of NH_4^+-regulated genes of *Herbaspirillum seropedicae* by random insertional mutagenesis. Arch Microbiol 187:379–386. doi:10.1007/s00203-006-0202-9

Serrato RV, Sassaki GL, Cruz LM, Carlson RW, Muszynski A, Monteiro RA, Pedrosa FO, Souza EM, Iacomini M (2010) Chemical composition of lipopolysaccharides isolated from various endophytic nitrogen-fixing bacteria of the genus *Herbaspirillum*. Can J Microbiol 56:342–347. doi:10.1139/W10-011

Setubal JC, dos Santos P, Goldman BS, Ertesvag H, Espin G, Rubio LM, Valla S, Almeida NF, Balasubramanian D, Cromes L, Curatti L, Du Z, Godsy E, Goodner B, Hellner-Burris K, Hernandez JA, Houmiel K, Imperial J, Kennedy C, Larson TJ, Latreille P, Ligon LS, Lu J, Marek M, Miller NM, Norton S, O'Carroll IP, Paulsen I, Raulfs EC, Roemer R, Rosser J, Segura D, Slater S, Stricklin SL, Studholme D, Sun J, Viana CJ, Wallin E, Wang B, Wheller C, Zhu H, Dean DR, Dixon R, Wood D (2009) Genome sequence of *Azotobacter vinelandii*, an obligate aerobe specialized to support diverse anaerobic metabolic processes. J Bacteriol 191:4534–4545. doi:10.1128/JB.00504-09

Sevilla M, Kennedy C (2000) Genetic analysis of nitrogen fixation and plant-growth stimulating properties of *Acetobacter diazotrophicus*, an endophyte of sugarcane. In: Triplett EW (ed) Prokaryotic Nitrogen fixation: a model system for the analysis of biological process. Horizon Scientific Press, Wymondham, UK, pp 737–760

Singh MK, Singh DP, Mesapogu S, Babu BK, Bontemps C (2011) Concomitant colonization of *nifH* positive endophytic *Burkholderia* sp. in rice (*Oryza sativa* L.) promotes plant growth. World J Microbiol Biotechnol 27:2023–2031. doi:10.1007/s11274-011-0664-z

Somers E, Srinivasan M, Vanderleyden J (2004) Rhizosphere bacterial signalling: a love parade beneath our feet. Crit Rev Microbiol 30:205–240

Spaepen S, Vanderleyden J, Remans R (2007) Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbiol Rev 31:425–448. doi:10.1111/j.1574-6976.2007.00072.x

Sprent JI, de Faria SM (1988) Mechanisms of infection of plants by nitrogen fixing organisms. Plant Soil 110:157–165. doi:10.1007/BF02226795

Strobel G (2006) Harnessing endophytes for industrial microbiology. Curr Opin Microbiol 9:240–244. doi:10.1016/j.mib.2006.04.001

Tadra-Sfeir MZ, Souza EM, Faoro H, Muller-Santos M, Baura VA, Tuleski TR, Rigo L, Yates MG, Waseem R, Pedrosa FO, Monteiro RA (2011) Naringenin regulates expression of genes involved in cell wall synthesis in *Herbaspirillum seropedicae*. Appl Environ Microbiol 77:2180–2183. doi:10.1128/AEM.02071-10

Taghavi S, van der Lelie D, Hoffman A, Zhang YB, Walla MD, Vangronsveld J, Newman L, Monchy S (2010) Genome sequence of the plant growth promoting endophytic bacterium *Enterobacter* sp. 638. PLoS Genet 6:e1000943. doi:10.1371/journal. pgen.1000943

Taule C, Mareque C, Barlocco C, Hackembruch F, Reis VM, Sicardi M, Battistoni F (2012) The contribution of nitrogen fixation to sugarcane (*Saccharum officinarum* L.), and the identification and characterization of part of the associated diazotrophic bacterial community. Plant Soil 356:35–49. doi:10.1007/s11104-011-1023-4

Terakado-Tonooka J, Ohwaki Y, Yamakawa H, Tanaka F, Yoneyama T, Fujihara S (2008) Expressed *nifH* genes of endophytic bacteria detected in field-grown sweet potatoes (*Ipomoea batatas* L.). Microbes Environ 23:89–93. doi:10.1264/jsme2.23.89

Tjamos SE, Flemetakis E, Paplomatas EJ, Katinakis P (2005) Induction of resistance to *Verticillium dahlia* in *Arabidopsis thaliana* by the biocontrol agent K-165 and pathogenesis-related proteins gene expression. Mol Plant Microbe Interact 18:555–561. doi:10.1094/MPMI-18-0555

Ueda T, Suga Y, Yahiro N, Matsuguchi T (1995a) Remarkable N_2-fixing bacterial diversity detected in rice roots by molecular evolutionary analysis of *nifH* gene sequences. J Bacteriol 177:1414–1417

Ueda T, Suga Y, Yahiro N, Matsuguchi T (1995b) Genetic diversity of N_2-fixing bacteria associated with rice roots by molecular evolutionary analysis of *nifD* library. Can J Microbiol 41:235–240

Urquiaga S, Xavier RP, de Morais RF, Batista RB, Schultz N, Leite JM, Sa JM, Barbosa KP, de Resende AS, Alves BJR, Boddey RM (2012) Evidence from field nitrogen balance and ^{15}N natural abundance data for the contribution of biological N_2-fixation to Brazilian sugarcane varieties. Plant Soil. doi:10.1007/s11104-011-1016-3

van Loon LC (2007) Plant responses to plant growth-promoting rhizobacteria. Eur J Plant Pathol 119:243–254. doi:10.1007/s10658-007-9165-1

van West P, Morris BM, Reid B, Appiah AA, Osborne MC, Campbell TA, Shepherd SJ, Gow NAR (2002) Oomycete plant pathogens use electric fields to target roots. Mol Plant Microbe Interact 15:790–798. doi:10.1094/MPMI.2002.15.8.790

Venieraki A, Dimou M, Pergalis P, Chatzipavlidis I, Katinakis P (2011) The genetic diversity of culturable nitrogen-fixing bacteria in the rhizosphere of wheat. Microb Ecol 61:277–285. doi:10.1007/s00248-010-9747-x

Verhagen BW, Trotel-Aziz P, Couderchet M, Hofte M, Aziz A (2010) *Pseudomonas* spp. induced systemic resistance to *Botrytis cinerea* is associated with induction and priming of defence responses in grapevine. J Exp Bot 61:249–260. doi:10.1093/jxb/erp295

Verma SC, Singh A, Chowdhury SP, Tripathi AK (2004) Endophytic colonization ability of two deep-water rice endophytes, *Pantoea* sp. and *Ochrobactrum* sp. using green fluorescent protein reporter. Biotechnol Lett 26:425–429. doi:10.1023/B:BILE.0000018263.94440.ab

Voorhorst WGB, Eggen RIL, Luesink EJ, de Vos WM (1995) Characterization of the *cel*B gene coding for b-glucosidase from the hyperthermophilic archaeon *Pyrococcus furiosus* and its expression and site-directed mutation in *Escherichia coli*. J Bacteriol 177:7105–7111

Webster G, Gough C, Vasse J, Bathchelor CA, O'Callaghan KJ, Kothari SL, Davey MR, Denarie J, Cocking EC (1997) Interactions of rhizobia with rice and wheat. Plant Soil 194:115–122. doi:10.1023/A:1004283819084

Weilharter A, Mitter B, Shin MV, Chain PSG, Nowak J, Sessitsch A (2011) Complete genome sequence of the plant growth-promoting endophyte *Burkholderia phytofirmans* strain PsJN. J Bacteriol 193:3383–3384. doi:10.1128/JB.05055-11

West ER, Cother EJ, Steel CC, Ash GJ (2010) The characterization and diversity of bacterial endophytes of grapevine. Can J Microbiol 56:209–216. doi:10.1139/W10-004

Widmer F, Shaffer BT, Porteous LA, Seidler RJ (1999) Analysis of *nifH* gene pool complexity in soil and little at Douglas fir forest sites of the Orgegon cascade mountain range. Appl Environ Microbiol 65:374–380

Winstanley C, Morgan JA, Pickup RW, Saunders JR (1991) Use of a *xylE* marker gene to monitor survival of recombinant *Pseudomonas putida* populations in lake water by culture on nonselective media. Appl Environ Microbiol 57:1905–1913

Xing T, Quellet T, Miki BL (2004) Towards genomic and proteomic studies of protein phosphorylation in plant-pathogen interactions. Trends Plant Sci 7:224–230. doi:10.1016/S1360-1385(02)02255-0

Yan Y, Yang J, Dou Y, Chen M, Ping S, Peng J, Lu W, Zhnag W, Yao Z, Li H, Liu W, He S, Geng L, Zhnag X, Yang F, Yu H, Zhan Y, Li D, Lin Z, Wang Y, Elmerich C, Lin M, Jin Q (2008) Nitrogen fixation island and rhizosphere competence traits in the genome of root-associated *Pseudomonas stutzeri* A1501. Proc Natl Acad Sci 105:7564–7569. doi:10.1073/pnas.0801093105

Yang J, Kloepper JW, Ryu C (2009) Rhizosphere bacteria help plants tolerate abiotic stress. Trends Plant Sci 14:1–4. doi:10.1016/j.tplants.2008.10.004

Yanni YG, Rizk RY, Corich V, Squartini A, Ninke K, Phillip-Hollingswoth S, Orgambide G, de Bruijn F, Stoltzfus J, Buckley D, Schmidt TM, Mateos PF, Ladha JK, Dazzo FB (1997) Natural endophytic association between *Rhizobium leguminosarum* bv. *trifolii* and rice roots and assessment of its potential to promote rice growth. Plant Soil 194:99–114. doi:10.1023/A:1004269902246

Yanni YG, Rizk RY, Abd El-Fattah FK, Squartini A, Corich V, Giacomini A, de Bruijn F, Rasemaker J, Maya-Flores J, Ostrom P, Vega-Hernandez M, Hollingsworth RI, Martinez-Molina E, Mateos P, Velazequez E, Wopereis J, Triplett E, Umali-Gracia M, Anarna JA, Rolfe BG, Ladha JK, Hill J, Mujoo R, Ng PK, Dazzo FB (2001) The beneficial plant growth-promoting association of *Rhizobium leguminosarum* bv. *trifolii* with rice roots. Aust J Plant Physiol 28:845–870. doi:10.1071/PP01069

Zehr JP, Mellon MT, Zani S (1998) New nitrogen-fixing microorganisms detected in oligotrophic oceans by amplification of nitrogenase (*nifH*) genes. Appl Environ Microbiol 64:3444–3450

Zehr JP, Jenkins BD, Short SM, Steward GF (2003) Nitrogenase gene diversity and microbial community structure: a cross-system comparison. Environ Microbiol 5:539–554. doi:10.1046/j.1462-2920.2003.00451.x

Zeyaullah M, Kamli MR, Islam B, Atif M, Benkhayal FA, Nehal M, Rizvi MA, Ali A (2009) Metagenomics-an advanced approach for noncultivable micro-organisms. Biotechnol Mol Biol Rev 4:49–54

Zhang X, Candas M, Griko NB, Taussig R, Bulla LA Jr (2006) A mechanism of cell death involving an adenylyl cyclase/PKA signaling pathway is induced by the Cry1Ab toxin of *Bacillus thuringiensis*. Proc Natl Acad Sci 103:9897–9902. doi:10.1073/pnas.0604017103

Zhang H, Xie X, Kim MS, Kornyeyev DA, Holaday S, Pare PW (2008) Soil bacteria augment *Arabidopsis* photosynthesis by decreasing glucose sensing and abscisic acid levels in planta. Plant J 56:264–273. doi:10.1111/j.1365-313X.2008.03593.x

Zhu B, Liu H, Tian WX, Fan XY, Li B, Zhou XP, Jin GL, Xie GL (2012) Genome sequence of *Stenotrophomonas maltophilia* RR-10, isolated as an endophyte from rice root. J Bacteriol 194:1280–1281. doi:10.1128/JB.06702-11

Crop and Soil Management Zone Delineation Based on Soil Property or Yield Classification

Michael S. Cox and Patrick D. Gerard

Abstract Site-specific crop and soil management has the potential to increase crop production efficiency and decrease environmental impact. Determination of management zones is essential for site-specific management to be successful. These zones delineate portions of the field with the same yield limiting factors such that these factors can be managed independent of the rest of the field. However, spatial and temporal variability can confound zone delineation, thus stability of the zones must be ensured. Zone delineation has typically followed one of two methods. The first method defines areas of the field using the variability in crop yield while the second uses the variability of soil properties that might influence yield. Cluster analysis has commonly been used as a pattern recognition method to identify stable zones based on yield data. After the yield zones have been classified, soil properties can be related to the zones and be managed on a site specific basis. These soil properties can be found using directed sampling, intense soil survey data or some form of remote sensing. The second method of determining management zones used the spatial variability of soil physical or chemical properties to define the management areas. The high cost of intensive soil sampling needed for this method proved to be a limitation. Soil electrical conductivity (EC_a) has emerged as an inexpensive way of identifying soil variability and has been used in many studies to relate soil differences to crop yield. These differences were typically related to changes in soil series or soil water availability. While zone delineation has shown to be successful in

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many crops, there is little information available on zones changes between crops in a rotation.

The objectives of this study were to determine if yield classes change between crops within a soybean-corn-soybean rotation and whether soil properties can predict the yield classes or the year-to-year changes. A percentile classification method was used to categorize 3 years of yield in two fields into low, medium and high yield classes. There was significant agreement in yield classifications between years in both fields. Linear discriminant analysis predicted yield class based on soil properties correctly 10–66% of the time in Field 1 and in Field 233–100% of the time. There was little consistency in soil properties used to make class predictions in Field 1 but soil apparent electrical conductivity did appear in four of the six discriminant functions. Field 2 had a great deal of consistency in soil parameters that classified yield from year to year. Soil test K and Mg were present in all discriminant functions while Ca, P, texture and EC_a were present in five of the six. Of the years where at least one variable was found to be useful in discrimination, EC_a was present in both fields. This suggests that the management zones based on these soil properties may increase production efficiency.

Keywords Soil fertility • Plant/soil interactions • Site-specific management

1 Introduction

Sustainable agriculture depends on the efficient use of soil inputs. Site specific crop management (SSCM) attempts to optimize production inputs based on the variability present in the field in such a way as to maximize economic return while minimizing the potential for environmental impact and assuring crop quality and traceability (Godwin et al. 2002). This process of developing an SSCM plan considers many different crop growth factors and the effect they have on crop yield and quality and can involve a number of decision support tools. Crop and soil management zone delineation is an important part of this process. Crop and/or soil management zones are intended to identify within-field areas that have the same or similar yield limiting characteristics such that they can be managed independently. These zones are intended to maximize the efficiency of inputs by only using the required amounts in those areas in need. However, spatial and temporal variability in crop growth and yield patterns can add to the difficulty in determining yield-limiting soil properties (Huggins and Alderfer 1995; Lamb et al. 1997; Schepers et al. 2004; Diker et al. 2004). Zone delineation techniques have typically followed one of two approaches. One option has been to identify zones from crop yield maps collected over a number of years. This method collects multiple years of yield history from a field or fields and then subjects the data to some form of pattern recognition analysis (Lark and Stafford 1997; Grenzedörffer and Gebbers 2001). The second option is to identify soil factors in the field which influence yield (Stafford et al. 1996). This approach investigates the spatial variability of soil chemical and physical properties in the field and attempts to delineate zones appropriate for some form of variable rate management. A potential limitation with this option is the amount of data required for zone delineation. In a review of soil variability studies, Beckett and Webster (1971) found that significant spatial correlation between samples can be tens of meters for some physical and chemical properties but that up to a half of that variability can occur within 1 m^2. Hence, the cost of measuring soil variability by traditional sampling, i.e. cores, can be prohibitive.

2 Defining Soil Management Zones Using Crop Yield

Several methods using data clustering have been proposed to identify zones based on yield patterns. Cluster analysis in some form has come to dominate the analysis methods. Clustering assigns data points into groups such that objects within the same group are more alike than those in other groups. When used in SSCM, the data points can represent some crop or soil characteristic that limits or potentially limits crop growth or yield. For instance, nutrient values may be clustered to determine field areas where growth or yield may be limited due to deficiencies. The data points may also be measures of growth or yield in order to determine limiting factors. In this scenario, yield values may be clustered to define lower or higher yielding areas of a field. These areas are then sampled for various crop growth factors to determine which factor or factors are limiting yield. Cluster analysis has been used in a number of studies to define SSCM management zones.

Lark and Stafford (1997) used a multivariate clustering method and 3 years of winter barley (*Hordeum vulgare*) yield data and found that patterns could be determined using this method. This study collected yield data from a 6 ha field located at the Silsoe Research Institute from 1993 to 1995. Data for local weather, soil series information and soil moisture, organic matter, pH and mineral N at two depths from around the field were also collected. The yield data was subjected to non-hierarchical "fuzzy" clustering as a pattern recognition method. This method resulted in two distinctive clusters representing high yield that were associated with individual soil series. It also found that soil moisture was related to one of these high yielding clusters. A third cluster was also found to be related to soil moisture. The researchers concluded that fuzzy classification could identify patterns of between season yield variation and the patterns were spatially coherent and the differences between the groups could be attributed to soil moisture.

Most research that uses crop yield to determine management zones measures yield values with yield monitors. Boydell and McBratney (2002) took a different approach using remotely sensed cotton yield estimates to develop stable yield areas applicable to management zones. Estimating yield from remotely sensed data is not limited to SSCM applications. Bauer (1975) discusses remote sensing applications in determining yield and distribution of crops. In the Boydell and McBratney study, the authors used Landsat TM data for two fields collected over 11 consecutive years. The remotely sensed data was analyzed for temporal stability using a fuzzy classification method discussed by de Gruijter and McBratney (1988). The classification found two options for each field. One option used two clusters (management zones) of spatially stable data while the other found with seven to nine clusters. It was noted that climatic factors (i.e. dryland years vs. irrigated) affected overall cluster means and could affect the presence of outliers. They concluded that stable yield patterns may emerge from multi-year estimates, water management resulted in different behavior of yield patterns, and 5-years of data gave reasonably stable estimates of yield zones.

In contrast to the findings of Lark and Stafford (1997) and Boydell and McBratney (2002), Dobermann et al. (2003) compared the fuzzy classification methodology used by Boydell and McBratney (2002), a hierarchal clustering method (Johnsoan and Wichern 1998) and four empirical methods to classify and delineate yield zones in two corn/soybean fields over 6 years. The empirical methods included one published method (Blackmore 2000) and three protocols based on the frequency distribution of the yield data. The authors concluded that the empirical methods accounted for less than 54% of the yield variability and were unable to define the high-yielding areas of the fields. The cluster analysis did only slightly better accounting for 60–66% of yield variation and there was little difference between the cluster analysis methods. However, the fuzzy-K classification method of de Gruijter and McBratney (1988) accounted for less yield variability if used with average yield or yearly yield data than if used with average yield and its standard deviation.

Cluster analysis coupled with discriminant analysis has also been used to determine potential management zones. Discriminant analysis has been used in a number of studies to relate categorical data (yield data) to soil or topographical data (Jaynes et al. 2003; Ping et al. 2005; Cox and Gerard 2007). Discriminant analysis can be used to determine relationships between categorical dependent variables (categories are known *a priori*) and quantitative independent variables (Hair et al. 1987). Jaynes et al. (2003) classified 224 yield plots into clusters and then predicted which yield cluster each plot would fit into based on 6 years of corn (*Zea mays*) yield data. They concluded that this method could be used to determine management zones if inputs vary among yield clusters.

Ping et al. (2005) found similar results in irrigated cotton (*Gossypium hirsutum*). They used cluster analysis to group lint yield and soil properties into high and low yield classes. This study found that soil pH, extractable Ca and Mg, K saturation, clay content and soil N:P ratio were related to cotton yield class, and two potential management zones were developed from these soil properties.

Empirical classification methods have also been used to determine yield patterns. Blackmore (2000) used 6 years of both winter wheat (*Triticum aestivum*) and oil seed rape (*Brassica napus* L.). yield data. The coefficient of variation (CV) determined for each data point mean over the years was used to determine the temporal stability. Coefficients of variation for the standardized yield means greater than 30% were regarded as temporally unstable when considering all crop years and removed from further analysis. The important outcome of this study was that stable yield zones could be identified even where the crop changed.

Cox and Gerard (2007) used Blackmore's method to define four yield classes and identify soil and or topographical properties that best predicted yield class for three fields growing soybean over 3 years. Cox and Gerard (2010) also used this method when attempting to determine yield zones in a soybean-rice rotation. They found that this method could be used to define yield zones but there was little consistency in the zones from year to year. These studies showed that multi-year yield data could be classified into zones and the zones related to soil or topographical properties.

3 Defining Soil Management Zones Using Soil Properties

Many attempts have been made to identify soil management zones based on soil chemical or physical properties (Kitchen et al. 1999; King et al. 2005; Cox et al. 2005, 2006). A recurring issue throughout these investigations however is the number of samples required to adequately define the variability in the soil properties is high and the cost becomes prohibitive to use in production systems. Hence alternative methods of delineation soil variability have been investigated.

Kitchen et al. (1999) explored the use of inexpensive and accurate methods for measuring soil properties that could aid in the interpretation of yield maps and aid in making SSCM decisions. In this study, the investigators chose four fields with claypan soils and measured EC_a in a vertical dipole mode resulting in a measurement depth of approximately 1.83 m (root zone). At the time of EC_a data collection, the depth of the Bt horizon (claypan depth) was also measured. The fields in question were also soil-sampled based on a grid with each grid size appropriate to the field being sampled to a depth of 15 cm and analyzed for P, K, Ca, Mg, CEC, organic matter pH, and neutralizable acidity. Grain yield was measured using a commercial yield monitor mounted on combines. Soil EC_a was converted to topsoil thickness through regression similar to Doolittle (1994). Kitchen et al. found little correlation between yield and soil measurements but did find that EC_a could be used to aid in management zone interpretation. High EC_a measurements were indicative of droughty soil areas with shallow topsoils, thus soil management though could increase water infiltration and/or water conservation would be beneficial.

Fraisse et al. (2001) used an unsupervised classification of topographic attributes (slope, profile curvature, tangential curvature, and compound topographic index (CTI)) and soil EC_a to define soil management areas then used yield data to determine the zone applicability. The soil data was subjected to principle component analysis to determine which variables were the major contributors to variability and hence, used in the unsupervised classification. The PCA results indicated elevation and soil EC_a as well as slope should be used in the unsupervised classification but that the slope variable may have less of an effect on the zone delineation. The classification procedure was used to delineate two to six management zones in each field. Yield data was collected over a period of 5 years for two fields and included three different crops. After normalizing the yield data, the maximum decrease in yield variance was explained by five zones when water stress occurred in the first field. However the number of zones required to decrease the yield variance was temporally (crop grown and weather) dependent.

In 2002, Anderson-Cook et al. attempted to classify mid-Atlantic coastal plain soil types within fields using EC_a and crop yield maps. They reasoned that soil types could serve as delineators for variable rate application maps (i.e. management zones) but using an Order 1 Soil Survey (prepared at scales ranging from 1:2,500 to 1:10,000) at the scale needed for this use would be prohibitively expensive. In this particular study, the soils at the experimental site were determined by an Order 1 soil survey (Nicholson et al. 1998). Georeferenced soil EC_a was collected in the horizontal dipole model (measurement depth of approximately 0.75 m). Yield for a variety of crops was collected with a commercial combine equipped with a yield monitor. Correct classification rates delineating soil types depended on crop and year but ranged from 85.3 to 95.5% when EC_a alone was used for classification, 83–88.8% when crop yield alone was used for classification and 90.3–96.4% when both EC_a and crop yield were used for classification. The researchers concluded EC_a and crop yield could be used to delineate broad differences in soil types (management zones) but that classification of more precise soil types was more difficult.

The findings of Anderson-Cook et al. (2002) were supported in the 2003 study of Earl et al. The objective of this study was to rate methods for determining spatial variability in soil series, fertility, and physical properties. Soil series maps were generated from soil samples collected on a 100 m grid on five representative fields in England and Wales. Soil pits were also used for profile determination in selected spots in each field. The authors then collected EC_a data and soil samples gathered with a mechanized soil sampler. In addition, soil samples were collected on a 50 m grid for nutrient analysis. Earl et al. found that the conventional method of soil sampling was appropriate for determining soil series at the location of sampling but could not delineate soil series boundary. The boundaries could be found when using the mechanized soil sampling method but the time required for core analysis was prohibitive. Soil variation found from the EC_a data appeared to reflect the variation in soil series and was cost effective method of measuring soil variability. A limitation of this study was that no crop yield data was included to determine if this soil variability contributed to yield limitations and would, thus serve as management zone delineation method.

The limitation of the Earl et al. (2003) study was address in a separate study by Taylor et al. (2003). Three fields used in Earl et al. (2003) were harvested for 2–3 years using a commercial combine equipped with a yield monitor. Soil EC_a was measured when the fields were at or near field capacity and detailed soil surveys were conducted using a tractor mounted soil sampling device. Soil EC_a variation was highly correlated to variations in soil series. Soil variability and yield variability agreement was higher in dry seasons while in wetter years the longer-range variability was smaller. The researchers did find that multivariate clustering of yield and EC_a data identified zones that coincided with differences in soil nutrient levels and yield differences in the following season. This could result in nutrient management zones for directed soil sampling.

A 2006 study in northeastern Colorado took a different approach and compared two management zone systems, one based on yield, the other based on soil color. Hornung et al. (2006) developed a delineation technique using bare soil imagery, topography and a producer's experience with the field and compared that with a technique using bare soil imagery, soil organic matter, soil cation exchange capacity, soil texture, and yield data from the previous year. Management zones were delineated based on both methods and N applied in accordance with the zones. Grain yields from the technique using bare soil imagery, topography and a producer's experience were significantly different between zones and were consistently higher than the more complex method. Thus, Hornung et al. considered the technique based on fewer inputs was relatively better than the yield based technique

4 Objectives

While many of the studies outlined above used multiple years of data and were successful in delineating management zones, few considered the effect of crop rotation and how management zones might change temporally from crop to crop. Cox and Gerard (2010) attempted to determine and predict management zones from year to year in a soybean (*Glycine max*)- rice (*Oryza sativa*) rotation and had limited success. One of the possible limitations of this study was the drastic change in cultural conditions as the rice was grown under flooded conditions while the soybean was grown under irrigated conditions. Hence the purpose of this study was to determine and predict management zones temporally using crops grown under similar cultural conditions.

The specific objectives of this study were to determine: (1) if yield classes change between crops within a soybean-corn-soybean rotation and (2) whether soil properties can predict the yield classes or the year-to-year changes.

5 Materials and Methods

5.1 Site Description

The study was conducted on two commercial fields (Field 1: 23 ha and Field 2: 17 ha) located near Shelby, Mississippi (33° 57′ 03.72 N, 90° 46′ 04.65 W). Field 1 is comprised of approximately 37% Forestdale (fine, smectitic, thermic Typic Endoaqualf), 35% Dubbs (fine-silty, mixed, active, thermic Typic Hapludalf), 14% Dowling (very-fine, smectitic, nonacid, thermic Vertic Endoaquept) and 13% Dundee (fine-silty, mixed, active, thermic Typic Endoaqualf) soils (Soil Survey

Staff 2011). Field 2 consists of approximately 40% Dundee, 39% Dowling and 20% Forestdale soils (Soil Survey Staff 2011). The weather conditions during this study were generally wet in the spring with dry to drought-like conditions common during the summer. Both fields were irrigated during the dry portions of the growing season. These fields were in a soybean (2007, 2009) – corn (2008) rotation during the course of this study.

5.2 Yield Collection and Analysis

A commercial yield monitor configured to record data at 1 s intervals and a differentially corrected geographical positioning system (DGPS) receiver mounted on a field combine were used to measure crop yields over the course of this study. The 1 s measurement interval resulted in a yield sample of approximately 1.5 by 6 m with the center point of the rectangle recorded as the yield location. Spatial and temporal changes in the yield data were then analyzed following Blackmore (2000): To remove the effects of years and create a dataset that could be considered relative yield, each yield value was standardized by dividing it by the yearly average for that field and then multiplying by 100 (Dobermann et al. 2003). ArcView geographical information system (GIS) (ESRI, Redlands, CA, USA) was then used to overlay the standardized yield data for each year. The three standardized yield values were averaged for each yield location in the field to give one mean standardized yield value. Coefficients of variation (CV) for each yield location in the field were determined as described by Blackmore (2000) to determine the amount of temporal variability in the data. Yield locations in a field with a CV >30% were considered to be unstable and removed from further analysis (Wollenhaupt et al. 1997). The majority of those points removed were located at the field edges. The remaining yearly yield data were separated into three yield classes according to Cox and Gerard (2010). Standardized yield data less than or equal to the 33rd percentile were classified as low yielding, those with values that were greater than the 33rd percentile but less than or equal to the 66th percentile were classified as average yielding, and those greater than the 66th percentile were classified as high yielding. Year to year consistency between yield classes was assessed using a frequency analysis (Proc Freq of SAS Ver. 9) (SAS Inst, Cary, NC, USA). A frequency analysis can determine if there is agreement or similarity between datasets (years in this case) using a Kappa coefficient. A Kappa coefficient of one indicates perfect agreement between years, whereas a value of zero represents the agreement expected by chance alone. A Kappa coefficient significantly different from zero indicates agreement. Although a Kappa coefficient might be statistically different from zero, it may not have much practicality because of the large sample size used in this study. For these large datasets, therefore, it is more meaningful to report the confidence intervals for the Kappa coefficient (Agresti 1990). If the confidence interval includes zero, then there is only agreement that is expected

Fig. 1 Apparent soil electrical conductivity (EC_a) is often determined using an EM38. Shown above is a similar unit (EM38-DD, Geonics, Mississauga, Ontario, Canada) designed to collect both horizontal (effective measuring depth is approximately 75 cm) and vertical (effective measuring depth is approximately 150 cm) (McNeill 1980)

by chance alone. If the confidence interval does not include zero, then there is evidence of a higher level of agreement.

5.3 Soil and Topography Characterization and Analysis

As in Cox and Gerard (2010) soil samples (0–15 cm) were taken in the spring of each year from a 10 m radius around the center point of a 1 ha square grid. Twenty six samples were collected from Field 1 and 20 samples from Field 2. The samples of soil were allowed to air dry and ground to pass through a 2-mm sieve. Soil samples were analyzed for pH in water (1:1) and soil test Ca, K, Mg and P by the Lancaster method (Cox 2001). Dry combustion (Vario EL *III* elemental analyzer, Elementar Inc., Mt. Laurel, NJ. USA) was used to determine total C and N (Nelson and Sommers 1996). Texture was determined by the pipette method (Gee and Orr 2002). Shallow (0–30 cm) and deep (0–100 cm) apparent soil electrical conductivities (EC_a) were recorded with a ground conductivity meter (EM38-DD, Geonics, Mississauga, Ontario, Canada) (Fig. 1). Elevation was recorded relative to a local benchmark at each soil sampling point using RTK-GPS (Topcon, Livermore, CA, USA). The standardized yield data were overlain with the soil sampling data to match the soil sample point to the relevant yield data for statistical analysis. The yield locations closest to the soil sampling point were used to represent the normalized yield at that sampling point. This distance was never more than 2 m. PROC UNIVARIATE of SAS Ver. 9 (SAS Inst., Cary, NC, USA) was used to compute descriptive statistics for the soil and yield data.

5.4 Statistics

Several studies have used linear discriminant analysis to relate categorical data, such as the yield classes used here, to soil or topographical data (Jaynes et al. 2003; Ping et al. 2005; Cox and Gerard 2007, 2010). This analysis is similar to

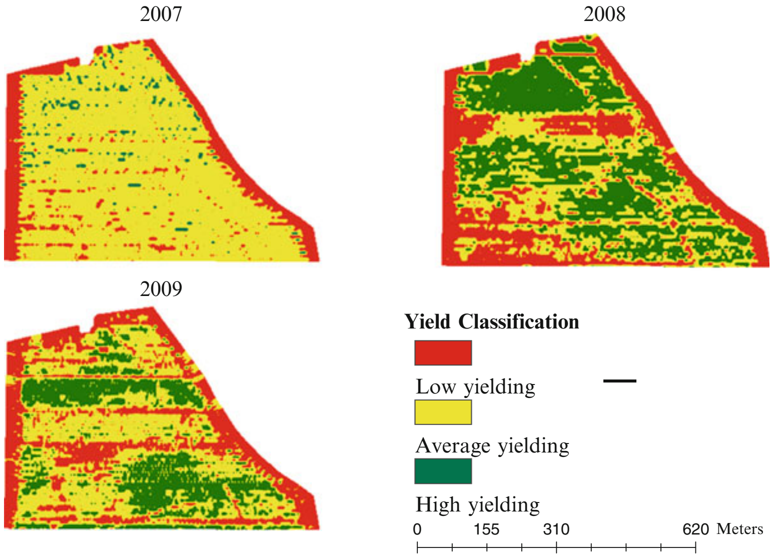

Fig. 2 Results of yield zone classification for Field 1. Crop rotation in this field was soybean (2007, 2009) – Corn (2008)

analysis of variance but can be used to determine relationships between categorical dependent variables (categories are known *a priori*) and quantitative independent variables (Hair et al. 1987). PROC STEPDISC Ver. 9 (SAS Inst., Cary, NC, USA) with the backward elimination option was used to determine which soil properties (0.15 level of significance to remain in the model) provided the best discrimination between the yield classes. PROC DISCRIM Ver. 9 with the crossvalidate option (SAS Inst., Cary, NC, USA) was used to develop a discriminant function from the main contributing soil properties that could place locations in each field into a predicted yield class. The predicted yield class was then compared to the actual yield class to determine the accuracy of the discriminating function for each year of this study.

6 Results and Discussion

6.1 General Yield Characteristics

For Field 1, the 33rd and 66th percentiles were 100 and 109 kg ha^{-1} in 2007 (soybean). For 2008, the 33rd and 66th percentiles were 97 and 116 kg ha^{-1} and in 2009 they were 92 and 120 kg ha^{-1}. For Field 2, pertinent percentiles for the 2007 soybean yield were 102 (33rd percentile) and 112 kg ha^{-1} (66th percentile). For 2008, the percentiles were 102 and 114 kg ha^{-1}. In the 2009 soybean year, the percentiles were 88 and 128 kg ha^{-1}. General yield patterns determined for each field and each year are shown in Fig. 2 (Field 1) and Fig. 3 (Field 2).

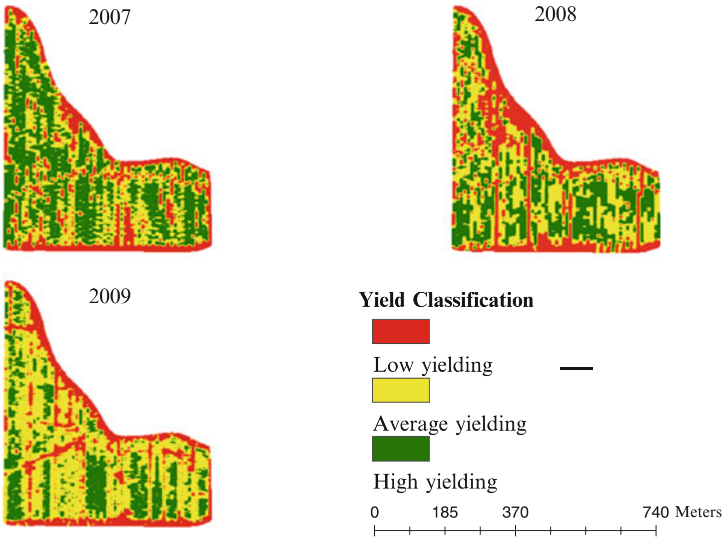

Fig. 3 Results of yield zone classification for Field 2. Crop rotation in this field was soybean (2007, 2009) – Corn (2008)

Table 1 Agreement between year-to-year yield class comparisons in Field 1 based on a frequency analysis

	% Agreement					
	2008			2009		
Yield Class	Low	Average	High	Low	Average	High
2007						
Low	65	18	17	58	21	21
Average	27	33	41	27	34	39
High	20	25	55	30	36	33
2008						
Low				43	23	34
Average				29	34	37
High				31	44	36

6.2 *Frequency Analysis*

A frequency analysis was used to determine if yield classes in each field remained consistent across years. Yield classes in Field 1 were marginally consistent. Agreement between classes ranged from 33% (2007/2008 average; 2007/2009 high) to 65% (2007/2008 2 low) (Table 1). Field 2 results were similar to those of Field 1. Yield class agreement ranged from 33% (2007/2009 average) to 52% (2007/2009 low) in this field (Table 2). All other combinations were within these ranges suggesting the yield classes were marginally stable from year to year.

Table 2 Agreement between year-to-year yield class comparisons in Field 2 based on a frequency analysis

	% Agreement					
	2008			2009		
Yield Class	Low	Average	High	Low	Average	High
2007						
Low	51	23	26	52	26	22
Average	26	34	39	26	33	41
High	26	34	40	24	36	40
2008						
Low				50	29	21
Average				27	34	40
High				23	34	42

None of the confidence intervals for any of the Kappa coefficients included zero and, therefore, the agreement between each classification was considered to be significant. The ranges of consistency are somewhat expected given the presence of a soybean-corn rotation but they do suggest that the yield classes may be useful in predicting management zones if these classes are related to yield-affecting soil properties.

6.3 Yield Class Prediction

Soil properties that could predict yield class for each year were identified using discriminant analysis. The analysis was successful in 49% of the total comparisons of actual and predicted yield class in Field 1 and 65% of the comparisons in Field 2.

In Field 1, the low yielding areas of the field in 2007 were classified correctly 43% (Table 3) of the time based on soil Ca and Mg and soil EC_a, whereas the only point representing a high yielding area was misclassified for the crop. The average yielding portions of the field had the most accurate predictions with 50% being classified correctly. In 2008, the low yielding areas of the field were classified correctly 29% of the time, the average yielding areas were correctly classified 66% of the time and the high yielding areas were accurate 50% of the time (Table 3). These classifications were based on soil Ca, K, and Mg, total C and N, pH, texture, and EC_a. Soil P, pH, EC_a and elevation were used to create a discriminant function for 2009. The low yielding areas of the field were classified correctly 22% of the time, the average yielding areas were correct 43% of the time and the high yielding areas 50% of the time using this function (Table 3).

As in Cox and Gerard (2010), we tried to predict crop yield classes in following years with soil data collected in prior years. A discrimination function predicting the 2008 corn yield classes based on total N, soil available P and K, soil texture and elevation determined in 2007 resulted in 0% (low yield), 44% (average yield)

Table 3 Classification accuracies of predicted versus actual yield for the linear discriminant functions developed for Field 1

	Predicted yield class								
	Percentage correct								
	2007			2008			2009		
Actual yield class	Low	Average	High	Low	Average	High	Low	Average	High
Low	43	57	0	29	14	57	22	33	44
Average	44	50	6	0	66	33	29	43	29
High	0	100	0	50	0	50	20	30	50

These functions were developed to determine potential soybean/corn yield classes from soil properties in Field 1

Table 4 Classification accuracies of predicted versus actual yield for the linear discriminant functions developed for Field 1

		Predicted yield class					
		Percentage correct					
Soil property year	Actual yield class	2008 Corn yield			2009 Soybean yield		
		Low	Average	High	Low	Average	High
2007	Low	0	57	43	0	56	44
	Average	33	44	22	29	57	14
	High	20	40	40	30	20	50
2008	Low						
	Average						
	High						

These functions were developed to determine potential soybean/corn yield classes from soil properties in Field 1

and 40% (high yield) correct classification (Table 4). It is notable that the 2007 soil data resulted in worse predictions in Field 1 for the areas requiring management decisions in 2008 (i.e. the low and average yielding areas) than did the soil data gathered in 2008. This was expected as the rotation changed from soybean in 2007 to corn in 2008. However, soil testing laboratories often give recommendations for 3 years. These results bring that practice into question. The accuracy of these predictions were 0% for low yielding areas, 57% for the average yielding areas and 50% for the high yielding areas (Table 4) based on Ca, K, Mg, P, total C and N, and texture. No variables were found to be useful when attempting to determine the 2009 yield classes using the soil measurements determined in 2008.

Results of the discriminant analysis were mixed for Field 2 also (Table 5). The discriminant procedure found soil Ca, K, Mg, P, pH, texture, elevation and EC_a useful in predicting the 2007 yield. The resulting function correctly predicted the low (50%), average (100%) and high (64%) crop yield categories (Table 5). The discriminant analysis found soil Ca, K, Mg, total C and N, pH, texture and EC_a could be used to

Table 5 Classification accuracies of predicted versus actual yield for the linear discriminant functions developed based on soil data in Field 2

Actual yield class	Predicted yield class Percentage correct 2007			2008			2009		
	Low	Average	High	Low	Average	High	Low	Average	High
Low	50	25	25	60	40	0	40	20	40
Average	0	100	0	33	67	0	13	75	13
High	18	18	64	33	0	67	29	0	71

These functions were developed to determine potential soybean/corn yield classes from soil properties

Table 6 Classification accuracies of predicted versus actual yield for the linear discriminant functions developed to predict yield class based on soil data from previous years in Field 2

Soil property year	Actual yield class	Predicted yield class Percentage correct 2008 Corn yield			2009 Soybean yield		
		Low	Average	High	Low	Average	High
2007	Low	60	0	40	40	0	60
	Average	0	67	33	0	63	38
	High	17	0	83	43	29	29
2008	Low				40	0	60
	Average				0	88	13
	High				57	0	43

The discriminating function that would predict the 2009 yield also contained these same soil properties with the exception of soil pH. The resulting function correctly predicted the low (40%), average (75%) and high (71%) crop yield categories (Table 5).

The soil properties recorded in 2007 and 2008 were moderately successful in predicting the subsequent yield classes (Table 6). Percentages of correct classification in these years ranged from 29% (2007/2009, high yield) to 88% (2008/2009, average yield) (Table 6) based on soil Ca, K, Mg, P, total C and N, soil pH, soil texture and elevation (2008) and soil K, Mg, P, total C and N, soil texture, elevation and EC_a (2009). The best classification resulted from using 2008 soil Ca, K, Mg, P, total C and N, soil texture, EC_a; to predict the average yield class of the 2009 soybean crop.

In Field 1, there was little or no consistency in the best soil properties for classifying yield from year to year. However, soil EC_a did appear in four of the six classification functions made for this field. This suggests that soil EC_a, while not a plant growth factor in itself, was related to yield class and could be used to direct soil sampling. In contrast to Field 1, Field 2 had a great deal of consistency in soil parameters that classified yield from year to year. Soil test K and Mg were present in all classification functions while Ca, P, texture and EC_a were present in five of the six. The presence of soil test K and P in the functions for this field suggest that variable rate application of these fertilizers may increase efficiency, while texture and EC_a may be used to direct soil sampling in this field as in Field 1. While there was no one soil factor in both fields for every year of the study, there was some consistency in the best soil properties for classifying in both fields. Of the years where a discriminating function could be found usable according to backward elimination, soil EC_a was a selected variable in both fields. This suggests that the management zones based on this soil property may increase production efficiency.

6.4 Yield Classification Change Between Years

Since the discriminant analysis of the relationship between yield classification and soil properties was not consistently successful, we tried to determine if soil properties could be used to predict the change in yield classification between years. To do this, the change in classification from year to year was determined and assigned a general categorical response of increase, no change or decrease (Cox and Gerard 2010). A discriminant analysis was again used to determine if the yield classification response could be related to the soil properties from the previous years.

In Field 1, no variables were found to predict changes in yield classification from 2007 (soybean) to 2008 (corn) or from 2007 (soybean) to 2009 (soybean). The changes between the 2008 corn and the 2009 soybean yield classes were predicted based on total C data collected in 2008 and soil EC_a. The success rate ranged from 13% for those areas with no change to 45% where yield class decreased.

In Field 2, the linear discriminant function was 78% successful when predicting the no change yield classification from 2007 (soybean) to 2008 (corn) using soil test Ca and K, total C and N, pH, soil texture and elevation. However, the function was only correct 50% of the time when calculating the increase in yield classification for these years. This same function was 71% accurate when predicting the decrease in yield classification. Generally, predictions between the 2007 soybean crop and the 2008 corn crop using the 2007 soil test Ca and Mg and soil EC_a data were worse than the yearly 2007 and 2008 estimations. The discriminant function accurately predicted the no change, increase and decrease yield classifications 40, 33 and 83% of the time, respectively. The discriminant analysis found pH and EC_a to be useful when using the 2008 soil parameters to predict changes in yield classification in the 2009 yield dataset. Accuracies for these predictions were 75, 86, and 44% when comparing no change, decreased yield, and increased yield respectively.

Successful management zone delineation has been achieved using monocrop systems (Lark and Stafford 1997; Hornung et al. 2006; Jaynes et al. 2003). However, information is limited for rotation systems. The results of this study indicate some success at zone definition in a soybean-corn rotation suggesting that this method could be used to delineate crop/soil management zones. These methods have the potential to direct soil management decisions resulting in correct placement of soil amendments for efficient crop production with minimal environmental impact.

7 Conclusion

In summary, yield data collected over 3 years from two producer fields in a soybean-corn-soybean rotation were normalized to remove the effect of years and then categorized into low-yielding, average-yielding, and high-yielding classes. In both fields, this classification resulted clusters of low, medium, and high yielding areas for all 3 years of the study. A frequency analysis found significant agreement in both fields when comparing year-to-year yield classification. Kappa values for the comparisons were significant due to the large number of data points, and were probably not meaningful. However, none of the confidence intervals for the values included zero, thus the agreements were deemed significant.

Linear discriminant analysis was also used to determine if yield class could be predicted by soil parameters. In Field 1, this analysis found relationships ranging from 0 to 66% of the time while in Field 2 the success rate ranged from 40 to 100% of the time. Linear discriminant analysis was also used to find soil parameters that could predict the change in yield class from year-to-year. In both fields, various soil parameters could be used to determine changes in yield classes among the crops.

This study found that management zone definition in a soybean-corn rotation could be achieved with some success. These zones have the potential to direct soil management decisions resulting in correct placement of soil amendments for efficient crop production with minimal environmental impact.

References

Agresti A (1990) Categorical data analysis, 1st edn. Wiley, Hoboken

Anderson-Cook CM, Alley MM, Roygard JKF, Khosla R, Novle RB, Doolittle JA (2002) Differentiating soil types using electromagnetic conductivity and crop yield maps. Soil Sci Soc Am J 66:1562–1570

Bauer ME (1975) The role of remote sensing in determining the distribution and yield of crops. Adv Agron 27:271–305

Becket PHT, Webster R (1971) Soil variability-a review. Soil Fert 34:1–15

Blackmore S (2000) The interpretation of trends from multiple yield maps. Comput Electron Agric 26:37–51

Boydell B, McBratney B (2002) Identifying potential within-field management zones from cotton-yield estimates. Precis Agric 3:9–23

Cox MS (2001) The Lancaster soil test method as an alternative to the Mehlich 3 soil test method. Soil Sci 166:484–489

Cox MS, Gerard PD (2007) Soil management zone determination by yield stability analysis and classification. Agron J 99:1357–1365

Cox MS, Gerard PD (2010) Changes in yield classification in a soybean-rice rotation. Precis Agric 11:507–519

Cox MS, Gerard PD, Reynolds DB (2005) Selected soil property variability and their relationships with cotton yield. Soil Sci 170:928–937

Cox MS, Gerard PD, Abshire MA (2006) Selected soil properties' variability and their relationships with yield in three Mississippi fields. Soil Sci 171:541–551

de Gruijter JJ, McBratney AB (1988) A modified fuzzy k-means method for predictive classification. In: Bock HH (ed) Classification and related methods of data analysis. Elsevier, Amsterdam, pp 97–104
Diker K, Heerman DF, Brodahl MK (2004) Frequency analysis of yield for delineating yield response zones. Precis Agric 5:435–444
Dobermann A, Ping JL, Adamchuk VI, Simbahan GC, Ferguson RB (2003) Classification of crop yield variability in irrigated production fields. Agron 95:1105–1120
Doolittle JA, Sudduth KA, Kitchen NR, Indorante SJ (1994) Estimating depths to claypans using electromagnetic induction methods. J Soil Water Conserv 49:572–575
Earl R, Taylor JC, Wood GA, Bradley I, James IT, Waine T, Welsh JP, Godwin RJ, Knight SM (2003) Soil factors and their influence on within-filed crop variability, part I: Field observation of soil variation. Biosyst Eng 84:425–440
Fraisse CW, Sudduth KA, Kitchen NR (2001) Delineation of site-specific management zones by unsupervised classification of topographic attributes and soil electrical conductivity. Trans ASAE 44:155–166
Gee GW, Orr D (2002) Particle size analysis. In: Dane JH, Topp GC (eds) Methods of soil analysis, part 4: physical methods. Soil Science Society of America, Madison, pp 255–294
Godwin RJ, Earl R, Taylor JC, Wood GA, Bradley RI, Welsh JP, Richards T, Blackmore BS, Carver MJ, Knight S, Welti B (2002) "Precision farming" of cereal crops: a five year experiment to develop management guidelines. HGCA project report No. 267, London UK. http://www.hgca.com/publications/documents/cropresearch/267_complete_final_report.pdf. Accessed 15 Feb 2012
Grenzedörffer GJ, Gebbers RIB (2001) Seven year of yield mapping-analysis and possibilities of multi -year yield mapping data. In: Grenier G, Blackmore S (eds) Proceeding of the third European conference on precision agriculture. Agro Montpellier Ecole Nationale Supérieure Agronomique, France pp 31–36
Hair JF, Anderson RE, Tatum RL (1987) Multivariate data analysis with readings, 2nd edn. Macmillan, New York
Hornung A, Khosla R, Reich R, Inman D, Westfall DG (2006) Comparison of site-specific management zones. Agron J 98:407–415
Huggins DR, Alderfer RD (1995) Yield variability within a long-term study: implications for precision farming. In: Robert PC et al (eds) Site-specific management for agricultural systems: proceedings of site-specific management for agricultural systems second international conference. ASA CSSA and SSSA, Madison, pp 417–426
Jaynes DB, Kaspar TC, Colvin TS, James DE (2003) Cluster analysis of spatiotemporal corn yield patterns in an Iowa field. Agron J 95:574–586
Johnson RA, Wichern DW (1998) Applied multivariate statistical analysis, 4th edn. Prentice Hall, Upper Saddle River
King JA, Dampney PMR, Lark RM, Wheeler HC, Bradley RI, Mayr TR (2005) Mapping potential crop management zones within fields: use of yield-map series and patterns of soil physical properties identified by electromagnetic induction sensing. Precis Agric 6:167–181
Kitchen NR, Sudduth KA, Drummond ST (1999) Soil electrical conductivity as crop productivity measure for claypan soils. J Prod Agric 12:607–615
Lamb JA, Dowdy RH, Anderson JL, Rehm GW (1997) Spatial and temporal stability of corn grain yields. J Prod Agric 4:87–101
Lark RM, Stafford JV (1997) Classification as a first step in the interpretation of temporal and spatial variation of crop yield. Annu Appl Biol 130:111–121
Nelson DW, Sommers LE (1996) Total carbon organic carbon and organic matter. In: Sparks DL (ed) Methods of soil analysis: part 3, 2nd edn. Soil Science Society of America, Madison, pp 961–1010
Nicholson J, Hammer G, Harper D, Crouch M (1998) Order 1 soil survey of the cropping systems research site, Camden Farm, Caroline County. Natural Resources Conservation Service Farmville, VA

Ping JL, Green CJ, Bronson KF, Zartman RE, Doberman A (2005) Delineating potential management zones for cotton based on yields and soil properties. Soil Sci 170:371–385

Schepers AR, Shanahan JF, Liebig MS, Schepers JS, Johnson SH, Luchiari A Jr (2004) Appropriateness of management zones for characterizing spatial variability of soil properties and irrigated corn yields across years. Agron J 96:195–203

Soil Survey Staff (2011) Web soil survey. Natural Resources Conservation Service, United States Department of Agriculture. http://websoilsurvey.nrcs.usda.gov/. Accessed 24 Feb 2012

Stafford JV, Ambler B, Lark RM, Catt J (1996) Mapping and interpreting yield variation in cereal crops. Comput Electon Agric 14:101–119

Taylor JC, Wood GA, Earl R, Godwin RJ (2003) Soil factors and their influence on within-filed crop variability, part II: Spatial analysis and determination of management zones. Biosyst Eng 84:441–453

Wollenhaupt NC, Mulla DJ, Gotway-Crawford CA (1997) Soil sampling and interpolation techniques for mapping spatial variability of soil properties. In: Pierce FJ, Sadler EJ (eds) The state of site specific management for agriculture. ASA CSSA and SSSA, Madison, pp 19–53

The Vine Functioning Pathway, A New Conceptual Representation

Cécile Coulon-Leroy, René Morlat, Gérard Barbeau, Christian Gary, and Marie Thiollet-Scholtus

Abstract Climate change, new regulations for preservation of the environment and demands of the markets for specific products, make it increasingly necessary to optimize the choice of cropping practices. The winegrowers take into account the combined influences of environmental factors, plant material and practices, to improve grapes and wine. However, this combined influence has been little studied. A general formalization of these multiple influences at the scale of viticultural terroirs is necessary to better characterize the growth of the vine and its impact on the yearly characteristics of grape and wine. In this article, following a global system approach we review current knowledge about the relationships between environmental factors, plant material, agricultural practices, the growth of the vine and the characteristics of grapes and wine. We propose a conceptual model formalizing the relationships between the systemic variables. The system can be represented as the new concept of 'the vine functioning pathway', which we define as the logical and ordered combination of the effects of environmental factors, plant material and agricultural practices on the levels of vigor and earliness of the vine and the final characteristics of the product. The resulting product of the system model is the grape and then the wine. This conceptual model is less accurate than a functional mathematical model but is the first which takes into account the whole complexity of the vine system. The conceptual model built and implemented by a computer can be used as a support tool for decisions aiding in the optimization of cropping practices based on environmental factors and specific products goals.

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1 Introduction

Recent issues, such as climate change, new regulations for preservation of the environment and demands of the markets for specific products, make it increasingly necessary to optimize the choice of cropping practices. This choice is done on the basis of production objectives and takes into account environmental factors such as soil, parent-rock, landscape and climate characteristics.

The choice of agricultural practices, in which we include the choice of the grape variety and the rootstock, is part of a 'sustainable wine industry'. The International Organization of Vine and Wine (OIV) includes in this concept the production of quality products, considering requirements of precision in viticulture, risks to the environment, products safety, and consumer health. The OIV concept of the 'sustainable wine industry' involves also heritage, historical, cultural, ecological and aesthetic aspects (OIV 2004).

The influences of the combination of environmental factors and agricultural practices, as well as oenological practices, on the sensory properties of wine have been known empirically for many years. Winegrowers empirically incorporate the effects of environmental factors, which lead to the notion of 'terroir'. The International Organization of Vine and Wine defines the terroir as "a concept which refers to an area in which collective knowledge of the interactions between the identifiable physical and biological environment and applied vitivinicultural practices develops, providing distinctive characteristics for the products originating from this area" (OIV 2010). Depending on the diversity of the physical environmental factors, a winegrower is required to manage each of the plots, tailoring the practices to the characteristics of the environment. Winegrowers and cooperatives produce a range of wines, and they implement a series of dedicated agricultural and oenological practices to obtain the wines they want to produce, depending on the legislation, technical or economic constraints (Cadot 2006). The concept of 'terroir' is at the base of the Protected Designation of Origin (equivalent to the French "Appellation d'Origine Contrôlées") organization. PDO organization allows to distinguish products and to attribute those specific sensory, technical and environmental dimensions (Casabianca et al. 2005).

From a conceptual point of view, the environment of the field and above all its soil characteristics, is the first element of the terroir system; practices are considered as less important and so less taken into account in research studies on terroir (Cadot et al. 2012). However, on their study, these authors show the prevalence of technical factors on the characteristics of the sensorial profiles of wines.

Here, following a global approach, we review the impact of environmental factors and winegrowers' practices on the grape and wine quality and the method of their evaluation. Additionally, we propose a systemic modelisation based on the vine functional pathway concept to best adapt agricultural practices to the environmental factors of the field and to the expected wine quality.

2 A Wine Style Well Identified as Objective of Production

Wine quality is perceived as multi-dimensional (Charters 2007). It is influenced by the attributes of the grapes and the oenological practices. Nevertheless, the concept of grape and wine quality is not much understood; and yet the identification and definition of key components is necessary to improve vineyard management and harvest decisions to produce grapes according to end-use specifications (Keller 2010). The quality of grapes refers to the chemical composition of berries and the proportion of skin, seed and flesh. Wines quality can be characterized by their chemical composition and sensorial profile (Ribéreau-Gayon et al. 1998; Landon et al. 2008). However, the integration of individual wine attributes into the overall wine quality remains a challenge (Soar et al. 2008).

In the aim of developing decision support aid tools for winegrowers, research works must be driven by the commercial reality of producing wines with specific style and quality as proposed by Holt et al. (2008). These authors considered a global quality, evaluated quantitatively by professionals. In their study, they used the Australian Wine Show 20-point system to evaluate wines (Rankine, 1990; Dunphy and Locksin 1998 in Holt et al. 2008). In the same country, Halidday J. (1991, 1998, 2003, 2006 in Sadras et al. 2007) proposed a 10-point scale. Nevertheless, professional judges are not always consensual to evaluate wines (Hodgson 2008 in Keller 2010; Cadot 2010; Lesschaeve 2003) and their concept of quality can differ from those of consumers (Lattey et al. 2010).

Types of wine can be identified and can be classified according to two different scales of details: Level I. The scale of the criteria is the nature of the end-product. For example, the end-product is the wine and a grape variety such as Cabernet franc from the middle Loire Valley (France) may produce a sparkling wine, a rosé or a red wine corresponding to several Protected Designation of Origin, Level II. The scale of the criteria is the types within the categories of the end-product. For example, Deloire et al. (2005) identified six types in the category of red wines, which range from wine with herbaceous to tannic wine.

The winegrower has end-products goals according to a winery strategy and a conceptual representation of the type of wine he wants to produce, which leads him to adapt his agricultural practices (Cadot et al. 2011). When the wine produced by the winegrower is assessed, the conceptual representation that he has of the type of wine does not always correspond to the perceptual representation of the wine he actually produced (Cadot et al. 2012). In one way, to characterize product styles consensually is necessary to define the output of a future model at the basis of a support aid tools to better adapt practices to the production objectives. And in another way linking the wine style with the functioning of the vine will allow to better monitor and control the type of product obtained.

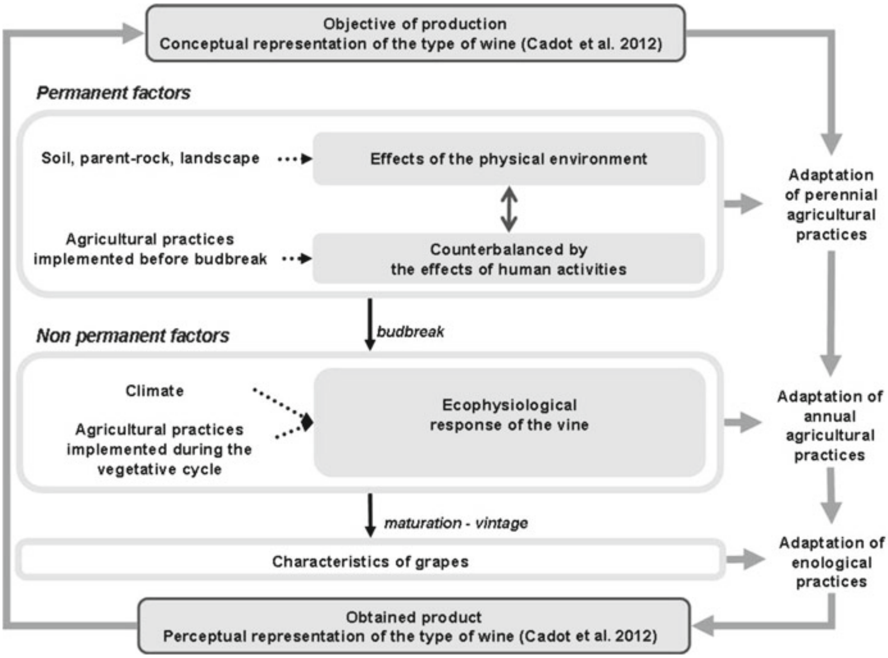

Fig. 1 Representation of the construction of the quality of grapes and wines

3 A Wine Style Determined by the Functioning of the Vine

3.1 The Construction of the Quality of Grapes and Wines

The characteristics of wines depend on the characteristics of the grapes and the wine-making practices that are implemented. Upstream characteristics of the grapes depend on the growth of the vine that is affected by environmental factors (soil, subsoil and landscape environment), the climate and the annual or perennial agricultural practices of the winegrowers (Fig. 1) (Morlat et al. 2001; Van Leeuwen et al. 2004; Ubalde et al. 2007; Reynolds and Heuvel 2009).

Winegrowers adapt their practices according to their production objectives and to the physical characteristics of their plots. In perennial crops such as grapevine, several agricultural practices are fixed at the time of planting; e.g., grape variety, type of rootstock and density. Other variable practices can be adapted each year during the growing season, depending on the climate or the performance level chosen; e.g., date of pruning, disbudding, intensity of leaf removal or grape thinning. If the fixed practices associated with environmental factors do not induce the desired ecophysiological comportment of the vineyard and consequently do not lead to the type of expected wine, the winegrower has to modify the variable practices in order to compensate the effect of fixed practices and environmental factors.

Goulet and Morlat (2010) noticed that the practices that are already in place in the vineyard are sometimes unsuitable. For example, in the vineyard of the Sarthe in Loire Valley (France), 72% of the plots have a too vigorous rootstock since the environmental factors induce already a very strong vigor. This observation suggests that if winegrowers know well the environmental factors of their plots and their effects on vine development, they can optimize their practices. A good adaptation of practices can help reducing the negative effects of the physical factors of the environment. Therefore, it is necessary to consider these effects in conjunction with those of agricultural practices to better understand the behavior of the vine. If the fixed practices were well-adapted to the environmental factors and to the objectives of production, the number of corrective actions during the growing season would be reduced, allowing for a more sustainable wine industry.

The winegrower considers the functioning of the vine at the plot scale. Morlat et al. (2001) defines the Basic Terroir Unit as the smallest useful area for vineyard management, in which the response of the vine is reproducible through the wine. It includes three natural components: the geological substratum, the soil and the landscape environment (Morlat et al. 2001). The Basic Terroir Unit can be studied using a model based on soil depth and the degree of weathering of the parent rock (Morlat et al. 2001). (Coipel et al. 2006) note that the effect of environmental factors on terroir is related to soil depth rather than to the type of soil itself. It should be noted that the field model of Morlat et al. (2001) cannot be applied in the case of soils that do not result from the degradation of bedrock, such as superficial formation soils (Vaudour et al. 2005).

Bodin and Morlat (2006) showed differences between the environments classified as 'Weakly Weathered Rock' and 'Strongly Weathered Rock' in terms of earliness of the vine vegetative cycle. On the 'Weakly Weathered Rock' environments, both vine earliness and water constraint are higher or more frequent than on 'Strongly Weathered Rock' environments, but the vigor is lower. However, the authors note that the water supply also varies depending on the type and hardness of bedrocks.

Carey et al. (2008) integrate the climatic parameters and define the Natural Terroir Unit as a landscape characterized by a relative homogeneity of geology, soil, topography and climate. Carbonneau (1993) integrates agricultural practices and defines the Viticultural Terroir Unit as a combination between the Basic Terroir Units and agricultural practices. Few studies on terroir characterization include human activities both in their individual and collective dimension. However, human activities can reveal the potential of a terroir through the implemented practices (Bérard and Marchenay 2006).

It should be noted that several Basic Terroir Units might be present in the same plot. Depending on the circumstances, the winegrower can adjust his agricultural practices at the intra-plot level (e.g., by the choice of rootstock). Work in precision viticulture shows the importance of intra-plot management (Bramley and Hamilton 2007).

Producers have a type of wine as an objective; for them, the soil is a major component. However, studies linking the conceptual representation of their product and the perceptual representation, that is to say, what they want to produce versus what they actually produce, highlight the role of viticultural and oenological practices, particularly oenological practices, on which winegrowers have a better control and can respond quickly (Cadot et al. 2012). Understanding the dynamic construction of yield and grape quality through the analysis of the functioning of the vine may allow producers to reduce the gap between the two representations by adapting their practices.

3.2 Vigor and Precocity: Two Variables Used by the Winegrowers to Monitor the Functioning of the Vine

The vine functioning can be characterized both by the earliness of the vine cycle and the quantity of biomass produced (Champagnol 1984). Vine **vigor** corresponds to the rhythm and intensity of growth of the shoot (Carbonneau et al. 2007). At the plant level, the 'vine vigor' term is commonly used both in practice and in the literature to characterize a general state of vine growth, but depending on age or size, a vine can have few but very vigorous shoots, or many shoots that are less vigorous (Dry and Loveys 1998). The vigor of the vine, equated with its vegetative growth, depends on interactions between the plant material, the physical environment and the agricultural practices, which together determine the access to water and nutrients.

The physical environment partly conditions the vigor of the vine. For the cartography of the Basic Terroir Units of a vineyard, Morlat et al. (2001) proposed a method to calculate a composite variable that characterized the vigor imparted by environmental factors, according to the soil water holding capacity, the stoniness of the soil and the hardness of the bedrock. These factors impact the maximal spatial distribution of the root system and consequently the vine vigor; the deeper and denser the root system, the more vigorous is the plant.

This level of vigor, however, is modified by the technical choices of the winegrower. There are differences in vigor depending on the choice of plant material. The vine variety 'Cabernet franc' is considered more vigorous than the variety 'Merlot' (Havinal et al. 2008). To fight against the damage caused by the insect *Philloxera vastratix*, the variety named 'scion' is grafted onto a rootstock. Both the rootstock itself and the combination of variety and rootstock induce strong effects on the vigor of the vine (Minet et al. 2000). The choice of the rootstock allows compensating for an excessive or deficient vigor imparted by the soil. The differences between rootstocks (e.g., rootstock SO4 is more vigorous than rootstock Riparia) are related to intrinsic genetic characteristics but can also be explained by differences on their response to water deficit (Marguerit et al. 2011), Goulet and Barbeau (2006) noted that Riparia is less effective at drawing water than SO4. However, soil properties seem to affect the distribution of the root system more significantly than does the rootstock (Smart et al. 2006).

Vine vigor depends on the distribution of the root system and the regime of water supply. It is modulated by the effect of environmental factors and the rootstock, but also by agricultural practices such as intercropping (Barbeau et al. 2006). Water availability for the vine decreases as the surface area of the intercrop increases (Tesic et al. 2007). Weed competition leads to a greater rooting depth of the vine in weeded rows (Goulet and Barbeau 2006). Interrow grass cover is often used as an alternative to chemical weed control, to reduce chemical pollution of the environment and to control soil erosion. Many studies, summarized by Morlat (2010), have shown the positive influence of an interrow grass cover on the physical characteristics of the soil, the control of the vine vigor and grape quality. This practice is fully consistent with a sustainable viticulture. However, weed competition with the vine depends on both the area covered by the grass in the inter-row and the grass species used (Delabays et al. 2006); they must be carefully tailored to the characteristics of the environment. Weeds compete with the vine for water and nitrogen (Celette et al. 2009). Nitrogen is a major mineral required for vine growth that also has an impact on the characteristics of grapes and wine (Bell and Henschke 2005).

The distribution of the root system of the vine will also depend on the planting density of the vine. It results from both the spacing between rows and the spacing between plants on the row and influences the vigor of the vine. Planting density is often determined by the decrees of the PDO that govern many French vineyards. Plots of Chenin planted at a density of 5,000 vines/ha have a lower vigor than plots planted at a density of 2,500 vines/ha, as evidenced by a lower weight of pruned wood (Morlat et al. 1984). Murisier and Zufferey (2006) also noted a lower weight of pruned wood by vine stock for the high-density vineyards of Chasselas. In low-density vineyards, from 2,500 to 3,000 vines/ha, the vigor of the plants is maximal because of the lack of competition. When density increases, competition between plants intensifies, resulting in a reduced growth of individual plants, up to the limit of 8,000 vines/ha according to Carbonneau et al. (2007).

The management of the vineyard throughout the control of vine vigor and of yield level determines the balance between vegetative growth and productivity (Kliewer and Dokoozlian 2005), This balance can be achieved through a rational management system involving the aerial shape of the vine (Reynolds and Heuvel 2009) and interaction practices in the planting of the plot, such as the choice of rootstock and planting density (Dry and Loveys 1998; Rives 2000). Annual practices used to limit vegetative growth, such as topping, disbudding or grape thinning, can be regarded as remedial practices and not as factors directly influencing plant vigor.

Precocity of the vine cycle equals to early vine development means an earlier date of onset of the main phenological stages (budbreak, flowering and veraison). Depending on the degree of earliness the optimal maturity of the grapes for a given type of wine will be reached sooner or later in the growing season. For example, in the northern vineyards of France, a late variety may not achieve optimal maturity; so harvest would eventually be decided to avoid excessive rot of grapes

Like vigor, earliness depends on the interactions between the plant, the physical environment and agricultural practices. Its level is modified by the agricultural practices of the winegrower, but to a lesser extent than vigor. Earliness is largely due to the macroclimate of the area where the vineyard is located, in relation with the temperature of the air (Pouget 1963; Riou 1994). But it is also due to the mesoclimate related to the landscape surrounding a patch of vines. The date of budbreak depends on the soil temperature at the rooting zone in relation with the water content, the soil texture and characteristics of the soil surface (Morlat et al. 2001), so earliness is also influenced by the soil climate. The water holding capacity is a major variable in the thermal behavior of the soil, having an impact on soil warming and therefore on the early establishment of the leaf area in spring and early summer (Asselin et al. 2001; Morlat et al. 2001; Cellier et al. 1996). Differences in earliness according to soil texture may exist in the absence of other limiting factors; for example, a stonier soil profile induces an earlier development of vine because of an improved nighttime heat retention (Trought et al. 2008).

Morlat et al. (2001) proposed a composite variable that characterizes the earliness of a vine plot imparted by environmental factors, taking into account soil depth, stoniness of the soil, hardness of the bedrock, humidity at the field capacity, natural drainage, color of the soil surface, depth of the zone of maximum root colonization, altitude above sea level, aperture of the landscape and aspect. For Nendel (2009), only the sum of the temperatures has an influence. Other authors note the dominating effect of air temperature and provide simulation models of the date of bud break based on air temperature (Riou 1994; Pouget 1963; Garcia de Cortazar et al. 2009).

Some practices have an impact on vine precocity. The date of pruning can be used to significantly alter the earliness imparted by environmental factors (Martin and Dunn 2000). The date of pruning is chosen by the winegrower, primarily as a consequence of the empiric knowledge he has on the risk of frost for each one of his plots. If the risk of frost is high, the plot will be pruned later to delay the start of vegetative growth and reduce the risk of damage on buds. The type of rootstock has a slight influence on the earliness; however, very little study has been done on its effect (Barbeau and Blin 2010).

Water supply during the vegetative and reproductive cycle of the vine impacts the vine vigor and the precocity. Many studies show that the characteristics of the grape are strongly influenced by the water availability (Koundouras et al. 1999). A water supply inducing a moderate drought is optimal for the production of high quality grapes (Morlat 2010; Van Leeuwen et al. 2009). Different levels of drought will lead to different types of wine (Deloire et al. 2005). A non-limiting water supply leads to a higher vigor and a prolonged vegetative growth over time. The yield is higher, but the compounds in the grapes namely sugars, organic acides and phenolic compounds are less concentrated which affect wine quality (Champagnol 1984; Tesic et al. 2002; Cortell et al. 2005). Conversely, a water supply regime that induces a very high water constraint will have an adverse effect on photosynthesis and will decrease the production of assimilates or even block the accumulation of sugars in the grapes (Ojeda 1999). The effect of drought on the vine depends on the timing of its appearance. Water stress that occurs before the veraison or that is higher after veraison has a strong negative effect on the composition of the grapes, while a moderate water stress after veraison has a positive effect (Girona et al. 2009; Hardie and Considine 1976; Matthews and Anderson 1988; Morlat et al. 1992; Ojeda et al. 2002). In addition, a strong water stress causes a decrease in the size of the berries, which may result in a high concentration of certain compounds (e.g., phenolic compounds in the skin) (Ojeda et al. 2002). An increase in the concentration of phenolic compounds results in a higher color intensity of the wine and a change in its structure (Chacon et al. 2009). However, it should be noted that some effects, such as an increase concentration of phenolic compounds in the skin, are independent of the size of the berries (Roby et al. 2004).

Water supply to the vine depends on the water holding capacity of the soil, the accessibility of the water by the roots of the vine, and the climate, as well as agricultural practices and plant material. Water supply to the soil and to the vines is linked to rainfall and possibly irrigation, while water losses are the result of runoff, direct soil evaporation and transpiration from the plant. Goulet et al. (2004) proposed a method for calculating the maximum useful water reserve in the soil as a function of soil depth, humidity at field capacity and wilting point, bulk density and soil texture. Next water supply during the vegetative cycle can be assessed through a water balance model (Riou and Lebon 2000). Celette et al. (2010) have recently proposed a new model that takes into account interrow cover crop. Gary et al. (2005) proposed an optimal trajectory of water supply during maturation period to ensure satisfactory quality of wine produced. Deloire et al. (2005) provided a trajectory according to the wine style.

The vine functioning variables are dependant. The variables affecting the growth of the vine are linked partly on water availability, which plays a major role in the early stages of the vegetative cycle (Tesic et al. 2002; Carbonneau et al. 2007). The hierarchy of the functioning variables may vary from one region to another. For example, the effect of a lack of water constraint tends to be more pronounced in the French northern vineyards because the cycle of the vine is later: the vine may still be in the vegetative stage of growth during the ripening of the grapes (Morlat et al. 1997).

4 How to Characterize the Joint Effects of Environmental Factors and Agricultural Practices on the Vine Functioning?

Vine functioning during the vegetative cycle can be evaluated through several measurements. Many methods, direct or indirect, destructive or undestructive, have been developed to quantify the vegetative development and the vine vigor (Tregoat et al. 2001): use of sensors such as the NDVI (Goutouly et al. 2006; Homayouni et al. 2008), direct measurements of leaf area or weight of prunes canes (Carbonneau et al. 2007), as well as expert estimation (Bodin and Morlat 2003; Carey et al. 2007). Precocity of the vegetative and reproductive cycle can be directly observed on vine plots and estimated using indices that combine the dates of the phenological stages (Barbeau et al. 1998). So measures do exist and can be used to make a diagnostic. They are integrated in research programmes to evaluate the impact of terroir factors. However, since they are available only after the vineyard is established and during the vine cycle, they do not allow for an *ex-ante* estimation of the functioning of the vine, that would ease the choice of perennial practices. To remedy this situation, models can be built to estimate the vine precocity and vigor. In most cases, researchers try to quantify the impact of soil or climatic factors, or that of one agricultural practice on the vine functioning and/or the composition of grapes, the other variables being fixed. Under these conditions, ranking and evaluating the interactions between variables to build an efficient model may be difficult.

4.1 A Systemic Modelisation

Vine functioning is determined at the same time by environmental factors and agricultural practices. We must think, therefore, in a systemic way by considering the interactions of agricultural practices between themselves and with the environment, as mentioned by Meynard (2008). This should help to better understand and manage the links between environmental factors, agricultural practices and the characteristics of grapes and wine. Consequently, it will be possible to provide tools to support the decisions taken by the winegrowers to optimize agricultural practices and produce the expected products as part of a sustainable wine production. A systemic approach in viticulture can benefit from modeling. The model is a conceptual object composed of assumptions, principles and relationships; next, tools incorporate the concept of use (Jeuffroy et al. 2008).

Models are constructed from an aggregation of variables (Girardin et al. 1999) and can be classified according to the method of aggregation. Two main types are identified: mechanistic models and engineering models Passioura (1996). This author indicates that mechanistic models aspire to improve our understanding of the physiology and environmental interactions of crops while engineering models, based on empirical relations, aspire to provide sound management advice to farmers and sound predictions to policy makers. However the engineering models can not be applied outside the range of environmental variables used for their calibration.

The variables included in a model and their level of detail should be determined according to the intended use of the model. Thus, in the case of a model that will be used as a support tool for decision-making, the input variables must be easily accessible in real situations, leading to a simplification of the modeling process (Cros et al. 2003). It should be noted that an increased level of precision does not necessarily improve the model predictions, as it simultaneously increases the uncertainty (Sinclair and Seligman 1996). These last authors suggest rules of conduct: to define the objectives that must stay modest, to evaluate the model quality according to these objectives, to built new models using previous approaches, to determine the model organization according to the level of the problem (tissue, organ plant or plot) and to use synthetic relations when they are sufficiently sound to represent a more complex theory.

De Turckheim et al. (2009) indicated that models conceived to represent and gather knowledge about the functioning of systems are not necessarily suited to the objectives of supporting a decision, as they must be coupled to a model representing the decision process. This coupling can sometimes be complicated because of the formalisms adopted to represent the complex dynamics of biological processes.

The new challenge is to construct a model that can be used directly in the development of a tool in support of decision-making. The design of such a model must ultimately combine several sub-models, taking into account the biophysical system, the agricultural practices, the economic costs and the processes of decision-making (Loyce et al. 2002; Le Gal et al. 2009).

4.2 Current Vine Models

Studies that deal with the prediction of quality according to the vine functioning include calculation of various indices (Smart and Robinson 1991; Gray et al. 1997; Martinez de Toda et al. 2007). These indices take directly into account observations of the vine functioning on plots such as the weight of prunes canes, an indicator of the vine vigor. Other indices allow predicting vine functioning according to permanent environmental factors but do not integrate the impact of cultural practices (Morlat et al. 2001).

Models, integrating all processes involved in defining fruit quality and reproducing plants response to climate and cultural practices exist for some perennial or annual crops but they still must be adapted to grapevine (Dai et al. 2010). These authors mentioned that, for grapevine, many models focus on shoot development (Lebon et al. 2004; Pallas et al. 2008), canopy structure (Louarn et al. 2008), response to pruning (Orlandini et al. 2006), water balance at the vineyard level (Lebon et al. 2003; Pellegrino et al. 2006; Celette et al. 2010), as well as dry mass accumulation and allocation at the canopy level (Gutierrez et al. 1985; Vivin et al. 2002; Poni et al. 2006). Some research works aim fat predicting berry ripening (Fernandez Martinez et al. 2012; Sadras and Petrie 2012). These models take into account some berry components and climatic parameters: most of the time sugar concentration in relation with air temperature.

A crop model has been developed for grapevine, the STICS-grapevine model (Brisson et al. 2001; Garcia de Cortazar 2006). This last author have introduced a new sub-model, named BRIN, to simulate the bud break stage, the first phenological stage (Garcia de Cortazar et al. 2005). BRIN takes into account varietal characteristics and the dormancy period. Some interests of the STICS model are its robustness to compare vineyards at a country scale and evaluate the impact of climate change. However parameters values are not sufficiently accurate to use the model to compare plots at the vineyard scale. It does not take into account the impact of some perennial practices such as the choice of the rootstock, nor the age of the vines and their carbohydrates reserve. We previously mentioned the important role of rootstock on vine development. STICS model can predict a yield level but does not simulate quality, except for the sugar content. Presently, the STICS-grapevine model can not be used to support decision at the plot scale. Other models have been built in this objective but for specific problems such as the adaptation to the soil salinity (Walker et al. 2005) or to optimize fertilization practices (Nendel and Kersebaum 2004).

4.3 Our Conceptual Model Based on the Vine Functioning Pathway

It is difficult to experimentally assess the impact of all factors involved in the composition of grapes. Therefore, building a working model capable of faithfully translating and explaining the influence of all environmental factors and agricultural practices and their interactions is a hard task. We wish to contribute to the modeling system involving environmental factors, agricultural practices, the growth of the vine and the type of product obtained, as there is a demand for such a model from the wine industry. We propose, here, a conceptual representation based on the concept of the vine functioning pathway. We rely on the literature previously cited and on the knowledge of our research team as suggested by Zhu et al. (2007). This representation will be further used to develop a decision support tool to adapt agricultural practices to environmental factors according to the production objectives.

As for crop management, defined as the logical and ordered combination of techniques implemented on a plot to obtain a crop (Sebillotte 1974), we define the **vine functioning pathway** as the logical and ordered combination of the effects of physical environmental factors, agricultural practices and climate on the annual level of vigor and earliness of the vine and the final characteristics of the product. The starting point of the vine functioning pathway corresponds to the characterization of the joint effects of environmental factors and agricultural practices implemented ahead of the growing season (before budbreak, which corresponds to the start of vegetation) *i.e.* the practices associated with the installation of the vineyard (e.g., planting density), those carried out over several growing seasons (e.g., interrow management) or implemented before budbreak (e.g. date of pruning). The same starting point can lead to several vine functioning pathways given the variability of climate and annual agricultural practices.

Our conceptual model is illustrated in Fig. 2. According to the weather of the year, the same plot can follow different pathways leading to different types of grapes (e.g., plot B – Grape Type 1 & Grape Type 2). The same types of grapes can be obtained through different pathways (e.g., grape type 2), so even if a plot has an higher water holding capacity (plot C), the introduction of a highly competitive intercrop can lead to a water supply similar to that of a non-intercropped plot with a lower maximum useful water reserve (plot B). With identical climatic conditions, different combinations of environmental factors and agricultural practices may provide the same potential for vigor or precocity, so, different pathways may still lead to a single type of grape and wine.

The climate is evolving, and agriculture must adapt (GIEC 2007; Trnka et al. 2011). The proposed model is part of a dynamic approach. Harvest dates are earlier and earlier, even if the climate change impacts differently across regions and on types of wines. Sadras and Petrie (2011) have shown that the onset of ripening in warmer climate is due to an earlier maturity of grapes rather than a faster accumulation of solutes. Jones et al. (2005) pointed out that one way to adapt to climatic change is to improve the modeling of the system by including the effect of the plant material choice and the adaptation of farming practices. A limiting environment for

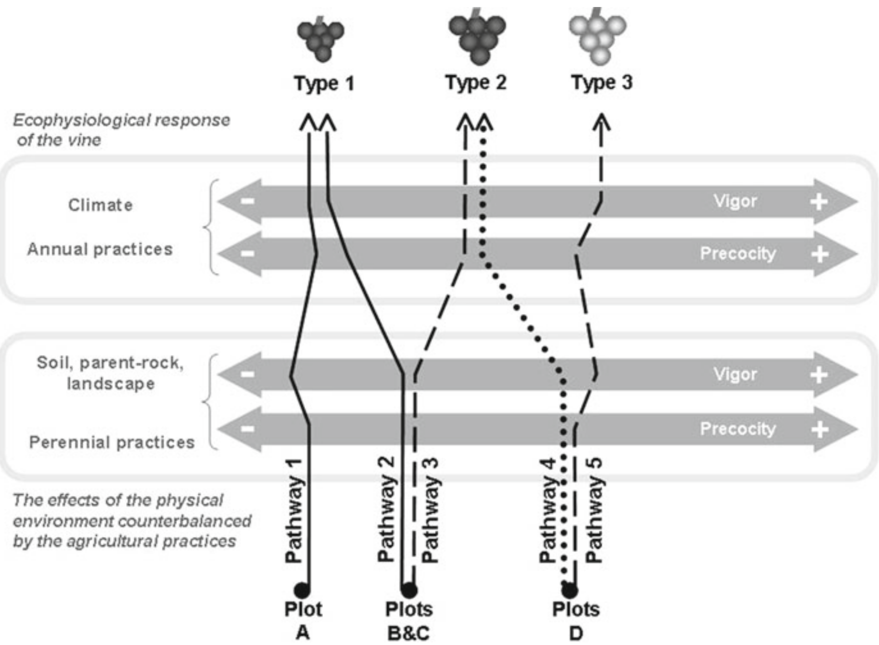

Fig. 2 Illustration of the model based on the vine functioning pathway concept. *Plot A*: shallow soil on hard rock, imparting low vigor with a medium vigor rootstock vigor. *Plot B*: shallow soil on hard rock imparting low vigor with a strong vigor rootstock vigor. *Plot C*: deep soil on river alluvium imparting a strong vigor with a low vigor rootstock. *Plot D*: deep soil on river alluvium imparting a strong vigor with a moderate vigor rootstock. Grapes and Wine *Type 1*: astringent, unbalanced, alcoholic wine. Grapes and wine *Type 2*: fruity, medium concentration, balanced wine. Grapes and Wine *Type 3*: vegetal, low concentration, high acidity wine (Deloire et al. 2005). The same plot can produce, according to the weather condition of the year, different types of grapes (e.g., *Plot B&C –Type 1* & *Type 2*). The same type of grapes can be obtained through different ecophysiological pathways (e.g., *Type 1*)

today's viticulture could become a favorable environment for a satisfactory vineyard operation in the future. The conceptual model presented here can serve as a support to the implementation of new practices in the frame of a changing climate and to reconsider plot localization.

Although our model does not include bio-aggressors, it can participate in the management of pests and diseases pressure by a better characterization of the effects of physical environment and agricultural practices on vine development. The vigor of the vine is generally favorable to certain diseases; thus, a vigorous vine will be more susceptible to downy mildew (*Plasmopara viticola*) given a more favorable microclimate for the development of the fungus and a higher proportion of susceptible young leaves on secondary stems (Calonnec et al. 2009; Valdes et al. 2005). Upstream management of vigor through modeling the functioning of the vine can help to move towards practices aligned with the recommendations of agro-ecology (Dalgaard et al. 2003).

The vine functioning pathway sets the foundations and the structure of a systemic model that takes into account all the vine system linking the environmental factors, the agricultural practices and the quality of wine.

4.4 Selection of Variables and Relations Taken into Account: An Application Case

The impact of permanent environmental factors on the functioning of the vine can be evaluated through the characterization of Terroir Unit database and the calculation of two composite variables (Morlat et al. 2001). One variable is used to characterize the vigor of the vine imparted by the permanent environmental factors. It is calculated according to the water holding capacity of the soil, the gravel percentage on the soil profile and the hardness of the parent-rock. Permanent environmental factors have an impact on the earliness of the phenological stages and especially on the beginning of the vegetative cycle with the period of budbreak. They influence the mesoclimate and the pedoclimate of the plots. Another variable is used to characterize earliness at the beginning of the vine cycle. It is calculated according to several parameters: gravel percentage on the soil profile, soil depth, hardness of the parent-rock, humidity at field capacity, natural drainage, color of soil surface, maximum rooting depth, altitude above sea level, landscape opening, and the exposure.

Protected designation of origin (PDO) regulations enforce some practices to obtain the best vine functioning of the vine according to the sensorial characteristics of the PDO expected. Other practices are not regulated and winegrowers need to optimize them according to the environmental factors of their plots and the products they want to elaborate. We take the example of the "Chinon" PDO, located in the middle Loire valley (France) (Table 1). Wines are mainly produced with the Cabernet franc variety. Winegrowers can choose the type of rootstock as well as the type of clone within the Cabernet franc or Cabernet Sauvignon varieties. As mentioned previously, the choice of the rootstock has an impact on the vigor and the earliness of the vine. Different clones of the same grapevine variety lead to different levels of yield and quality of grapes. Next, the type of soil management is a decision of the producers. They can sow a cover crop in between the vine rows; that will affect the vine development by reducing its vigor. The type of winter pruning determines the canopy structure and the yield potential. It is enforced through the PDO regulations but producers still can decide the date of pruning and therefore adjust the budbreak period according to the risk of spring frost. During the vegetative and reproductive cycle of the vine, winegrowers can adapt some annual practices to limit the development of the vine and better protect it against pest and fungus attacks.

Annual practices carried out during the vine vegetative cycle are considered as corrective practices and are not taken into account in our model. Our objective is to propose a model to optimize the choice of perennial and semi-perennial practices according to the environmental factors of the plots and the wines that the winegrowers want to elaborate. In the Chinon case, two perennial practices should firstly be looked at: the choice of the rootstock and the clone. Secondly, the impact of the soil

Table 1 Regulated and non-regulated practices for Chinon PDO wines (decree n°2011-1557). PDO: protected designation of origin

	Regulated practices	Non-regulated practices
Perennial practices	Grapevine variety: Cabernet franc (>90%); Cabernet Sauvignon (<10%) Density of plantation >4,500 vine/ha Row spacing <2.1 m Plant spacing on the row >1 m	Type of rootstock Type of clone for each grapevine variety
Semi-perennial practices	Pruning: single Guyot system Yield level <9,500 kg/ha	Soil management: cover crop choice, organic and/or mineral fertilization
Annual practices	Pruning: 7–10 buds on the cane preserved and one spur for the generation of the replacement cane Ratio between foliage height and row spacing: >0.6 that conditioning the minimal topping height Irrigation prohibited Minimal sugar content of grapes (g/l) Vintage permit	Day of pruning Topping frequency Disbudding Leaf removal Remove green bunches of grapes Pesticide protection

management on the functioning of the vine needs to be determined. Thirdly, the date of winter pruning needs to be considered.

Typology of climatic years of the past can be used to select contrasting weather databases. In the middle Loire valley, four types have been identified (Coulon et al. 2011). Weather data such as the air temperature can be modeled to calculate the dates of the phenological stages. Rainfall and water supply to the vine can be computed for contrasted climatic years in order to model the highest variability of climatic factors.

The output of the model needs to be determined. Based on expert evaluations, Courtin et al. (2006) distinguish wines of Chinon according to their structure. This classification is coherent with the two styles of Chinon wines, characterized as light or full-bodied (Vins de Loire). In this application case, the typology proposed by Courtin et al. (2006) can be used. The wine styles used as output can be defined by several experts of the area studied.

The conceptual framework is presented Fig. 3. It links the variables influencing wine style on the Chinon area. It sets a basis for a model development, providing a structure for the future mathematical implementation.

4.5 Methodology of Model Implementation in a Systemic Approach

A computer implementation of the conceptual model must be conducted. Knowledge-based and artificial intelligence techniques can be used to model environmental systems (Chen et al. 2008). Models build, more particularly those

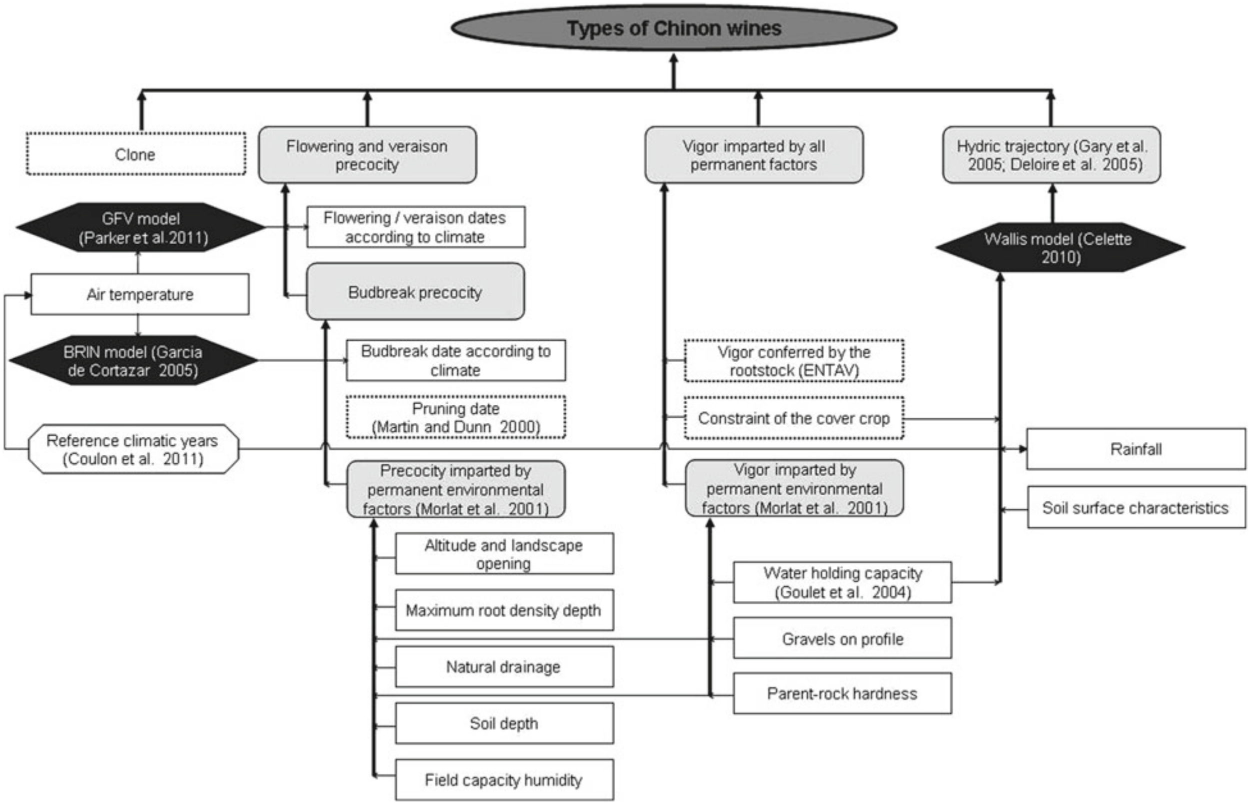

Fig. 3 Conceptual framework to model the vine functioning pathway. Application to a middle Loire valley vineyard: Chinon PDO wines. PDO: protected designation of origin

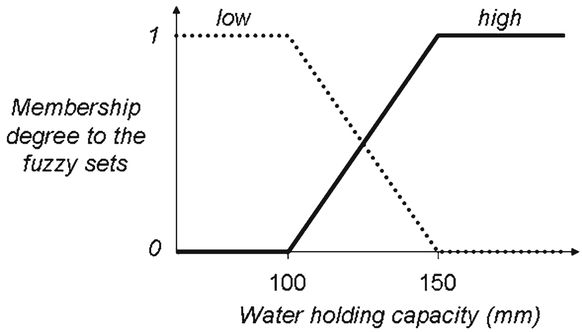

Fig. 4 Partitioning of the input variables of the model using fuzzy logic. In this example the water holding capacity is totally *low* for values lower than 100 mm and totally *high* for values higher than 150 mm

based on fuzzy inference systems, should be able to represent complex and imprecise systems that are not very well described by complex mathematical models.

In practice, the wine consultant or researcher characterizes the management of the vine in relation to average parameters; e.g., low or high water holding capacity, early or late development using qualitative terms. The output of our conceptual model is a type of product (grape/vine). The modeling approach is thus qualitative. Modeling in the form of an expert system based on decision rules such as "if… then…" associated with fuzzy logic seems the most appropriate procedure (Jackson 1998). This method allows to represent specific information (e.g., a maximal water holding capacity expressed in millimeters) in a lexical form (e.g., 'low' or 'high water holding capacity') that can be integrated into an expert system (Zadeh 1965). Furthermore, fuzzy logic is able to deal with uncertain data. Studies using this methodology have been conducted in viticulture (Grelier et al. 2007; Paoli et al. 2005; Coulon et al. 2010; Thiollet-Scholtus 2004) and show the usefulness of this approach.

Firstly, decision rules aggregated variables, for example "If Water holding capacity is *high* and the percent of gravels on the soil profile is *low* and the parent-rock hardness is *crumbly* then the vine vigor imparted by the permanent environmental factors is *high", and* rules conclusion values must be defined, for example the value 3 on a [1;3] scale for the previous decision rule given as an example (Coulon et al. 2010). Kaufman et al. (2009) mention that some experts considered the need for compensation if one parameter would indicate good conditions for plant growth and another one bad conditions; other experts suppose that plant growth is limited by the parameter with the worst value and allow no compensation. An accurate and interpretable system can be built by integrating expert knowledge and knowledge induced from data (Guillaume and Magdalena 2006).

Secondly, each quantitative variable must be partitioned in fuzzy sets (Fig. 4). Even if fuzzy sets allow a progressive transition between linguistic labels of a variable such as between 'low' and 'high', crisp parameters need to be determined. Model parameters can be adapted to local environment but could also make sense to compare areas or regions using the same set of parameters.

Modeling the vine system with a qualitative approach is an original method complementary to the functional approaches developed in complex crop models. The conceptual model proposed here is less accurate than a functional mathematical model but it takes into account all the vine system.

5 Conclusion

In one hand, the review of the current knowledge about the relationships between environmental factors, agricultural practices, the growth of the vine and the characteristics of grapes and wine and in another hand, the review of current model formalizing these relations allow us to propose a conceptual model to better manage the choice of agricultural practices according to the environmental factors and the type of wine expected.

Our model is based on the concept of "the vine functioning pathway", which we define as the logical and ordered combination of the effects of environmental factors and agricultural practices on the level of vigor and earliness of the vine and the final characteristics of the product. We proposed to implement the conceptual model using fuzzy inference systems. This method allows representing complex and imprecise systems; that are not very well described by complex mathematical models. The implemented model will be less accurate than a functional mathematical model but it will take into account all the vine system.

Once implemented, the model based on the concept of the 'vine functioning pathway' can serve as a support tool for decision making in order to optimize the choice of practices in relation to environmental factors. In the short term, the tool can help to adapt annual practices. In the medium-term, it can contribute to sustainable practices. In the long term, it can assess the impacts of climate change on the behavior of the vine and the evolution of terroirs.

Modeling the functioning of the vine in the vineyard at the scale of the plot is in line with a process of sustainable production. It allows optimizing the choice of agricultural practices and reduces those with a corrective function, reducing at the same time production costs while promoting the production of quality products and maximizing the potential value of soils.

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References

Asselin C, Barbeau G, Morlat R (2001) Climatic components approach to diverse scales in viticulture zoning (in French). Bull OIV 74(843–844):301–318

Barbeau G, Blin A (2010) Rootstock influence on agronomic comportement of the vine (cv. Cabernet franc) in the middle Loire valley (in French). Available at http://www.techniloire.com/documents/124963587/Essai%20porte-greffe%20CF_vf.pdf. Accessed 23 Mar 2011

Barbeau G, Asselin C, Morlat R (1998) Estimate of the viticultural potential of the Loire valley "terroirs" according to a vine's cycle precocity index (in French). Bull OIV 71(805/806):247–262

Barbeau G, Goulet E, Ramillon D, Rioux D, Blin A, Marsault J, Panneau JPP (2006) Effect of the interaction rootstock-grass cover on the agronomical response of the grapevine *Vitis vinifera L.,* cvs. Cabernet Franc et Chenin (in French). Progrès Agric Vitic 123(4):80–86

Bérard L, Marchenay P (2006) Local products and geographical indications: taking account of local knowledge and biodiversity. Int Soc Sci J 58(187):109–116

Bell SJ, Henschke PA (2005) Implications of nitrogen nutrition for grapes, fermentation and wine. Aust J Grape Wine Res 11(3):242–295. doi:10.1111/j.1755-0238.2005.tb00028.x

Bodin F, Morlat R (2003) Characterizing a vine terroir by combining a pedological field model and a survey of the vine growers in the Anjou region (France). J Int Sci Vigne Vin 37(4):199–211

Bodin F, Morlat R (2006) Characterization of viticultural terroirs using a simple field model based on soil depth I. Validation of the water supply regime, phenology and vine vigour, in the Anjou vineyard (France). Plant Soil 281(1/2):37–54. doi:10.1007/s11104-005-3768-0

Bramley RGV, Hamilton RP (2007) Terroir and precision viticulture: are they compatible? J Int Sci Vigne Vin 41(1):1–8

Brisson N, Gary C, Justes E, Roche R, Mary B, Ripoche D, Zimmer D, Sierra J, Bertuzzi P, Burger P, Bussiere F, Cabidoche YM, Cellier P, Debaeke P, Gaudillere JP, Henault C, Maraux F, Seguin B, Sinoquet (2001) An overview of the crop model STICS. Paper from the 2nd international symposium on modeling cropping systems, Florence, Italy

Cadot Y (2006) Le lien du vin au terroir: complexité du concept de typicité. (in French). Revue des œnologues 119:9–11

Cadot Y (2010) Influence de la date de vendange sur les composés phénoliques de la baie de raisin; conséquences pour la typicité du vin. Ph.D. thesis, Centre International d'Études Supérieures en Sciences Agronomiques Montpellier Supagro, France, 229pp

Cadot Y, Caille S, Samson A, Thiollet-Scholtus M, Barbeau G, Casabianca F, Cheynier V (2011) Sensory typicality related to terroir: from conceptual to perceptive representation. Are wine-making practices decisive ? In: Dunod (ed) Actualités Oenologiques 2011, paper from the '9e symposium international d'œnologie', Bordeaux, 15–17 Juin 2011, Paris, France

Cadot Y, Caille S, Thiollet-Scholtus M, Samson A, Barbeau G, Casabianca F, Cheynier V (2012) Characterisation of typicality for wines related to terroir by conceptual and by perceptual representations. An application to red wines from the Loire Valley. Food Qual Pref 24(1):48–58. doi:10.1016/j.foodqual.2011.08.012

Calonnec A, Cartolaro P, Chadoeuf J (2009) Highlighting features of spatiotemporal spread of powdery mildew epidemics in the vineyard using statistical modeling on field experimental data. Phytopathology 99(4):411–422. doi:10.1094/phyto-99-4-0411

Carbonneau A (1993) Unité de terroir viticole. (in French). Progrès Agric. Vitic 2:29–30

Carbonneau A, Deloire A, Jaillard B (2007) La vigne. Physiologie, terroir, culture. (in French). Dunod (ed), Paris, France

Carey V, Archer E, Barbeau G, Saayman D (2007) The use of local knowledge relating to vineyard performance to identify viticultural terroirs in stellenbosch and surrounds. Paper from the international workshop on advances in grapevine and wine research. International Society Horticultural Science, pp 385–391

Carey VA, Saayman D, Archer E, Barbeau G, Wallace M (2008) Viticultural terroirs in Stellenbosch. South Africa. I. The identification of natural terroir units. J Int Sci Vigne Vin 42(4):169–183

Casabianca F, Sylvander B, Noël Y, Béranger C, Coulon J-B, Roncin F (2005). Terroir et typicité: deux concepts-clés des Appellations d'Origine Contrôlée. Essai de définitions scientifiques et opérationnelles. (in French). Paper from an international symposium on territory and regional development issues, Lyon, 9–11 Mar 2005

Celette F, Findeling A, Gary C (2009) Competition for nitrogen in an unfertilized intercropping system: the case of an association of grapevine and grass cover in a Mediterranean climate. Eur J Agron 30(1):41–51. doi:10.1016/j.eja.2008.07.003

Celette F, Ripoche A, Gary C (2010) WaLIS-a simple model to simulate water partitioning in a crop association: the example of an intercropped vineyard. Agric Water Manage 97(11):1749–1759. doi:10.1016/j.agwat.2010.06.008

Cellier P, Jacquet A, Bautrais P, Morlat R, Delanchy P (1996) Modélisation du régime thermique des sols de vignoble du Val de Loire: Relations avec des variables utilisables pour la caractérisation des terroirs. (in French). Paper from the first International Terroir Congress 17–18 July 1996, Angers, France, pp 107–112

Chacon JL, Garcia E, Martinez J, Romero R, Gomez S (2009) Impact of the vine water status on the berry and seed phenolic composition of 'Merlot' (Vitis vinifera L.) cultivated in a warm climate: consequence for the style of wine. Vitis 48(1):7–9

Champagnol (1984) Eléments de physiologie de la vigne et de viticulture générale. (in French). Champagnol (ed), St-Gely du Fesc, France

Charters S, Pettigrew S (2007) The dimensions of wine quality. Food Qual Pref 18(7):997–1007. doi:10.1016/j.foodqual.2007.04.003

Chen SH, Jakeman AJ, Norton JP (2008) Artificial intelligence techniques: an introduction to their use for modelling environmental systems. Math Comput Simul 78(2–3):379–400

Coipel J, Rodriguez Lovelle B, Sipp C, Van Leeuwen C (2006) "Terroir" effect, as a result of environmental stress, depends more on soil depth than on soil type (Vitis vinifera L. cv. Grenache noir, Côtes du Rhône, France, 2000). J Int Sci Vigne Vin 40(4):177–185

Cortell JM, Halbleib M, Gallagher AV, Kennedy JA, Righetti TL (2005) Influence of vine vigor on grape (Vitis vinifera L. cv. Pinot Noir) and wine proanthocyanidins. J Agric Food Chem 53(14):5798–5808. doi:10.1021/jf0504770

Coulon C, Rioux D, Guillaume S, Charnomordic B, Barbeau G, Thiollet-Scholtus M (2010) Design of an indicator of vine vigor potential conferred by soil (VIPOS), using a fuzzy expert system. Paper from the VIIIe international Terroir Congress, Soave, Italy, pp 87–92

Coulon C, Ganenco A, Neethling E, Thiollet-Scholtus M (2011) Méthode de typologie d'années climatiques de référence à l'usage de la modélisation. Application à la moyenne Vallée de la Loire. (in French). Progrès Agric. Vitic 17–2011:347–353

Courtin, V, Rioux D, Boutin D, Cesbron S, Kasprik A-C (2006) Typologie des vins en rapport avec les facteurs environnementaux du terroir et le cépage. Application au vignoble de Chinon. (in French). Paper from the VIe International Terroir Congress, Bordeaux-Montpellier, France

Cros MJ, Duru M, Garcia F, Martin-Clouaire R (2003) A biophysical dairy farm model to evaluate rotational grazing management strategies. Agronomie 23(2):105–122. doi:10.1051/agro:2002071

Dai ZW, Vivin P, Barrieu F, Ollat N, Delrot S (2010) Physiological and modelling approaches to understand water and carbon fluxes during grape berry growth and quality development: a review. Aust J Grape Wine Res 16:70–85

Dalgaard T, Hutchings NJ, Porter JR (2003) Agroecology, scaling and interdisciplinarity. Agric Ecosyst Environ 100(1):39–51. doi:10.1016/S0167-8809(03)00152-X

de Turckheim E, Hubert B, Messean A (2009) Concevoir et construire la décision. Quae (ed), Paris, France

Delabays N, Spring JL, Mermillod G (2006) Essai d'enherbement de la vigne avec des espèces peu concurrentielles: aspects botaniques et malherbologiques. Revue suisse Vitic Arboric Hortic 38(6):343–354

Deloire A, Ojeda H, Zebic O, Bernard N (2005) Influence de l'état hydrique de la vigne sur le style de vin. Progrès Agric Vitic 21:455–462

Dry PR, Loveys BR (1998) Factors influencing grapevine vigour and the potential for control with partial rootzone drying. Aust J Grape Wine Res 4(3):140–148. doi:10.1111/j.1755-0238.1998.tb00143.x

Fernandez Martinez R, Lostado Lorza R, Fernandez Ceniceros J, Martinez-de-Pison Ascacibar FJ (2012) Comparative analysis of learning and meta-learning algorithms for creating models for predicting the probable alcohol level during the ripening of grape berries. Comput Electron Agric 80:54–62

Garcia de Cortazar Atauri I, Brisson N, Seguin B, Gaudillere JP, Baculat B (2005) Simulation of budbreak date for vine. The BRIN model. Some applications in climate change study. Paper from the XIV International GESCO Viticulture Congress, 23–27 Aug 2005, Geisenheim, Germany, pp 485–490.

Garcia de Cortazar Atauri I (2006) Adaptation du modèle STICS à la vigne (Vitis vinifera L.) utilisation dans le cadre d'une étude d'impact du changement climatique à l'échelle de la France. Ph.D. thesis, Ecole Nationale Supérieure Agronomique de Montpellier, 292 pp

Garcia de Cortazar Atauri I, Brisson N, Gaudillere JP (2009) Performance of several models for predicting budburst date of grapevine (Vitis vinifera L.). Int J Biometeorol 53(4):317–326. doi:10.1007/s00484-009-0217-4

Gary C, Payan J-C, Kansou K, Pellegrino A, Wery J (2005) Un outil de diagnostic du vécu hydrique de parcelles viticoles, en relation avec des objectifs de rendement et de qualité. Paper from the XIV International GESCO Viticulture Congress, 23–27 Aug 2005, Geisenheim, Germany, pp 449–454

GIEC (2007) Contribution of working groups I, II and III to the fourth assessment report of the intergovernmental panel on climate change, Genève, Sweden

Girardin P, Bockstaller C, Van der Werf H (1999) Indicators: tools to evaluate the environmental impacts of farming systems. J Sustain Agric 13(4):5–21. doi:10.1300/J064v13n04_03

Girona J, Marsal J, Mata M, Del Campo J, Basile B (2009) Phenological sensitivity of berry growth and composition of Tempranillo grapevines (Vitis vinifera L.) to water stress. Aust J Grape Wine Res 15(3):268–277. doi:10.1111/j.1755-0238.2009.00059.x

Goulet E, Barbeau G (2006) Apports des mesures de résistivité électrique du sol dans les études sur le fonctionnement hydrique du système sol/vigne. J Int Sci Vigne Vin 40(2):57–69

Goulet E, Morlat R (2010) The use of surveys among wine growers in vineyards of the middle-Loire Valley (France), in relation to terroir studies. Land Use Policy 28:770–782. doi:10.1016/j.landusepol.2011.01.003

Goulet E, Morlat R, Rioux D, Cesbron S (2004) A calculation method of available soil water content: application to viticultural terroirs mapping of the Loire valley. J Int Sci Vigne Vin 38(4):231–235

Goutouly J-P, Drissi R, Forget D, Gaudillère J-P (2006) Caractérisation de la vigueur de la vigne par l'indice NDVI mesuré au sol. Paper from the International Terroir Congress, Bordeaux-Montpellier, France

Gray JD, Gibson RJ, Coombe BG, Iland PG, Pattison SJ (1997) Assessment of winegrape value in the vineyard—survey of cv. Shiraz from South Australian vineyards in 1992. Aust J Grape Wine Res 3(3):1–8

Grelier M, Guillaume S, Tisseyre B, Scholasch T (2007) Precision viticulture data analysis using fuzzy inference systems. J Int Sci Vigne Vin 41(1):19–31

Guillaume S, Magdalena L (2006) Expert guided integration of induced knowledge into a fuzzy knowledge base. Soft Comput 10(9):773–784

Gutierrez AP, Williams DW, Kido H (1985) A model of grape growth and development: the mathematical structure and biological considerations. Crop Sci 25(5):721–728

Hardie WJ, Considine JA (1976) Response of grapes to water-deficit stress in particular stages of development. Am J Enol Vitic 27(2):55–61

Havinal MN, Tambe TB, Patil SP (2008) Comparative studies on vine vigour and fruitfulness of grape wine varieties. Asian J Hort 3(1):180–182

Holt HE, Francis IL, Field J, Herderich MJ, Iland PG (2008) Relationships between berry size, berry phenolic composition and wine quality scores for Cabernet Sauvignon (Vitis vinifera L.) from different pruning treatments and different vintages. Aust J Grape Wine Res 14(3):191–202. doi:10.1111/j.1755-0238.2008.00019.x

Homayouni S, Germain C, Lavialle O, Grenier G, Goutouly JP, Van Leeuwen C, Da Costa JP (2008) Abundance weighting for improved vegetation mapping in row crops: application to vineyard vigour monitoring. Can J Remote Sens 34:S228–S239

Jackson J (1998) Introduction to expert systems, 3rd edn. Addison Wesley, Harlow

Jeuffroy M-H, Bergez J-E, David C, Flenet F, Gate P, Loyce C, Maupas F, Meynard J-M, Reau R, Surleau-Chambenoit C (2008) Utilisation des modèles pour l'aide à la conception et à l'évaluation d'innovations techniques en production végétale: bilan et perspectives. In: Système de culture innovants et durables. Educagri (ed), Paris, pp 109–128

Jones GV, White MA, Cooper OR, Storchmann K (2005) Climate change and global wine quality. Clim Chang 73(3):319–343. doi:10.1007/s10584-005-4704-2
Kaufman M, Tobias S, Schulin R (2009) Quality evaluation of restored soil with a fuzzy logic expert system. Geoderma 151(3–4):209–302.
Keller M (2010) Managing grapevines to optimise fruit development in a challenging environment: a climate change primer for viticulturists. Aust J Grape Wine Res 16:56–69. doi:10.1111/j.1755-0238.2009.00077.x
Kliewer WM, Dokoozlian NK (2005) Leaf area/crop weight ratios of grapevines: influence on fruit composition and wine quality. Am J Enol Vitic 56(2):170–181
Koundouras S, Van Leeuwen C, Seguin G, Glories Y (1999) Influence of water availability on vine vegetative growth, berry ripening and wine characteristics in the Mediterranean zone (example of Nemea, Greece, variety Saint-George, 1997). J Int Sci Vigne Vin 33(4):149–160
Landon JL, Weller K, Harbertson JF, Ross CF (2008) Chemical and sensory evaluation of astringency in Washington State red wines. Am J Enol Vitic 59(2):153–158
Lattey KA, Bramley BR, Francis IL (2010) Consumer acceptability, sensory properties and expert quality judgements of Australian Cabernet Sauvignon and Shiraz wines. Aust J Grape Wine Res 16(1):189–202
Le Gal PY, Merot A, Moulin CH, Navarrete M, Wery J (2009) A modelling framework to support farmers in designing agricultural production systems. Environ Model Softw 25(2):258–268. doi:10.1016/j.envsoft.2008.12.013
Lebon E, Dumas V, Pieri P, Schultz HR (2003) Modelling the seasonal dynamics of the soil water balance of vineyards. Funct Plant Biol 30(6):699–710
Lebon E, Pellegrino A, Tardieu F, Lecoeur J (2004) Shoot development in grapevine (Vitis vinifera) is affected by the modular branching pattern of the stem and intra- and inter-shoot trophic competition. Ann Bot 93:236–274
Lesschaeve I (2003) Evaluating wine "typicité" using descriptive analysis. Paper from the 5th Pangborn sensory science symposium
Louarn G, Dauzat J, Lecoeur J, Lebon E (2008) Influence of trellis system and shoot positioning on light interception and distribution in two grapevine cultivars with different architectures: an original approach based on 3D canopy modeling. Aust J Grape Wine Res 14(3):143–152
Loyce C, Rellier JP, Meynard JM (2002) Management planning for winter wheat with multiple objectives (1): the BETHA system. Agric Syst 72(1):9–31. doi:10.1016/S0308-521X(01)00064-6
Marguerit E, Brendel O, Van Leeuwen C, Delrot S, Ollat N (2011) Grapevine rootstock genetically determine scion transpiration and its response to water deficit: an integrated approach using ecophysiology and quantitative genetics. In: Systems approaches to crop improvement, Harpenden, United Kingdom, pp 89
Martin SR, Dunn GM (2000) Effect of pruning time and hydrogen cyanamide on budburst and subsequent phenology of Vitis vinifera L. variety Cabernet Sauvignon in central Victoria. Aust J Grape Wine Res 6(1):31–39. doi:10.1111/j.1755-0238.2000.tb00159.x
Martinez de Toda F, Tardaguila J, Sancha JC (2007) Estimation of grape quality in vineyards using a new viticultural index. Vitis 46(4):168–173
Matthews MA, Anderson MM (1988) Fruit ripening in Vitis vinifera L.: responses to seasonal water deficits. Am J Enol Vitic 39(4):313–320
Meynard JM (2008) Produire autrement: réinventer les systèmes de culture. In: Systèmes de culture innovants et durables. Educagri, Paris, pp 11–27
Minet F, Asselin C, Duc D, Barbeau G, Cosneau M, Bouvet MH (2000) Etude de l'adaptation de dix porte-greffe sur cépage Chenin, en Val de Loire, pour la production de vin de l'AOC "Coteaux du Layon". Progrès Agric Vitic 117(19):410–422
Morlat R (2010) Traité de viticulture de terroir. Lavoisier (ed), Paris, France
Morlat R, Remoue M, Pinet P (1984) The influence of the planting density and the method of soil management on root growth in a vineyard planted on good soil. Agronomie 4(5):485–491
Morlat R, Penavayre M, Jacquet A, Asselin C, Lemaitre C (1992) The effects of soils on water status and photosynthesis of grapevines during an exceptionally dry year (1990). Effects on grape ripening. J Int Sci Vigne Vin 26(4):197–220

Morlat R, Jacquet A, Asselin C (1997) Variabilité de la précocité de la vigne en Val de Loire: Rôle du terroir et du millésime, conséquences sur la composition de la baie. Revue Française Oenologie 165:11–22

Morlat R, Barbeau G, Asselin C, Besnard E (2001) Terroir Viticole: de la recherche à la valorisation par le vigneron. In: Un raisin de qualité: de la vigne à la cuve. J Int Sci Vigne Vin (Special issue): 21–33

Murisier F, Zufferey V (2006) The influence of plant density and hedgerow height on grape and wine quality. Trial on chasselas vines in leytron (Wallis, CH). Revue suisse Vitic Arboric Hortic 38(5):271–276

Nendel C (2009) Grapevine bud break prediction for cool winter climates. Int J Biometeorol 54(3):231–241. doi:10.1007/s00484-009-0274-8

Nendel C, Kersebaum KC (2004) A simple model approach to simulate nitrogen dynamics in vineyard soils. Ecol Model 177(1/2):1–15

OIV (2004) Development of sustainable viticulture. Résolution CST 01/2004. International organisation of vine and wine

OIV (2010) Définition of vitivinicultural terroir. Résolution VITI 333/2010. International organisation of vine and wine

Ojeda H (1999) Influence de la contrainte hydrique sur la croissance du péricarpe et sur l'évolution des phénols des baies de raisin (Vitis vinifera L.) cv. Syrah. Ph.D. thesis, University of Montpellier, France

Ojeda H, Andary C, Kraeva E, Carbonneau A, Deloire A (2002) Influence of pre- and post-veraison water deficit on synthesis and concentration of skin phenolic compounds during berry growth of Vitis vinifera cv. Shiraz Am J Enol Vitic 53(4):261–267

Orlandini S, Dalla Marta A, Matti GB (2006) Modelling grapevine responses to different training systems. Acta Hortic 707:49–56

Pallas B, Louarn G, Christophe A, Lebon E, Lecoeur J (2008) Influence of intra-shoot trophic competition on shoot development in two grapevine cultivars (Vitis vinifera). Physiol Plant 134(1):49–63

Paoli J-N, Tisseyre B, Zebic O, Guillaume S (2005) Détermination et cartographie des potentialités viticoles: une approche experte. Progrès Agric Vitic 23:508–511

Parker AK, Garcia de Cortazar Atauri I, Van Leeuwen C, Chuine I (2011) General phenological model to characterise the timing of flowering and veraison of Vitis viniferaL.

Passioura JB (1996) Simulation models: science, snake oil, education or engineering? Agron J 88:690.694

Pellegrino A, Goze E, Lebon E, Wery J (2006) A model-based diagnosis tool to evaluate the water stress experienced by grapevine in field sites. Eur J Agron 25(1):49–59

Poni S, Casalini L, Bernizzoni F, Civardi S, Intrieri C (2006) Effects of early defoliation on shoot photosynthesis, yield components, and grape composition. Am J Enol Vitic 57(4):397–407

Pouget R (1963) Recherches physiologiques sur le repos végétatif de la vigne (Vitis vinifera): la dormance des bourgeons et le mecanisme de sa disparition. Ann Amelior Plantes 13(special issue 1):1–27

Reynolds AG, Heuvel JEV (2009) Influence of grapevine training systems on vine growth and fruit composition: a review. Am J Enol Vitic 60(3):251–268

Ribéreau-Gayon P, Dubourdieu D, Donèche B, Lonvaud A (1998) Traité d'oenologie. 1. Microbiologie du vin. Vinifications (ed) DUNOD, Paris

Riou C (1994) The effect of climate on grape ripening: application to the zoning of sugar content in the European community. Office Publ. Officielles des CE, EUR 15863 FR/EN, Luxembourg, 322pp

Riou C, Lebon E (2000) Application d'un modèle de bilan hydrique et de la mesure de la température de couvert au diagnostic du stress hydrique de la vigne à la parcelle. Bull OIV 73(837–838):755–764

Rives M (2000) Vigour, pruning, and cropping in the grapevine (Vitis vinifera L.). II. Experiments on vigour, pruning and cropping. Agronomie 20(2):205–213. doi:10.1051/agro:2000120

Roby G, Harbertson JF, Adams DA, Matthews MA (2004) Berry size and vine water deficits as factors in winegrape composition: anthocyanins and tannins. Aust J Grape Wine Res 10(2):100–107. doi:10.1111/j.1755-0238.2004.tb00012.x

Sadras VO, Petrie PR (2011) Climate shifts in south-eastern Australia: early maturity of Chardonnay. Shiraz and Cabernet Sauvignon is associated with early onset rather than faster ripening. Aust J Grape Wine Res 17(2):199–205. doi:10.1111/j.1755-0238.2011.00138.x
Sadras VO, Petrie PR (2012) Predicting the time course of grape ripening. Aust J Grape Wine Res 18(1):48–56. doi:10.1111/j.1755-0238.2011.00169.x
Sadras VO, Soar C, Petrie PR (2007) Quantification of time trends in vintage scores and their variability for major wine regions of Australia. Aust J Grape Wine Res 13(2):117–123. doi:10.1111/j.1755-0238.2007.tb00242.x
Sebillotte M (1974) Agronomie et agriculture. Essai d'analyse des taches de l'agronome. Cahier ORSTOM 24:3–25
Sinclair TR, Seligman NG (1996) Crop modeling: from infancy to maturity. Agron J 88(5):698–704. doi:10.2134/agronj1996.00021962008800050004x
Smart R, Robinson M (1991) Sunlight into wine. Winetitles (ed), Broadview, Australia
Smart DR, Schwass E, Lakso A, Morano L (2006) Grapevine rooting patterns: a comprehensive analysis and a review. Am J Enol Vitic 57(1):89–104
Soar CJ, Sadras VO, Petrie PR (2008) Climate drivers of red wine quality in four contrasting Australian wine regions. Aust J Grape Wine Res 14(2):78–90. doi:10.1111/j.1755-0238.2008.00011.x
Tesic D, Woolley DJ, Hewett EW, Martin DJ (2002) Environmental effects on cv Cabernet Sauvignon (Vitis vinifera L.) grown in Hawke's Bay, New Zealand. 1. Phenology and characterisation of viticultural environments. Aust J Grape Wine Res 8(1):15–26. doi:10.1111/j.1755-0238.2002.tb00208.x
Tesic D, Keller M, Hutton RJ (2007) Influence of vineyard floor management practices on grapevine vegetative growth, yield, and fruit composition. Am J Enol Vitic 58(1):1–11
Thiollet-Scholtus M (2004) Construction d'un indicateur de qualité des eaux de surface vis-à-vis des produits phytosanitaires à l'échelle du bassin versant viticole. Ph.D. thesis, Institut national Polytechnique de Lorraine, Colmar, France. Available from http://www.inra.fr/theses/these-integrale/Theses/thiollet/pdf/these.pdf
Tregoat O, Ollat N, Grenier G, Leeuwen CV (2001) Comparative study of the accuracy and speed of various methods for estimating vine leaf area. J Int Sci Vigne Vin 35(1):31–39
Trnka M, Brazdil R, Dubrovsky M, Semeradova D, Stepanek P, Dobrovolny P, Mozny M, Eitzinger J, Malek J, Formayer H, Balek J, Zalud Z (2011) A 200-year climate record in Central Europe: implications for agriculture. Agron Sustain Dev. doi:10.1007/s13593-011-0038-9
Trought MCT, Dixon R, Mills T, Greven M, Agnew R, Mauk JL, Praat JP (2008) The impact of differences in soil texture within a vineyard on vine vigour, vine earliness and juice composition. J Int Sci Vigne Vin 42(2):67–72
Ubalde JM, Sort X, Poch RM, Porta M (2007) Influence of edapho-climatic factors on grape quality in Conca de Barbera vineyards (Catalonia, Spain). J Int Sci Vigne Vin 41(1):33–41
Valdes H, Celette F, Fermaud M, Cartolaro P, Clerjeau M, Gary C (2005) How to evaluate the influence of vegetative vigour in vine sensitivity to cryptogamic diseases? Paper from the XIV International GESCO Viticulture Congress, Geisenheim, Germany, pp 832–838
Van Leeuwen C, Friant P, Chone X, Tregoat O, Koundouras S, Dubourdieu D (2004) Influence of climate, soil, and cultivar on terroir. Am J Enol Vitic 55(3):207–217
Van Leeuwen C, Tregoat O, Chone X, Bois B, Pernet D, Gaudillere JP (2009) Vine water status is a key factor in grape ripening and vintage quality for red bordeaux wine. How can it be assessed for vineyard management purpose ? J Int Sci Vigne Vin 43(3):121–134
Vaudour E, Morlat R, Van Leeuwen C, Doledec A-F (2005) Terroirs viticoles et sols. Dunod (ed), Paris, France
Vins de Loire. http://www.vinsdeloire.fr/SiteGP/FR/. Accessed 23 Mar 2011
Vivin P, Castelan M, Gaudillère J-P (2002) A source/sink model to simulate seasonal allocation of carbon in grapevine. Acta Hortic 584:43–56
Walker RR, Zhang X, Godwin DC, White R, Clingeleffer P (2005) Vinelogic growth and development simulation model – rootstock and salinity effects on vine performance. Paper from the XIV International GESCO Viticulture Congress, Geisenheim, Germany, 23–27 Aug 2005
Zadeh LA (1965) Fuzzy sets. Info Control 8:338–353
Zhu Y, Cao WX, Dai TB, Tian YC, Yao X (2007) A knowledge model system for wheat production management. Pedosphere 17(2):172–181. doi:10.1016/S1002-0160(07)60023-X

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