Chapter 8

Proteomics Potential and Its Contribution toward Sustainable Agriculture


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Sustainable agriculture has run its course during the past decades and “food security” issues are not what they used to be in the past. Due to the ever-changing global climate and population growth, food security is now one of the hottest topics of discussion around the world. To overcome food shortages, hunger, and starvation, a breakthrough in cutting-edge research and translation of the research findings to the field are desperately needed. Technologies including high-yielding varieties, intensive use of agrochemicals, and so on, used in the green revolution, helped to feed billions of people. However, they caused significant environmental degradation and seem unable to meet the future demand for food security. The impacts of global climate and environmental change mean that crops are prone to damage on an unprecedented scale. Therefore, there is a tremendously increased demand for research efforts and scientific collaboration to address the need for enhanced food production by minimizing the impacts of climate change in crop production. In this chapter, we not only discuss the past and future of sustainable agriculture but also try to bring to the forefront the necessity of expediting research using high-throughput omics technologies such as genomics, proteomics, and metabolomics. Among these, proteomics is the main focus of this review, in which we describe its use in crops, giving examples from cereals to legumes and fruits. We also give an insight into potential biomarkers and their exploitation in screening natural genetic resources, alone or in combination with other technology-derived results, for generating the next-generation crop plants for the twenty-first century and beyond.

We begin with the Industrial Revolution. In human history, the Industrial Revolution starting in the eighteenth century, followed by the green revolution in the mid-twentieth century, did not only incredibly increase human capacity but also accelerated world population growth. However, both revolutions have impacted on acceleration of changes in the global environment, biodiversity, and climate. Although the green revolution helped to feed billions of people, this heavy input-based technology seems unable to meet the future demands for food supply, especially in developing countries, due to deterioration of soil health and environmental pollution. It has been estimated that, over the last century, the atmospheric concentrations of carbon dioxide (CO₂), nitrous oxide (N₂O), and methane (CH₄) have increased by 25%, 16%, and 100%, respectively (Houghton et al. 2001; Hoffert et al. 2002; IPCC AR4 WGI 2007). As a result, the temperature on the earth’s surface has increased by about 0.76°C (IPCC 2007a). However, it is now known that over the last 50 years the rate of warming (0.13°C ± 0.03°C per decade) has increased nearly twofold compared with the last 100 years, which poses a serious threat to biodiversity, agricultural productivity, and food security in many countries. For example, it has been reported that global warming is causing receding and thinning of glaciers in the Himalaya at an accelerated rate (Hasnain 2002; IPCC 2007b). The Himalayan glaciers form a unique reservoir supporting the perennial rivers Indus, Ganga, and Brahmaputra, which are considered the lifeline of millions of people in the South Asian countries of Pakistan, Nepal, Bhutan, India, and Bangladesh.

Over the past three decades, and especially in the last few years, climate change has become one of the most heavily researched subjects in science (Lamb 1995). The theoretical analysis of most of the modelling approaches to climate change prediction does not consider potential evolutionary changes of living organisms. Recently published work has shown that many species are capable of relatively rapid genetic changes, which enhances their ability to invade new areas in response to anthropogenic ecosystem modification (Clements and Ditommaso 2011). Therefore, climate change represents a major challenge to maintenance of biodiversity and richness of plant species (Sommer et al. 2010). Soil microbes, including probiotic bacteria, play a key role in the ecosystem by driving major biogeochemical processes directly contributing to the maintenance of plant productivity. Climate change generally affects organisms either directly via physiological stress...
or indirectly via changing relationships among species (Harley 2011). As mentioned by Harley (2011), there is some evidence suggesting that anthropogenic climate change can alter interspecific interactions and produce unexpected changes in species distributions, community structure, and diversity. The changed climate will create some common problems for wildlife, such as loss of habitat due to inundation, shortage of food due to breaks in the food chain, loss of breeding grounds, altered breeding patterns, and so on. In this situation many wild species may find it difficult to maintain their existence. Therefore, it is important to understand whether and how climate change impacts on the biodiversity of organisms, including microorganisms, and how such changes might feed back to influence changes in plant productivity (Thuiller 2007; van der Heijden et al. 2008).

As well as biodiversity and plant productivity, climate change is also posing a challenge to the interconnected development goals such as supply of food, energy, and water (Beddington 2009; Godfray et al. 2010).

It is now well accepted that climate change is posing a threat to agricultural production systems worldwide, and more severely in tropical and subtropical countries (Rosenzweig and Parry 1994). Although several studies suggest that doubling of the atmospheric CO₂ concentration will lead to only a small decrease in total global crop production, developing countries are likely to bear the brunt of the problem, including a considerable decrease in crop production (Rosenzweig and Parry 1994). Recently, Lobell et al. (2011) analyzed the climate trends and global crop production since 1980. Those authors found that, in the cropping regions and growing seasons of most countries, temperature trends from 1980 to 2008 exceeded one standard deviation of historic year-to-year variability. Further, their analysis indicates that global maize and wheat production has declined by 3.8% and 5.5%, respectively, relative to a counterfactual without climate trends (Lobell et al. 2011). Rice production has also decreased, but to a lesser extent than the other cereals. It has been noted that the climate trends were large enough in some countries to offset a significant portion of the increases in average yields that arose from technology, CO₂ fertilization, and other factors (Lobell et al. 2011).

It must be emphasized that climate change has already led to altered distributions of species, phenotypic variation, and allele frequencies (Bradshaw and Holzapfel 2001; Franks et al. 2007; Lobell et al. 2011; Lynch and Lande 1993; Umina et al. 2005), and that the impact of changing climate is expected to intensify (Hancock et al. 2011). Hoffmann and Sgrò (2011) have reported that the capacity to respond to changing climate is likely to vary widely as a consequence of variation among species in their degree of phenotypic plasticity and their potential for genetic adaptation, which in turn depends on the amount of standing genetic variation and the rate at which new genetic variation arises. Understanding the genetic basis and modes of adaptation to current climatic conditions will be essential to accurately predict responses to future environmental change.

*Arabidopsis thaliana* (hereafter called *Arabidopsis*) is an excellent model for investigating the genetic basis and mode of adaptation to climate. One reason is the extensive climatic variation across its native range. The other reason is the availability of genome-wide single-nucleotide polymorphism data among a geographically diverse collection. Recently, Hancock et al. (2011) conducted a genome-wide scan to identify climate-adaptive genetic loci and pathways in *Arabidopsis*. Amino acid-changing variants were significantly enriched among the loci strongly correlated with climate, suggesting that our scan effectively detects adaptive alleles. Their findings predicted relative fitness among a set of geographically diverse *Arabidopsis* accessions when grown together in a common environment and provide a set of candidates for dissecting the molecular bases of climate adaptations, as well as insights about the prevalence of selective sweeps, which has implications for predicting the rate of adaptation. Findings of their study also suggested that species such as *Arabidopsis* may reach adaptive limits under rapid climate change, due to the constraints imposed by waiting for new mutations. In another study on *Arabidopsis*, Fournier-Level et al. (2011) have identified candidate loci for local adaptation from a genome-wide association study of lifetime fitness in geographically diverse accessions. The fitness-associated loci were found to exhibit both geographic and climatic signatures of local adaptation. Relative to genomic controls, high-fitness
alleles were generally distributed closer to the site where they increased fitness, occupying specific and distinct climate spaces. Independent loci with different molecular functions contributed most strongly to fitness variation in each site, which suggested that independent local adaptation by distinct genetic mechanisms may facilitate a flexible evolutionary response to changing environment across a species range.

Rice (*Oryza sativa* L.) is the cereal crop and monocot genome model (Goff et al. 2002; Yu et al. 2002), and, as it is a food for life, research into rice biology has progressed greatly over the years since its genome sequence became available in 2002. In particular, proteomics has progressed at an unexpected pace since 2000, establishing and understanding the proteomes of tissues, organs, and organelles under normal and adverse environmental conditions (for review see Agrawal and Rakwal 2011, and references therein). Established proteomes have also helped in reannotating the rice genome and revealing new roles of previously known proteins. Proteomics-based discoveries in rice are likely to be translated into improving crop plants, and vice versa, against ever-changing environmental factors. A major role of rice proteomics is envisioned in addressing the basic global problem of food security under threat from climate change and to meet the demands of the human population, which is projected to reach 6–9 billion by 2040. Moreover, rice, together with wheat and maize, provides 50% of the total calories consumed by the human population worldwide (Maclean et al. 2002). Thus, rice is a crop that requires our utmost attention in both fundamental and applied research.

More research is required to estimate the likely scale and timing of climate change impacts on different sectors of the economy, for informed planning of future investment strategies. Advanced technologies such as biotechnology and genetic engineering should be applied to develop cropping systems that are resilient to climate change (including crop varieties tolerant of flooding, drought, and salinity, and also those based on indigenous cultivars and other varieties suited to the needs of resource-poor farmers), fisheries, and livestock systems to ensure food security. Applying emerging genetic techniques holds great promise for understanding how biodiversity in soil and environments will respond to global changes (Chakraborty et al. 2008; Roesch et al. 2007).

### 8.2 SUSTAINABLE AGRICULTURE: PAST AND EXISTING ISSUES

#### 8.2.1 Issues that Brought the First Green Revolution

Increasing population and issues such as environmental pollution and climate change brought the need for enhancing agricultural production and food security. In the 1940s, people in Mexico, under the leadership of the great scientist Norman Borlaug, started agricultural practices that combined Borlaug’s wheat varieties with new mechanized agricultural technologies. This resulted in Mexico producing more wheat than was needed by its own citizens, and becoming an exporter of wheat by the 1960s. Due to the success of this improved practice, very soon agricultural practitioners around the world adapted this practice for increasing their respective agricultural productions. This movement, including a series of research, development, and technology transfer initiatives in agriculture for increasing agriculture production around the world during the 1960s, was named the green revolution by former United States Agency for International Development (USAID) director, William Gaud, in 1968 (Gaud 1968). The initiatives, led by Norman Borlaug, also called the “father of the green revolution,” were credited with saving over a billion people from starvation. They involved the development of high-yielding varieties of cereal grains; expansion of irrigation infrastructure; modernization of management techniques; and distribution of hybridized seeds, synthetic fertilizers, and pesticides to farmers. Borlaug was later awarded the Nobel Peace Prize.

The green revolution spread technologies that had already existed before, but had not been widely used outside industrialized nations. These technologies include modern irrigation projects,
pesticides, synthetic nitrogen fertilizer, and improved crop varieties developed through the conventional, science-based methods available. The technological advancements that led to the success of the green revolution were based largely on molecular breeding and genetic engineering, which produced high-yielding varieties/cultivars of cereal crops, especially wheat (Norin 10, Japanese semi-dwarf wheat cultivar) (De Datta et al. 1968), rice (IR8) (IRRI 1962), Golden Rice (Ye et al. 2000), maize (Bt corn), and so on. It should be noted that these high-yielding varieties were able to significantly outperform traditional varieties in the presence of adequate irrigation, pesticides, and fertilizers. Thus, production of crops increased dramatically around the world during 1961–1985.

8.2.2 The “Second” Green Revolution and Climate Change

Since the 1960s, world per capita agricultural production has increased by 25% and per capita food production in Asia has increased by 76%. That was an era when we needed to ensure food for all, but now the scenario has changed. We have developed strategies to achieve food security for all. The green revolution played a central role in this achievement. However, this success has been at the expense of the natural resource base, by the overuse of natural resources or through their use as a sink for pollution. These practices eroded crop biodiversity and gave rise to other ecological problems, such as soil infertility, chemical pollution of land and water resources, pesticide poisoning, pest infestation, and so on. Also, due to the ever-increasing population, global demand for associated agricultural products is projected to rise by at least 50% over the next two decades (UN Millennium Project 2005). The key challenges now are assurance of quality food and sustainability where the green revolution failed, despite the challenges of increasing population, decreasing fertility, pollution, and, most of all, climate change.

Climate change is an ongoing process, but the pace of this change is very slow. Issues such as global warming and dimming resulting from environmental pollution, and other human-induced as well as natural factors, have increased the rate of climate change. As global warming increases, we can see that species and their habitats will be or are on the decrease. This will affect the chances for ecosystems to adapt naturally, leading to an imbalance in biodiversity. So, in the present scenario, a major challenge for agricultural production is to manage biotic and abiotic stresses with perpetually rising numbers of pests and pathogens due to the changing climate. Thus, climate change has become a major issue, and an intensive drive will be needed to overcome its effects (Adams et al. 1998).

Significant effort to assess the impending impacts of climate change on agriculture began in 1978, when the National Defense University assembled an international group of climate experts to predict the probabilities of various climate change events and the resulting impacts on agriculture. In 1988, the Intergovernmental Panel on Climate Change (IPCC) was created by the United Nations Environment Programme (UNEP) and the World Meteorological Organization (WMO) to assess the scientific knowledge on global warming. The IPCC concluded in 1990 that there was broad international consensus that climate change was anthropogenic. That report led to an international convention for climate change, the United Nations Framework Convention on Climate Change (UNFCCC), signed by over 150 countries at the Rio Earth Summit in 1992. Since then, more structured scientific studies have resulted in a growing consensus on the interactions between climate change and agriculture (Pachauri and Reisinger 2007), culminating in the 2007 Fourth Assessment Report of the IPCC. Environmental pollution, deforestation, and increased emission of greenhouse gases, especially CO₂, have resulted in its accumulation in the atmosphere, leading to global warming and ultimately climate change. Some of the harmful effects of elevated levels of CO₂ have been observed recently, such as reduction in the sea ice in the Arctic and acidification of oceans, both leading to a decline in biodiversity (Yamamoto et al. 2012). So we need to tackle climate change and warming by introducing the use of carbon sinks to soak up CO₂, reforestation, and at the same time adapting sustainable agriculture practices.
We are on the verge of achieving a second green revolution to ensure food security to the ever-increasing population, but at the same time climate change is an important issue, which stresses the need for sustainability. Therefore, it is necessary to introduce agricultural practices that can fulfill the increasing demand for food and at the same time protect the climate. The scientific community has responded remarkably well and has taken initiatives to reduce the pace and the effects of climate change using modern biotechnological techniques and better equipment. Newer molecular biology disciplines, such as genomics, transcriptomics, metabolomics, and proteomics, along with systems biology, are being utilized today to address issues in agriculture and its sustainability.

### 8.2.2.1 How Genetically Engineered Plants (Enhanced Salt Tolerance in Plants by Ion Homeostasis and Osmoprotectant) Have Been Integrated into Agriculture and Their Contribution to Sustainable Agriculture

Adverse environmental conditions, such as high salinity, water deficit, and temperature extremes, are found in many agricultural areas and limit crop productivity worldwide. Plants have evolved various responses to these stresses at the molecular and cellular levels (Hasegawa et al. 2000; Shinozaki et al. 2003; Zhu 2002; Hakeem et al. 2012). So far, numerous genes related to plant response to abiotic stress have been identified and characterized (Gaxiola et al. 1999; Shi et al. 2000; Song et al. 2004; Waditee et al. 2003). Complete information is available on various plant genomes and advances in omics technologies are proceeding at a great pace, thus providing opportunities for the identification of transcriptional, translational, and posttranslational mechanisms, including signalling pathways that regulate the plant stress response.

To breed or genetically engineer plants to enhance abiotic stress tolerance would be part of a comprehensive strategy for sustainable agriculture. Manipulation of genes that affect specific targets, such as expression of genes encoding enzymes associated with the accumulation of compatible solutes, ion transport, stress proteins, and enzymes involved in scavenging oxygen radicals, has been used to generate transgenic plants (Pardo 2010; Umazawa et al. 2006; Yang et al. 2010). It has been shown that various compatible solutes enable plants to tolerate abiotic stress, and glycine betaine is one of the most potent compatible solutes found in nature. Many transgenic lines that overexpressed genes for the biosynthesis of glycine betaine have shown enhanced abiotic stress tolerance (see review, Chen and Murata 2011): for example, \textit{codA}-transgenic rice (Sakamoto and Murata 2001) and \textit{ApGSMT-DMT}-transgenic \textit{Arabidopsis} (Waditee et al. 2005). However, all transgenic plants accumulate only low levels of glycine betaine compared with natural accumulators of glycine betaine.

Manipulation of genes encoding ion transport for improving plant tolerance to salinity stress has been reported in both model and crop plants. In \textit{Arabidopsis}, physiological roles of the genes \textit{AtNHX1}, \textit{AtNHX7} (\textit{SOS1}), \textit{AtCHX17}, \textit{AtCHX23}, and \textit{AtHKT}, which contribute to salt tolerance, have been described (Apse et al. 1999, 2003; Berthomieu et al. 2003; Cellier et al. 2004; Shi et al. 2003; Song et al. 2004). Numerous investigations have led to the conclusion that these genes play important roles in expelling Na\(^+\) across the plasma membrane, sequestrating Na\(^+\) into a tonoplast, recirculating or removing large amounts of Na\(^+\) from shoots, or a combination of these. Overexpression of an \textit{Arabidopsis} vacuolar Na\(^+\)/H\(^+\) antiporter, \textit{AtNHX1}, in \textit{Arabidopsis} (Apse et al. 1999) and its ectopic expression in \textit{Brassica} (Zhang et al. 2001), tomato (Zhang et al. 2001), cotton (He et al. 2005), wheat (Xue et al. 2004), and \textit{Beta vulgaris} (Yang et al. 2005) resulted in improved salt tolerance. In addition, overexpression of the rice ortholog, OsNHX1, in rice and its ectopic expression in maize enhanced salt stress tolerance of transgenic rice cells and plants (Apse et al. 2003; Chen et al. 2007). Recently, ectopic expression of the \textit{Arabidopsis} \textit{AtNHX5} resulted in enhanced salt and drought tolerance in rice seedlings (Bassil et al. 2011).

High-altitude plant habitats are very specific because of their niche area of extreme environmental conditions. Their genes and proteins are being used as molecular tools for engineering
crop and other plants for better stress tolerance and adaptability against the present scenarios of climate change. Ectopic expression of copper zinc superoxide dismutase (CuZnSOD/PaSOD) from a high-altitude plant, *Potentilla australis*, improved copper and salt stress tolerance in *Arabidopsis* (Gill et al. 2010b, 2012). Later, it was also found that the ectopic expression of *PaSOD* improved stress tolerance in *Arabidopsis* because of overaccumulation of lignin in vascular bundles (Gill et al. 2010a). Apparently, overexpression of the same SOD in potato also enhanced photosynthetic performance under drought stress (Pal et al. 2012). These studies confirmed that the antioxidant genes from specific niche areas can be successfully utilized for engineering abiotic stress tolerance in crop plants without disturbing the native physiology of the plants. Recently, Dogra et al. (2013) and Rana and Sreenivasulu (2013) reported the role of cell wall hydrolases in seed germination of the high-altitude plants *Podophyllum hexandrum* and *Aconitum heterophylloyllum*. These cell wall hydrolases, due to their comparatively smaller size and enzymatic activity over a broad temperature range, can be utilized for manipulating seed germination problems. β-1,3-glucanase, a germination-related cell wall–degrading enzyme from *P. hexandrum*, reduced mean germination time and induced early seed germination even at low temperature (5°C) in *Arabidopsis* (unpublished data, personal communication with Vivek Dogra).

The results described above suggest that engineering plants by overexpressing genes encoding the accumulation of compatible solutes and ion transport appears to be a useful technology for various kinds of agriculturally important crops. To date, it has been reported that over 30 genetically engineered crops are being grown in 25 countries (Clive 2009). These efforts would be part of a comprehensive strategy for sustainable global agriculture that can meet the need for food production.

### 8.2.2.2 Plant Probiotics as Biofertilizers and Biopesticides

Probiotics are living microorganisms, which, when administered in adequate amounts, confer a health benefit on the host (Lilly and Stillwell 1965; Parker 1974). The term *probiotics* is widely used for bacteria such as *Lactobacillus* and *Bifidobacterium* that pass through the gastrointestinal tract of animals and humans and might prevent, or even cure, diarrhea and other gastrointestinal diseases (Haas and Defago 2005). Similarly, the term *plant probiotics* has been used to describe plant-associated elite bacteria that, when applied, promote the growth of the host plant (Maheshwari 2012). The promotion of plant growth by beneficial bacteria is achieved in several ways, including better nutrition, disease suppression, and hormonal activity (Borriss 2011; Haas and Defago 2005; Islam 2011). Understanding the mechanisms of interaction between plant probiotics and their host is considered important for their large-scale application in agriculture.

Our ability to provide adequate plant nutrition and pest control to increase crop yields and reduce land requirements will not be able to keep pace with the growing demand of the world's population using conventional high input-based agriculture. Hence, this has led to higher chemical inputs to promote plant nutrition and control insect pests and plant diseases. Consequently, an undue burden is placed on the planet’s ecosystems, along with serious pressure on the depleting natural resources used for making agrochemicals. The costs associated with the rapid development of modern fertilizers and pesticides include environmental pollution (Zaidi et al. 2009), unpredicted human health consequences, and deleterious effects on wildlife and other nontargeted organisms in the food chain. The realization that a sustainable agricultural system must be compatible with environmental concerns has developed into the philosophy of green agriculture. Microbial biofertilizers (Islam and Hossain 2012a) and biopesticides (Haas and Defago 2005; Islam et al. 2005; Islam and Hossain 2012b) represent an alternative to hazardous synthetic chemicals for sustainable green agriculture. Several lines of evidence suggest that probiotic bacteria can be used as alternatives to synthetic fertilizers such as nitrogenous (e.g., urea) and phosphatic fertilizers (e.g., super phosphates) (Borriss 2011; Islam and Hossain 2012a; Maheshwari 2012). This section reviews the current knowledge and trends of using plant probiotics as biofertilizers and biopesticides in low-input sustainable agriculture.
From the global viewpoint, agriculture is confronted with huge problems resulting from deterioration of the environment and depletion of natural resources. For example, we must be concerned about depletion of phosphate rock and energy sources (fossil fuels, natural gas, etc.), which are the important raw materials for production of phosphorus and nitrogenous fertilizers (Zaidi et al. 2009). Low levels of soluble soil phosphorus (P) are a serious constraint to crop production in tropical and subtropical soils, as most of the inorganic soil P forms complexes of iron, aluminum, and calcium phosphates that are adsorbed on clay particles. The solubility of these inorganic P compounds, as well as organic P, is extremely low, and only very small amounts of soil P are in solution at any one time. Probiotic bacteria that are known to enhance the solubilization of fixed soil P and applied phosphate fertilizer, resulting in better P nutrition in crop plants, are known as phosphate-solubilizing bacteria (PSB) (Abd-Alla 1994; Islam and Hossain 2012a). An important trait in plant growth-promoting bacteria is the ability of PSB to convert insoluble forms of phosphorus to an accessible form for increasing plant yields (Islam et al. 2007; Richardson and Simpson 2011; Islam and Hossain 2012a).

Bacteria from diverse taxonomic groups such as *Pseudomonas*, *Bacillus*, *Klebsiella*, *Rhizobium*, *Acinetobacter*, and so on have been shown to solubilize soil-insoluble P, and some of them have shown high promise for P nutrition in crop plants (Bianco and Defez 2010; Borriss 2011; Islam et al. 2007; Islam and Hossain 2012a; Naik et al. 2008; Richardson and Simpson 2011). The widely recognized mechanisms of phosphate solubilization mediated by these plant-associated bacteria are production of organic acids (such as gluconic, citric, oxalic), secretion of hydrolytic enzymes (such as phytases, phosphatases), or both (Abd-Alla 1994; Islam and Hossain 2012b). The production of organic acids by PSB appears to be independent of their genetic relatedness, and each strain has its own ability to produce organic acids during solubilization of inorganic phosphates (Vyas and Gulati 2009). Despite their potential as low-input practical agents for plant P nutrition, application of PSB has been hampered by their inconsistent performance in the field, which is usually attributed to their rhizosphere competence (Islam et al. 2007; Richardson and Simpson 2011). Hence, the full potential of PSB for P nutrition in the production of major crops has not yet been achieved (Islam and Hossain 2012a; Lugtenberg and Kamilova 2009). In fact, our knowledge of bacterial quorum sensing and ongoing complex molecular cross-talk within the rhizosphere after inoculation of a certain PSB is limited (Naik et al. 2008; Richardson and Simpson 2011). Application of plant probiotics such as PSB for improving P nutrition in crop plants is strongly connected to our better understanding of bacterial diversity, host specificity, mode of action, appropriate formulation, and method of application (Bianco and Defez 2010; Islam and Hossain 2012a; Richardson and Simpson 2011). Recent advances on whole genome sequencing of several important crop plants and PSB strains will provide a future basis for better understanding of PSB–plant interactions and development of improved strains as effective biophosphorus fertilizer for eco-friendly low-input sustainable agriculture. Several good reviews have recently been published on plant growth-promoting microorganisms (Haas and Defago 2005; Lugtenberg and Kamilova 2009) or soil microorganisms mediating phosphorus availability (Islam and Hossain 2012a; Richardson and Simpson 2011).

Similarly to P nutrition, a large body of literature reveals that bacteria from diverse genera such as *Acetobacter*, *Azoarcus*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Herbaspirillum*, *Klebsiella*, *Rhizobium*, and *Pseudomonas* are capable of N fixation from the atmosphere (Dixon and Kahn 2004; Doty 2011). The process of N fixation by bacteria is known as biological nitrogen fixation (BNF). BNF by a variety of symbiotic, associative, and free-living microorganisms has tremendous importance for the environment and for world agriculture. Nitrogen fixation is considered one of the key steps of the nitrogen cycle, as it replenishes the overall nitrogen content of the biosphere and compensates for the losses that are incurred due to denitrification. Application of diazotrophic bacteria substantially supplements the N requirement and promotes the growth of crop plants. The fixed N that is provided by BNF is less prone to leaching and volatilization as it is utilized in situ.
Therefore, this biological process contributes as an important and sustainable input into agriculture (Dixon and Kahn 2004).

Although symbiotic nitrogen fixation by *Rhizobium* spp. and their molecular cross-talk with leguminous plants have been well studied, nitrogen fixation by nonsymbiotic free-living or endophytic bacteria and their interactions with plants are poorly understood (Dixon and Kahn 2004; Doty 2011; Vyas and Gulati 2009). The discovery of symbiosis between N₂-fixing bacteria and legumes raises the eventual question of whether such a relationship is possible for nonleguminous plants (Ladha et al. 1997). More research is needed to discover the molecular mechanisms of BNF in nonleguminous crop species based on our understanding of nitrogen fixation biology in legumes (Godfray et al. 2010). Recently, Markmann et al. (2008) have found that several genes, including the so-called “symbiosis-receptor-kinase-gene” (SYMRK), are involved in a genetic program that links arbuscular mycorrhiza (AM) and one form of bacterial nodule symbiosis. The analysis of SYMRK in several species of plant provided striking evidence that most plants have a short version of SMYRK, which is required for AM symbiosis, while a longer variant was found only in plants involved in symbiotic relationships with nitrogen-fixing bacteria. This finding can be considered as an important step toward understanding the evolution of nitrogen fixation in plants, and even whether plants that do not form a symbiosis with nitrogen-fixing bacteria could be engineered to do so, thus increasing their N nutrition to ensure higher productivity. Making crop plants capable of fixing their own nitrogen via a close interaction with diazotrophic bacteria may be used as an alternative strategy for solving nitrogen nutrition in economically important crops such as rice, wheat, maize, and so on (Ladha et al. 1997). Although providing nitrogen nutrition to nonleguminous crops through BNF is a novel approach, its potential has a considerable payoff in term of increasing the production of these crops, which would not only help resource-poor farmers reduce the cost of production but also significantly reduce environmental pollution (Ladha et al. 1997). Several good reviews have recently been published on diazotrophic bacteria in nitrogen nutrition in plants (Beatty and Good 2011; Borriss 2011; Dixon and Kahn 2004; Doty 2011; Mia et al. 2012). In concert with plant nutrition, some elite strains exert multiple effects to promote plant growth through phytohormone production, enzyme activity, siderophore production, antagonisms against phytopathogens, or a combination of these (Bianco and Defez 2010; Duarah et al. 2011; Islam 2011; Islam and Hossain 2012a; Naik et al. 2008; Zaidi et al. 2009).

Biological control involves the use of organisms, their products, genes, or gene products to control undesirable organisms (pests) and favor desirable organisms, such as crops, trees, beneficial organisms, and insects. The organism that suppresses the disease or pathogen is referred to as the biological control agent (BCA). In the last three decades, we have witnessed a dramatic development in research on biological control of plant diseases caused by fungi, bacteria, nematodes, and peronosporomycetes (Cook 1993; Islam et al. 2005; Islam 2011; Islam and Hossain 2012b). Plant pathologists have been fascinated by the perception that disease-suppressing soil or antagonistic plant-associated bacteria could be used as environment-friendly BCA (Haas and Defago 2005). The concept of biological control by plant probiotic bacteria is becoming popular because of not only increasing public concern about the use of hazardous chemical pesticides, but also the uncertainty or inefficiency of current disease control strategies against phytopathogens (Cook 1993; Islam et al. 2005). Biological control strategies attempt to enhance the activities of BCA either by introducing high populations of a specific bacterium or by enhancing the conditions that enable the bacterium in its natural habitat to suppress the diseases (Nelson 2004). In fact, bacteria are easy to deliver, increase biomass production and yield, and improve soil and plant health (Islam and Hossain 2012b). Isolation and characterization of new potential bacteria and understanding their ecology, behavior, and mode of action are considered as major foci in current biocontrol research (Cook 1993; Islam et al. 2005, 2011). The rapid development of convenient techniques in molecular biology has revolutionized this field by facilitating the identification of the underlying molecular mechanism of pathogen suppression (Islam et al. 2005, 2011; Islam 2008; Islam and von Tiedemann...
2011), and by providing means for construction of “superior” bacteria through genetic engineering (Bainton et al. 2004).

A large body of literature indicates that bacterial antagonists can significantly suppress disease caused by peronosporomycete and fungal phytopathogens and increase the yield of crops. Bacterial antagonists commonly studied and deployed for the control of peronosporomycete diseases include *Pseudomonas, Bacillus, Burkholderia, Lysobacter, Actinobacter, Enterobacter, Paenibacillus*, and *Streptomyces* (Cook 1993; Haas and Defago 2005; Islam et al. 2005, 2011; Islam 2011; Islam and Hossain 2012b). Suppression of pathogens or diseases by the biocontrol agents is accomplished in several ways, such as production of antibiotics or lytic enzymes (Islam et al. 2005, 2011; Islam 2008; Islam and von Tiedemann 2011; Osburn et al. 1995; Perneel et al. 2008), competition for specific nutrients (e.g., iron or carbon) (Lee et al. 2008), induction of systemic resistance in the host plants (Islam et al. 2005; Khan et al. 1997). Several good reviews on biocontrol of plant diseases by plant probiotic bacteria have been published (Borriss et al. 2011; Haas and Defago 2005; Islam 2011; Islam and Hossain 2012b; McSpadden Gardener and Fravel 2002).

### 8.3 HIGH-THROUGHPUT APPROACHES: NECESSITY IN EXPEDITING SUSTAINABLE AGRICULTURE

The twenty-first century has seen the unravelling of the genomes of various plant species, including the most important food crops (Feuillet et al. 2010). It is these crop genomes that are bringing a paradigm shift in the approach to plant biology and crop breeding to meet future global food demand through crop improvement (Flavell 2010). With this genomic information, scientists are able to efficiently utilize the high-throughput technologies—transcriptomics (expression of genes genome-wide), proteomics (expression of proteins), and metabolomics (metabolites)—in order to systematically reveal the function of each gene in the genome (Fukushima et al. 2009; Weckwerth 2011). These technologies will not only address fundamental biological questions but also help create new resources in terms of plants that will be able to withstand adverse climatic conditions, which is one of the main goals of plant biologists and breeders in particular.

#### 8.3.1 Proteomics Approach and Its Importance among Omics Technologies

In the functional genomics era, proteomics is undoubtedly one of the rapidly emerging and expanding fields of study, dealing with the large-scale and systematic study of the protein population in the cell. Proteomics is absolutely necessary, and the statement of Watson and coworkers presents the reason—“as we seek to better understand the gene function and to study the holistic biology of systems, it is inevitable that we study the proteome”—highlighting the power of proteomics to address important physiological questions (Watson et al. 2003). Plant proteomics followed the advances in proteomics of mammalian systems, and readers are referred to the comprehensive book *Plant Proteomics: Technologies, Strategies, and Applications* for an in-depth reading on the subject (Agrawal and Rakwal 2008). Examples of proteomics research in plants/crops are given in Section 8.4.

#### 8.3.2 Molecular Breeding for Crop Improvement

Plant breeding describes methods for the creation, selection, and fixation of superior plant phenotypes in the development of improved cultivars suited to the needs of farmers and consumers. The primary goal of plant breeding is to improve yield, nutritional qualities, and other traits of commercial value. The integration of advances in biotechnology, genomic research, and molecular marker
applications with conventional plant breeding practices has created the foundation for molecular plant breeding. Methods for marker-assisted backcrossing were developed rapidly for the introgression of desirable traits and reduction of linkage drag, by which molecular markers were used in genome scans to select those individuals that contained both the transgene and the greatest proportion of favorable alleles from the recurrent plant genome (Ragot et al. 1995). The continued development and application of plant biotechnology, molecular markers, and genomics has established new tools for the creation, analysis, and manipulation of genetic variation and the development of improved cultivars during the past 25 years (Collard and Mackill 2008; Sharma et al. 2002; Varshney et al. 2006). Although molecular markers and other genomic applications have been successful in characterizing existing genetic variation within species, plant biotechnology generates new genetic diversity that often extends beyond species boundaries (Gepts 2002). For genes that cannot be transferred through crossing, molecular tools have made it possible to create an essentially infinite pool of novel genetic variation. Genes may be acquired from existing genomes spanning all kingdoms of life, or designed and assembled de novo in the laboratory. Biotechnology also facilitates the molecular stacking of transgenes that control a trait or suite of traits into a single locus haplotype defined by a transgenic event. Examples include development of Golden Rice (Ye et al. 2002), or the combination of transgenes that simultaneously increase synthesis and decrease catabolism of lysine in maize seeds (Frizzi et al. 2008). The combination of phenotypic data and molecular marker scores increase selection gains for maize grain yield and resistance to European corn borer (Johnson 2004). A breeding population of 250 corn lines obtained from the use of multiple trait indices and marker-assisted selection (MAS) showed that the use of molecular markers increased breeding efficiency approximately twofold relative to phenotypic selection alone (Eathington et al. 2007). MAS can also significantly enhance genetic gain for traits where the phenotype is difficult to evaluate because of its expense or its dependence on specific environmental conditions. The probability of identifying truly superior genotypes has been made possible by the use of molecular markers (Knapp 1998). Molecular markers for identification of plant resistance to soybean cyst nematode (Young 1999), resistance to cereal diseases (Varshney et al. 2006), and drought tolerance in maize (Tuberosa et al. 2007) have been used successfully. Molecular markers are being employed for detection and exploitation of naturally occurring DNA sequence polymorphisms. A number of molecular markers have been developed and used for the detection of polymorphisms. This started with the use of restriction endonuclease digestion of total genomic DNA followed by hybridization with a radioactively labelled probe, revealing differently sized hybridized fragments. This type of polymorphism, termed restriction fragment length polymorphism (RFLP), has been used extensively for genetic studies. However with the development of polymerase chain reaction (PCR), the field of molecular biology was revolutionized. A number of PCR-based molecular marker techniques have been developed, beginning with randomly amplified polymorphic DNAs (RAPDs) and simple sequence repeats (SSRs). These PCR-based procedures have the advantages that they are technically simple, are quick to perform, require only small amounts of DNA, and do not involve radioactivity (Waugh and Powell 1992). However, the gel-based assays that are needed for most molecular markers are time-consuming and expensive, limiting their utility at times. The new-generation molecular markers, single-nucleotide polymorphisms (SNPs), do not always need these gel-based assays. They are the most abundant of all marker systems known so far. A beginning has been made in the development and use of SNPs in higher plants, including some crops and tree species. Several approaches can be used for the discovery of new SNPs, and about a dozen different methods are also suitable for automation and high-throughput approaches. These methods, in principle, make a distinction between a perfect match and a mismatch (at the SNP site) between a probe of known sequence and the target DNA containing the SNP site. The target DNA in most of the methods is a PCR product, except in some cases such as “invasive cleavage assay” and “reduced representation shotgun (RRS)” (Gupta et al. 2001). SNPs are the only new-generation molecular markers for individual genotyping needed for MAS. There is some evidence that the
stability of SNPs and, therefore, the relative fidelity of their inheritance are higher than those of the other molecular systems such as SSRs and AFLPs. SNPs at a particular site in the DNA molecule should, in principle, involve four possible nucleotides, but in practice only two of these four possibilities have been observed at a specific site in a population. Consequently, SNPs are biallelic. However, the extraordinary abundance of SNPs largely offsets the disadvantage of their being biallelic and makes them the most attractive molecular marker developed so far. According to recent estimates, one SNP occurs every 100–300 bp in any genome, thus making SNPs the most abundant molecular markers known so far. One can only hope that SNPs will be developed expeditiously in all major crops in a variety of crop improvement programs, although the nonavailability of adequate sequence data may limit this activity.

The other high-throughput technique, diversity arrays technology (DArT), is an upcoming technology that does not require sequence information. DArT discovers a large number of markers in parallel and does not require further development of an assay once markers are discovered. DArT was developed to provide a practical and cost-effective whole genome fingerprinting tool (Jaccoud et al. 2001). Development of DArT starts with assembling a group of DNA samples representative of the germplasm anticipated to be analyzed with the technology. This group of samples usually corresponds to the primary gene pool of a crop species, but can be restricted to the two parents of a cross or expanded to secondary or even tertiary gene pools. The DNA mixture representing the gene pool of interest is processed using a complexity reduction method—a process by which reproducibility selects a defined fraction of genomic fragments, called representation, is then used to construct a library in *Escherichia coli*. The inserts from individual clones are amplified and used as molecular probes on DArT assays. DArT markers are biallelic markers that are either dominant or hemidominant. DArT has been successfully developed for 16 plant species and two species of plant pathogenic fungi. Rice was used for the initial proof-of-concept work of DArT (Jaccoud et al. 2001).

The choice of molecular marker technology depends strongly on the intended application. It is likely that the science of genomics in plants will follow the path of human genetics: increasing the resolution of analysis through increased marker density.

### 8.4 PROTEOMICS CONTRIBUTION IN CEREAL CROPS: RICE AND WHEAT

Although rice, like other plants, faces numerous challenges from abnormal environmental factors during its growth and development toward the final product (seed), it is more prone to abiotic factors such as high and low temperatures, drought, flooding, ozone (O₃), and so on (for review see Agrawal et al. 2006, 2009; Agrawal and Rakwal 2006, 2011; Rakwal and Agrawal 2003). These major abiotic stress factors also cause numerous changes in plants at the level of the proteome (for review see Kosová et al. 2011). Physiology, molecular biology, and genetics have greatly improved our understanding of the responsiveness of rice to stress. Recently a lot of effort has been applied to proteomic analysis in rice, and systematic studies have been performed on the functional identification of proteins present in different tissues at different developmental stages. In the last couple of years some interesting high-throughput proteomic approaches have been undertaken to understand the proteomic response against drought, high and low temperatures, and salinity. Rice, being a crop that requires excellent water management to achieve the desired yields, is particularly susceptible to water shortage or drought conditions. Drought, a meteorological event causing absence of rainfall for a period of time, can cause the soil to be depleted of moisture, and the water deficit results in a decrease of water potential in plant tissue (Hadiarto and Tran 2010; Mitra 2001; Zhang 2007). A recent paper by the group of Paul Haynes at Macquarie University, New South Wales, Australia, has recently assessed changes in the physiology and proteome of rice (cv. Nipponbare) leaf due to drought stress (Mirzaei et al. 2012a). These authors used a controlled regime of drought lasting for two weeks, which was then followed by rewatering and rapid recovery in order to understand
the gene-level events as plants experienced drought and recovery. Proteins were identified from leaf samples after moderate drought, extreme drought, and three and six days of rewatering by a combination of label-free quantitative shotgun proteomic and spectral counting using normalized spectral abundance factors, which resulted in the identification of 1548 nonredundant proteins (Mirzaei et al. 2012a). Results revealed that proteins were downregulated in the early stages of drought, and, as the drought became severe, proteins were found to be upregulated. When the stressed plants were rewatered, proteins were downregulated, suggesting that stress-related proteins were being degraded. The dominant identified proteins were found to be involved in signalling and transport under severe drought and decreased on rewatering. Mirzaei and coworkers speculated that water transport and drought signalling are critical elements of the overall response to drought in rice and might be the key to biotechnological approaches to drought tolerance (Mirzaei et al. 2012a). Similar studies in rice plants grown in split root systems to analyze long-distance drought signalling within root systems indicated a general upregulation of pathogenesis-related (PR), heat shock proteins (HSPs), and oxidation–reduction proteins in drought-exposed roots (Mirzaei et al. 2012b). PR proteins are known to play an important role in general adaptation to stress, along with HSPs, which act as molecular chaperones. Several peroxidases, superoxide dismutases, and catalases were also identified, which perhaps helped to suppress the levels of reactive oxygen species (ROS) in response to drought. Interestingly, plant microtubules and chitinases were also shown to be affected by transmissible defense-inducing signals in response to drought, thus suggesting that there is no single class of proteins unaffected by stress. Quantitative tandem mass tag proteomic analysis of drought-stressed parental and drought-tolerant near isogenic lines (NILs) for a rice quantitative trait locus (QTL) for yield under drought were also studied to understand the proteomic response to severe reproductive-stage drought stress. Systematic analysis of protein profiling in three different tissues of the same plant (flag leaf, panicles, roots), suggested effective carbon–nitrogen remobilization in flag leaf, panicle, and roots and enhanced detoxification of ROS species during stress as the key contributing factor to the remarkable performance of NILs (unpublished data, personal communication with Ajay Kohli). The proteomic response of rice plants to other abiotic stress factors such as high and low temperature has also been studied. Quantitative label-free shotgun proteomic analysis of 850 stress-responsive proteins from cultured rice cells exposed to high and low-temperature stress provided molecular insights into thermal stress response in plants (Gammulla et al. 2010). This study mainly concluded that there was a higher abundance of proteins involved in stress response, carbohydrate metabolism, lipid metabolism, cell redox homeostasis, cell wall modification, and cell division in response to high-temperature stress. Low-temperature stress elicited proteins involved in protein metabolism and cellular component organization. The same authors conducted a similar successor study to investigate the effect of high and low-temperature stress on the leaves of rice seedlings. Contrasting responses were observed in leaves and cell culture studies. Proteins involved in protein metabolism, such as eukaryotic initiation factors and elongation factors, were seen to be upregulated in leaves more than in cell culture (Gammulla et al. 2011). The authors hypothesized that this difference was due to the rapid cell turnover and coexistence of dividing and senescent cells in the case of cell cultures. This study also led to the identification of 20 novel stress-response proteins (Gammulla et al. 2011). They also provided an initial functional annotation to these novel proteins. The study also highlighted for the first time the presence of chloroplast ribosomal proteins and translation releasing factors in response to cold stress (Gammulla et al. 2011). Differentially expressed ubiquitinated proteins were identified in rice roots by nanospray liquid chromatography/tandem mass spectrometry in response to the initial phase of salt stress responses in rice seedlings. The expression of ubiquitination on pyruvate phosphate dikinase 1, heat shock protein 81–1, probable aldehyde oxidase 3, plasma membrane ATPase, cellulose synthase A catalytic subunit 4 (UDP-forming), and cyclin-C1-1 was identified and analyzed before and after salt treatment (Liu et al. 2012). Anther proteomic patterns for two contrasting rice genotypes under salt stress were also compared using matrix-assisted laser desorption/ionization
time-of-flight/time-of-flight (MALDI-TOF/TOF) analysis to understand the basis of salt tolerance. Several proteins were identified that might increase plant adaptation to salt stress by modulating important metabolic or biochemical processes such as carbohydrate/energy metabolism, anther and pollen wall remodelling and metabolism, and protein synthesis and assembly (Sarhadi et al. 2012). Knowledge of protein alterations under biotic and abiotic stresses should help us understand the molecular mechanism of stress tolerance in rice at the translational/posttranslational instead of the transcriptional level. This is imperative for understanding complex environmental signalling responses and engineering cultivars tolerant to stress, further leading toward sustainable agriculture.

In wheat, let us look at one of the less investigated components of climate change, O₃ stress, which is also a major environmental pollutant affecting plant growth and productivity (Cho et al. 2011). A recent study examining for the first time proteomics aspects of O₃-exposed wheat plants was carried out by Sarkar and coworkers at Banaras Hindu University, Varanasi, India (Sarkar et al. 2010). This study was designed to evaluate the impact of elevated concentrations of O₃ on phenotypical, physiological, and biochemical traits in two high-yielding cultivars of wheat, and also to analyze the leaf proteome using one/two-dimensional gel electrophoresis (1-/2-DGE) in conjunction with immunoblotting and mass spectrometry (MS) analyses under near-natural conditions using open-top chambers. The O₃ exposure caused specific foliar injury in both the wheat cultivars. Results also showed that O₃ significantly decreased photosynthetic rate, stomatal conductance, and chlorophyll fluorescence kinetics ($F_{v}/F_{m}$) in test cultivars. Biochemical evaluations also showed a greater loss of photosynthetic pigments. A significantly induced antioxidant system under elevated O₃ concentrations indicates the ability of O₃ to generate oxidative stress. 1-DGE analysis showed drastic reductions in the abundantly present ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) large and small subunits. Immunoblotting with specific antibodies confirmed induced accumulation of antioxidative enzymes such as superoxide dismutase and ascorbate peroxidase protein(s) and common defense/stress-related thaumatin-like protein(s). 2-DGE analysis revealed a total of 38 differentially expressed protein spots, common to both the wheat cultivars. It was found that some leaf photosynthetic proteins (including RuBisCO and RuBisCO activase) and important energy metabolism proteins (including ATP synthase, aldolase, and phosphoglycerate kinase) were drastically reduced. On the other hand, some stress/defense-related proteins (such as harpin-binding protein and germin-like protein) were found to be induced. The study reveals an intimate molecular network, provoked by O₃, affecting photosynthesis and triggering antioxidative defense and stress-related proteins, culminating in accelerated foliar injury in wheat plants (Sarkar et al. 2010).

### 8.5 PROTEOMICS CONTRIBUTION IN MODEL LEGUME SPECIES: A CASE STUDY IN SEEDS

Legumes, including Medicago truncatula, soybean, and peanut are among the most important crops worldwide, playing a major role in agriculture, the environment, and human and animal nutrition and health (Graham and Vance 2003). Legume seeds provide a valuable source of edible oils and proteins for feeding both animals and humans (Mitchell et al. 2009), although the levels of protein, oils, and starch vary between legume species. The protein content of legume seeds ranges from 20% to 40%, depending on the species. Seeds of soybean and M. truncatula exhibit high protein content, whereas the starch content is very low.

Seed development is a key step of the plant life cycle and is a complex process that determines the nutrient value of seeds for proteins and fatty acids. Particularly during the seed filling period, drastic changes in protein and oil composition occur. Due to the importance of seed filling, systematic studies of this phase of seed development are beginning in legumes. Comparative analysis during seed development is also being studied, with considerable attention to understanding protein and allergen accumulation and metabolic regulation at the level of the proteome.
Proteomic analyses provide a powerful tool to address biochemical and physiological aspects, not only of plant responses to abiotic and biotic stresses, but in various tissues at different developmental stages (leaf senescence, root symbiosis, seed development and germination). In addition, advances in 2-DGE coupled with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and nano-electrospray ionization liquid chromatography-tandem mass spectrometry (nESI-LC-MS/MS) and bioinformatics for protein identification, has been applied to understand gene function and characteristics of various genotypes in many legume species, including *Glycine max* (soybean), peanut (*Arachis hypogaea*), *Lotus japonicus* (Japanese trefoil) and *Medicago truncatula* (barrel medic). During the past decade, several proteomic studies during seed development or in mature seeds have been carried out for different legume species, such as *L. japonicus* (Dam et al. 2009), *M. truncatula* (Gallardo et al. 2003, 2007), *G. max* (Agrawal et al. 2008; Hajduch et al. 2005; Krishnan et al. 2009), and *A. hypogaea* (Kottapalli et al. 2008). These data, along with the reports on other legume proteomics, will be useful for future analysis of legume seed proteins. Furthermore, an interactive web database for proteomics data for seed filling in soybean and other legume oilseeds studied has been established (http://oilseedproteomicsmissouri.edu), which is freely available on publication of individual datasets.

**8.5.1 Proteomic Studies in Soybean**

Soybeans are responsible for approximately $12 billion in annual crop value and more than $5 billion in annual export value to the U.S. economy (Gunstone 2001). Soybeans are one of the important sources of fatty acids and proteins for human and animal nutrition (for review, see Thelen and Ohlrogge 2002) as well as other legumes. As mentioned above, seed filling during seed development is a period that largely determines the relative levels of storage reserves in seeds in accordance with significant changes in protein and oil composition. Particularly at this stage, soybean seed accumulates 40% storage reserves, mainly comprising the two abundant seed storage proteins, glycinin and β-conglycinin (Hill and Breidenbach 1974). So far, almost 80 storage proteins have been identified in soybean seeds. These results suggested that the relative proportion of storage components in seeds should vary dramatically among different plant species. Therefore, research on mechanisms of soybean development is not only useful for acquiring a basic understanding of plant developmental biology, but also valuable for improving agronomic traits, such as improvements in nutritional quality and nonallergenic proteins, that could influence other traits of agronomic value.

To gain insight into the complex process of seed development, identification of proteins and their expression profiles was comprehensively investigated, resulting in the establishment of metabolic biosynthetic flow during seed filling. An investigation on soybean (var. Maverick) seed filling (Hajduch et al. 2005) successfully profiled 679 2-DGE-separated protein spots at five sequential developmental stages (2–6 weeks after flowering). Analysis of each of these protein spot groups by MALDI-TOF MS yielded the identity of 422 of these proteins, representing 216 protein activities. These proteins were classified into 14 functional classes, according to a revised classification scheme originally used for the *Arabidopsis* genome. Overall, metabolism-related proteins were decreased, while proteins associated with destination and storage were increased. The accumulation of unknown proteins, sucrose transport and cleavage enzymes, cysteine and methionine biosynthesis enzymes, 14-3-3-like proteins, lipoxygenases, storage proteins, and allergenic proteins during seed filling is also identified and discussed to elucidate whether they are involved in seed development. Agrawal et al. (2008) also performed an in-depth investigation of proteins expressed during seed filling in soybean using 2-DGE and semicontinuous multidimensional protein identification technology (MudPIT) (Sec-MudPIT) in combination with nESI-LC-MS/MS. 2-DGE and Sec-MudPIT analyses were conducted on five sequential seed stages (two–six weeks after flowering) and identified 478 proteins out of 675 protein spots on high-resolution 2-D gel reference maps. The identified proteins were mainly protein components of metabolic biosynthetic pathways of...
carbohydrates, fatty acids, and proteins in soybean, and they were compared with a parallel study of rapeseed using high-quality and integrated datasets. These studies may provide knowledge about the importance of metabolic pathways involved in legume seed development, especially the synthesis of storage globulins. Furthermore, these results suggest that a detailed analysis of the proteomes of seeds could contribute to efforts aimed at increasing the nutritional value of seeds marketed for human consumption.

Different positions of seed storage proteins in the amount of separated proteins on 2-D gels have been detected by the variation in different genotypes (genetically determined quantitative variations). Several proteomic studies have demonstrated that seed storage protein content and composition (glycine subunits, trypsin inhibitors, major seed allergens, low abundant metabolic proteins) in soybean vary in accordance with genetic variability (Mahmoud et al. 2006; Natarajan et al. 2007a, b, 2012; Xu et al. 2007). For example, Natarajan et al. (2007a) characterized that the wild genotypes have a higher number of glycycin protein spots (G1, G2, G3, G4, and G5), which are high-abundant seed storage proteins, when compared with the other three genotypes. Major variation was observed in acidic polypeptides of G3, G4, and G5 compared with G1 and G2, and minor variation was observed in basic polypeptides of all subunits. These data indicated that there are major variations of glycycin subunits between wild and cultivated genotypes rather than within the same groups. Thus, approaches combining plant proteomics and plant genetics can help us elucidate the genetic control of seed protein composition, which can be exploited for legume crop improvement. These results give us important knowledge about the variability of protein and protein subunit accumulation among various cultivars, which is useful for improving both quantity (high-yield proteins) and quality (allergen-free proteins) of soybean seed proteins, which will benefit the overall utilization of soybean in the food and feed industries.

There have been technical advances in the fractionation of high-abundant seed proteins and high-resolution 2-D gels of soybean seeds. Technical advances related to 2-DGE and MS for protein identification have improved the sensitivity, reproducibility, and accuracy of proteome analysis (Rabilloud and Lelong 2011). It is possible to characterize various complex protein samples, since the 2-DGE-MS strategy has proven to be a reliable and efficient means of proteome analysis. However, this 2DE-MS strategy has its own difficulties in resolving low-abundant proteins (LAPs) due to the limited loading capacity of isoelectric focusing (IEF) gels (Rothemund et al. 2003). For instance, proteomic assessment of the LAPs within soybean seed is difficult when the overwhelming majority, sometimes 60%–80%, is made up of storage proteins (Krishnan et al. 2009). Basically, cultivated soybean seed storage proteins consist primarily of two major storage protein complexes, glycycin and β-conglycinin. Glycinin, accounting for roughly 40%–60% of the total seed protein, is a hexameric protein, ranging from 320 to 375 kDa. There have been attempts to establish an extraction method for storage proteins, including CuSO4 (Krishnan et al. 2009) and isopropanol fractionation (Natrajan et al. 2009). More recently, a simplified extraction method to improve the detection of LAPs by a protamine sulfate precipitation (PSP) method (unpublished data, personal communication with SunTae Kim) has been developed. These fractionation methods are simple, fast, economic, and reproducible for seed storage protein fractionation and suitable for downstream in-depth proteomics analysis.

Soybean possesses about 15 proteins recognized by IgEs from soy-sensitive people. So far, it has been reported that soybean seed contains 16 known protein allergens, including the seed storage proteins glycycin, beta conglycinin and alpha subunit, Gly m Bd 30K (vacuolar thiol protease), and Kunitz trypsin inhibitor (KTI), with differing degrees of severity (Thelen 2009). Glycinin, the most abundant seed storage protein in soybean seed, consists of five subunits, G1, G2, G3, G4, and G5. Among these subunits, only G2 and acidic polypeptides of G1 are reported to be allergens (Ogawa et al. 2000). The well-known soybean allergenic protein is P34, a member of the papain superfamily of cysteine proteases, and more than 65% of soy-sensitive patients react to the P34 protein (Wilson et al. 2005). Xu et al. (2007) have conducted comparative studies of allergen proteins

AU: The meaning of the sentence beginning “Different positions” is not clear. Please rewrite if possible.
in 16 cultivated and wild soybean genotypes, revealing that considerable heterogeneity of the \( \alpha \) subunit of \( \beta \)-conglycinin distribution exists among these 16 soybean genotypes. The data may serve as a 2-D reference map for comparison of soybean allergenic proteins in various soybean genotypes, and may also be useful for the modification of soybean to improve its nutritional value.

8.5.2 Proteomic Studies in Peanut

Peanut is an important food legume and is also one of the most important leguminous crops in the world, both for vegetable oil and as a protein source. Several research groups have reported studies of peanut proteomics. To date, proteomics has enabled the identification and characterization of methionine-rich protein (MRP) from cultivated peanut (Basha and Pancholy 1981; Sathanoori and Basha 1996), the establishment of genetic variation among peanut cultivars (Kottapalli et al. 2008), and the discovery of protein markers that are able to distinguish given subspecies (Liang et al. 2006).

Kottapalli et al. (2008) first profiled total seed proteins isolated from mature seeds of four peanut cultivars (New Mexico Valencia C (NM Valencia C), Tamspan 90, Georgia Green, and NC-7) using 2-DGE coupled with nESI-LC–MS/MS. Among 20 abundant protein spots showing differences in relative abundance among these cultivars, 14 nonredundant proteins were identified by nESI-LC–MS/MS, suggesting that the major proteins belong to arachin (glycinin and Arah3/4) and conarachin seed storage proteins, as well as other allergenic proteins. Some of these proteins showed cultivar-specific expression patterns. For example, New Mexico Valencia C showed low levels of the antinutritive proteins, such as lysyl oxidase (LOX) and galactose-binding lectin. Conversely, Arah3/h4, an allergen with decreased allergenic properties, was highly abundant in Tamspan 90, suggesting that the identified proteins might serve as potential markers for cultivar differentiation. It may be implied that no single cultivar has all the desirable traits for breeding a cultivar to increase seed quality in hypoallergenic peanut lines.

8.6 PROTEOMICS CONTRIBUTION IN HORTICULTURAL CROPS

8.6.1 Strawberry

Strawberries are a rich source of vitamins and have immense economic value due to their unique flavor. The strawberry proteome was analyzed to investigate the developmental changes during berry ripening (Bianco et al. 2009). Two-dimensional difference gel electrophoresis (2-D DIGE) gels of three different stages of berry development (immature, turning, and red) showed 568, 622, and 520 spots, respectively. Alternatively, a shotgun approach was also followed to identify the proteins involved in berry development. The identified proteins mainly belonged to the defense, energy, and secondary metabolism categories. Citric acid is an important constituent of the strawberry. Interestingly, many enzymes of the citric acid cycle, including citrate synthase, aconitase, isocitrate dehydrogenase, 2-oxoglutarate dehydrogenase complex, succinyl-CoA synthetase, succinate dehydrogenase, fumarase, and malate dehydrogenase, were identified. In addition, ripening-related proteins showed accumulation from immature to red berries. Four allergens were also detected in the strawberry proteome, suggesting a plausible explanation of strawberry allergy in some people. A comparison of protein sequences of strawberry Fra a 1 allergen with birch pollen allergen Bet v 1 and corresponding apple allergen showed 54% and 77% identity, respectively. Decreased abundance of allergens and enzymes involved in red pigment synthesis was observed in white strawberries, further confirming why red strawberry-allergic persons are not allergic to white strawberries (Hjernø et al. 2006). A detailed comparison of proteome profiles of different varieties of strawberries (red and white) showed that the existence of the allergen Fra a 1 varied between varieties, suggesting why some people are allergic to a particular variety of strawberry and not to others (Alm et al. 2007).
8.6.2 Grape

Wine grapes are an important horticultural crop due to their high nutrient content and high demand in the wine industry. Although analysis of the grape berry proteome is relatively difficult due to the high content of secondary metabolites and sugars that interferes with the protein extraction and separation methods, a number of reports have been published regarding the proteome analysis of the grape berry.

The proteomics of berry development has been studied in all parts of the berry, including skin (Deytieux et al. 2007; Negri et al. 2008a), pulp/flesh (Giribaldi et al. 2007; Martinez-Esteso et al. 2011), and whole berries (Sarry et al. 2004). In addition, grape berry proteome has been analyzed during postharvest withering (Carli et al. 2010). Results obtained from these studies reveal the accumulation of PR proteins such as thaumatin-like protein, different isoforms of chitinase, β-1,3-glucanase, abscisic stress ripening protein, polyphenol oxidase, and so on, in all the berry parts during berry maturation and withering. Increased activity of chitinase and β-1,3-glucanase was also reported in berry skin as the berry ripens. However, decreased abundance of HSPs and energy and general metabolism-related proteins was observed in berry skin from onset of ripening to color change. Besides, proteins associated with anthocyanin biosynthesis and cell wall loosening showed increased abundance during berry maturation (Deytieux et al. 2007).

In berry flesh, the majority of the identified proteins were associated with sugar and organic acid metabolism, indicating a vast reprogramming of sugar and acid content of the berries during ripening. In contrast to berry skin, proteins related to energy metabolism showed increased abundance during ripening. In addition, enzymes of protein synthesis and regulation also showed higher expression in flesh, suggesting a significant modulation of the flesh proteome during ripening (Martinez-Esteso et al. 2011). During maturation and withering, dehydration of berries takes place, and wax deposition in berries is observed to minimize this dehydration. An increased abundance of lipid metabolism-related proteins such as dienelactone hydrolase and 3-ketoacyl-(acyl-carrier-protein)-reductase was observed in matured and withered berries. The increased abundance of these enzymes can be correlated with wax synthesis, which minimizes water loss. Proteins associated with the cytoskeleton showed accumulation during ripening (Carli et al. 2010; Giribaldi et al. 2007) and remained similar up to withering (Carli et al. 2010).

The effect of water-deficit stress was analyzed in grape pericarp, seeds, and skin. Water deficit affected the abundance of approximately 7% of the grape pericarp proteins. In skin, water-deficit stress increases the abundance of proteosome subunit and proteases, ROS-detoxification enzymes, and selected enzymes involved in flavonoid biosynthesis, whereas the pulp showed increases in isoflavone reductase, glutamate decarboxylase, and an endochitinase. In contrast, the seed proteome was least affected by water deficit, and mainly comprised seed storage, maturation, and late embryogenesis abundant (LEA) proteins. Analysis of the metabolome in these tissues under water-deficit conditions showed accumulation of caffeic acid, proline, shikimate, and gluconate in skin and alanine, catechine, myo-inositol, shikimate, and sucrose in pulp (Grimplet et al. 2009).

Identification of herbicide (flumioxazin)-treated grape wine berry proteins showed degradation of RuBisCO, suggesting a negative effect of the herbicide on photosynthesis. However, several isoforms of PR-10 showed increased abundance, indicating their pivotal roles in decreasing the negative effects of herbicide stress. In addition, accumulation of enzymes involved in the photorespiration and antioxidant system was observed, suggesting activation of photorespiration and ROS as a result of herbicide stress (Castro et al. 2005).

The effect of abscisic acid (ABA) was analyzed before veraison in deseeded berries and at veraison in berry flesh and skin. ABA treatment affects the abundance of 60 protein spots of the berries, out of which 40 (15 from whole berries treated before veraison, 9 from berry flesh, and 16 from berry skins of berries treated at veraison) were identified by LC-MS/MS. Results showed that mainly ripening-related proteins, such as vacuolar invertase and NADP-dependent malic enzyme,
were affected by ABA treatment. An increased abundance of oxidative stress-related proteins such as ascorbate peroxidase was also identified before and at veraison (Giribaldi et al. 2010).

The effect of methylated cyclodextrins (MBCD) and methyl jasmonate (MeJA) elicitors was analyzed in grapevine cell suspension culture. Out of 233 spots detected in Coomassie Brilliant Blue (CBB)-stained gel, 39 were differentially modulated upon elicitation. Peptide mass fingerprinting (PMF) and MS/MS identification of 25 differentially modulated spots showed that they were involved in plant defense. The proteins included class III secretory basic peroxidase, class III chitinase, β-1,3-glucanase, thaumatin-like protein, and so on. Interestingly, some of the identified proteins are involved in systemic acquired resistance (SAR), suggesting that elicitation with MBCD mimics the effect of SAR in plants (Martinez-Esteso et al. 2009). In another similar study in which grapevine cell suspension culture was elicited with MBCD and MeJA, a total of 1031 spots were detected in the 2D-DIGE gel, of which 67 showed altered abundance upon elicitation. The enzymes involved in the trans-resveratrol (tr) biosynthetic pathway showed increased abundance with either MBCD or combined MBCD and MeJA but not with MeJA alone. In addition, accumulation of stilbenoids was observed in response to elicitation (Martinez-Esteso et al. 2011).

For analysis of the cell wall proteome of the skin and seeds of the grape berry, four different methods for cell wall protein extraction were tried. Out of the three methods tried for protein extraction (extraction in LiCl2 (lithium chloride), CaCl2 (calcium chloride), and phenol), the phenol method showed the best results, as seen by the highest number of detectable spots and greatest spot resolution. A total of 904 spots were observed in the cell wall proteome extracted by the phenol method, of which 47 were identified. The identified proteins included β-1,3-glucanase, several isoforms of chitinase, and ROS-detoxifying enzymes such as catalase and CuZnSOD. Several proteins related to carbohydrate metabolism were also observed in the cell wall fraction, which was devoid of signal peptide. These proteins included phosphoglyceromutase, glyceraldehyde-3-phosphate dehydrogenase, fructose-biphosphate aldolase, and so on, and might be involved in cell wall biosynthesis (Negri et al. 2008b).

The effect of the developmental stage was also analyzed in the plasma membrane of the grape berry. Silver-stained 2-D gels of the pre-veraison, veraison, and post-veraison berries showed 119, 98, and 86 spots respectively, of which 12 showed developmental stage-specific expressions. MALDI-TOF-MS identification of the differentially modulated proteins showed their involvement in transportation, metabolism, protein synthesis, and signalling. An increased abundance of ubiquitin proteolysis and cytoskeletal proteins at the veraison and post-veraison stages indicates protein degradation during berry ripening, which could be correlated with the decreased number of spots in these stages (Zhang et al. 2008).

8.6.3 Pear

After Vitis and Malus, pear is the third most economically important fruit produced in the temperate regions. A proteome analysis of pear fruit was conducted to analyze the effect of core breakdown disorder (Pedreschi et al. 2007), extreme gas conditions (Pedreschi et al. 2009), storage in a controlled atmosphere (Pedreschi et al. 2008), and bagging treatment (Feng et al. 2011). To analyze the effect of core breakdown disorder, proteins were extracted from healthy, sound, and brown tissue and were separated on 2-D gels. The amount of protein was highest in the healthy tissue, followed by sound and brown tissue, suggesting protein degradation during core breakdown. Identification of the spots showing differential abundances in healthy and brown tissue by MS/MS showed their involvement in energy metabolism, ROS detoxification, and ethylene biosynthesis. Proteins associated with energy metabolism and defense showed increased abundance in the brown tissue (Pedreschi et al. 2007). DIGE technology was employed to analyze the effect of anoxia and air on pear fruit slices. Upregulation of pentose phosphate pathway (PPP) enzymes was observed during anoxic conditions, suggesting that PPP is activated as an alternate source of energy production.
during low-oxygen conditions. In addition, accumulation of PR proteins was also observed, indicating their involvement in overcoming the effect of anoxia in addition to other abiotic and biotic stress conditions (Pedreschi et al. 2009). Proteome analysis of the skin tissue of natural and bagged pear showed decreased abundance of photosynthesis; signalling; and protein, carbohydrate, and acidity metabolism. Regarding photosynthesis, targets related to PS II and RuBiSCO were identified, suggesting their probable degradation during fruit bagging (Feng et al. 2011).

8.7 PROTEOMICS IN COMBINATION WITH OTHER OMICS APPROACHES TOWARD NEXT-GENERATION CROPS

8.7.1 Genomes, Proteomes, Transcriptomes: Application of Computational Approaches for Reconstruction, Modelling, and Analysis of -Omes

Plants are an important source of food and various multipurpose products for humans. Traditional studies have focused on reductionist approaches to characterize plant functions and to probe their molecular basis. The complexity of plant functions and responses is not adequately addressed by focusing on the functional details of DNA, RNA, proteins, and metabolites alone. For better understanding of plant functions, one must invoke the complex and subtle details of interactions among these constituent molecules. Lately, in addition to proteomics, there has been a tremendous rise in the exploration of plant systems-level genomes (AGI 2000; IRGSP 2005; Paterson et al. 2009; Schnable et al. 2009), protein interactomes (Dreze et al. 2011; Lee et al. 2010; Uhrig 2006), and transcriptomes (Yamada et al. 2003). The understanding of the plant protein–protein interaction network and other interactomes is expected to provide crucial insights into the regulation of plant developmental, physiological, and pathological processes (Lin et al. 2011; Morsy et al. 2008; Umezawa et al. 2006; Xhang et al. 2010).

Analysis of complex biological systems and their macromolecular interaction networks may be central in characterizing genotype-to-phenotype relationships in plants. Advances in graph theoretical analysis provide methods of functional interpretation of plant protein interactomes (Uhrig 2006). Identification of network modules as putative cellular modularity and network motifs that represent cores of functional modules could lead us to better identification and control of systems-level features specifying various biological processes (Uhrig 2006). It has been indicated that evolutionary processes have left their imprint on protein interactomes (Dreze et al. 2011). Network analysis has also been used to show how pathogens may exploit protein interactions to manipulate a plant’s cellular machinery (Mukhtar et al. 2011).

Thus, macromolecular interaction networks help us draw detailed maps of cellular networks reflecting the architecture and dynamic interplay of cellular dynamics. These interaction network models could be used to infer functionally important proteins and regulatory motifs that specify plant processes such as biotic and abiotic stresses, plant defense, plant–pathogen interactions, and so on, which have a strong bearing on sustainable agriculture. As a theoretically oriented question, which could lead to ways of controlling molecular mechanisms, it would be enriching to construct models of evolution of plant interactomes that address evolutionary mechanisms and features specific to plants.

Constructing comprehensive plant–protein interaction maps is a vast challenge, given the estimated number of proteins and interactions among them. There are three chief types of approaches used for construction of plant protein interactomes: in vitro, in vivo, and in silico. In vitro approaches consist of experimental methods such as protein microarrays, surface plasmon resonance, immunopurification, and so on, and are limited by the need for sophisticated instruments and procedures. In vivo approaches consist of experimental methods such as yeast two hybrid (Y2H) and protoplast Y2H. A high false positive rate is one of the major shortcomings of these methods. Interactions
could also be predicted by *in silico* analysis methods. One of the methods used is that of predicted orthologs. Predicted interologs identification has been used as a predictor of protein interaction on the premise that orthologous proteins, which are known to interact in one organism, can interact in the organism under study.

Several protein interaction databases have been compiled, including IntAct (Kerrien et al. 2012), Molecular Interaction Database (MINT) (Zanzoni et al. 2002), *Arabidopsis thaliana* Protein Interaction Networks (AtPID) (Cui et al. 2007), Database of Interacting Proteins (DIP) (Xenarios et al. 2000), Biomolecular Interaction Network Database (BIND) (Bader et al. 2001), and Biological General Repository for Interaction Datasets (BioGRID) (Stark et al. 2006).

So, in this era of integrative sciences, systems biology is being used for analysis and better understanding of complex interactome data generated through wet lab experiments. It has been inferred that using novel combinatorial approaches employing computational expertise, along with knowledge gained from genomics, transcriptomics, metabolomics, and proteomics-based studies, would lead to identification of targets for resolving the issues in agriculture and sustainability.

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