CHAPTER ONE

Food, Nutrition and Agrobiodiversity Under Global Climate Change

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Contents

Abstract

Available evidence and predictions suggest overall negative effects on agricultural production as a result of climate change, especially when more food is required by a growing population. Information on the effects of global warming on pests and pathogens affecting agricultural crops is limited, though crop–pest models could offer means to predict changes in pest dynamics, and help design sound plant health management practices. Host-plant resistance should continue to receive high priority as global warming may favor emergence of new pest epidemics. There is increased risk, due to climate change, to food and feed contaminated by mycotoxin-producing fungi. Mycotoxin biosynthesis gene-specific microarray is being used to identify food-born fungi and associated mycotoxins, and investigate the influence of environmental parameters and their interactions for control of mycotoxin in food crops. Some crop wild relatives are threatened plant species and efforts should be made for their in situ conservation to ensure evolution of new variants, which may contribute to addressing

new challenges to agricultural production. There should be more emphasis on germ-plasm enhancement to develop intermediate products with specific characteristics to support plant breeding. Abiotic stress response is routinely dissected to component physiological traits. Use of transgene(s) has led to the development of transgenic events, which could provide enhanced adaptation to abiotic stresses that are exacerbated by climate change. Global warming is also associated with declining nutritional quality of food crops. Micronutrient-dense cultivars have been released in selected areas of the developing world, while various nutritionally enhanced lines are in the release pipeline. The high-throughput phenomic platforms are allowing researchers to accurately measure plant growth and development, analyze nutritional traits, and assess response to stresses on large sets of individuals. Analogs for tomorrow's agriculture offer a virtual natural laboratory to innovate and test technological options to develop climate resilience production systems. Increased use of agrobiodiversity is crucial to coping with adverse impacts of global warming on food and feed production and quality. No one solution will suffice to adapt to climate change and its variability. Suits of technological innovations, including climate-resilient crop cultivars, will be needed to feed 9 billion people who will be living in the Earth by the middle of the twenty-first century.

1. INTRODUCTION

The world's population will be ~9 billion in 2050, when the concentration of carbon dioxide (CO_2) and ozone will be 550 ppm and 60 ppm, respectively and the climate will be warmer by 2 °C (Jaggard et al., 2010). To sufficiently feed these 9 billion people, the total food production will have to be increased by 70% within 2011–2050 to meet a net demand of ~1 billion t of cereals for food and feed and 200 million t of meat (WSFS, 2009). The evidence accumulated also suggests crop yield decline at temperatures above 30 °C (Boot et al., 2005; Schlenker and Roberts, 2009). Likewise crop quality will be likely less nutritious, thereby spreading more malnutrition in the developing world (Dwivedi et al., 2012 and the references therein).

Climate models predict that warmer temperatures and increases in the frequency and duration of drought during the twenty-first century will have negative impact on agricultural productivity (Lobell and Field, 2007; Kucharik and Serbin, 2008; Battisti and Naylor, 2009; Schlenker and Lobell, 2010; Roudier et al., 2011; Thornton et al., 2011; Lobell et al., 2011a,b). For example, maize production in Africa could be at risk of significant yield losses as researchers predict that each degree-day that the crop spends above 30 °C reduces yields by 1% if the plants receive sufficient water (Lobell et al., 2011a); these predictions are similar to those reported for maize yield

in the USA (Schlenker and Roberts, 2009). Lobell et al. (2011a) further showed that maize yields in Africa decreased by 1.7% for each degree-day the crop spent at temperature of over 30 °C under drought. Wheat production in Russia decreased by almost one-third in 2010, largely due to the summer heat wave (http://www.faostat.fao.org); similarly, wheat production declined significantly in China and India in 2010, largely due to drought (http://www.fao.org/giews/english/alert/index.htm) and sudden rise in temperature respectively, thereby causing forced maturity (Gupta et al., 2010). Warming at +2 °C is predicted to reduce yield losses by 50% in Australia and India (Asseng et al., 2011; Lobell et al., 2012). Likewise, the global maize and wheat production, as a result of warming during the period from 1980 to 2008, declined by 3.8% and 5.5%, respectively (Lobell et al., 2011b).

Climatic variation and change are already influencing the distribution and virulence of crop pest and diseases, but the interactions between the crops, pests and pathogens are complex and poorly understood in the context of climate change (Gregory et al., 2009). There is a growing awareness among academicians and policy makers to better appreciate the degree of health risk posed by climate change and formulate strategies that minimize adverse impacts. We need to integrate plant biology into the current paradigm with respect to climate change and humans and animals health to succeed in defeating emerging pests and pathogens posing a new threat to agriculture due to climate change (Patz and Kovats, 2002; McMichael et al., 2006; Ziska et al., 2009).

The evidence to-date suggests that global warming is significantly impacting human and livestock health (McMichael et al., 2006; Patz and Olson, 2006; Jones et al., 2008; Campbell-Lendrum et al., 2009). Mycotoxins of greatest concerns are aflatoxins, deoxynivalenol (DON), fumonisins, and ergot in food crops (Russell et al., 2010; Magan et al., 2011). Climate is a key driving force for fungal colonization and mycotoxin production (Magan et al., 2003) with potential to cause severe economic losses to growers. For example, the annual losses to the US growers from mycotoxin contamination exceed US$ 1 billion, with maize growers bearing the largest burden (Vardon et al., 2003). Both pre- and postharvest factors contribute to mycotoxin contamination in food and feed crops. The ability of the fungi to produce mycotoxins is largely influenced by temperature, relative humidity, insect attacks and stress conditions of the plants (Miraglia et al., 2009). Worldwide, mycotoxins cause a large number of diseases and human death annually (Lewis et al. 2005; Liu and Wu, 2010; Williams et al., 2004, 2010).

The largest outbreak of aflatoxicosis has been reported from rural Kenya, resulting in 125 deaths, due to consumption of maize contaminated with mycotoxin (Lewis et al., 2005).

Agrobiodiversity consists of the biological resources that are important for food production, including plants, animals, fisheries, and microorganisms that sustain the functioning of agroecosystems. Climate change poses a serious threat to species fitness (Bell and Collins, 2008; Kelly and Goulden, 2008), and to ecosystem services essential to food production (Shanthi Prabha et al., 2011). The latest database on world plant genetic resources highlighted that there are still large gaps, more specifically in crop wild relatives (CWR) and landraces, in ex situ gene bank collections preserved across the globe (Maxted et al., 2012). There is continuing need to assemble and screen germplasm strategically and discover new sources of variation that will enable developing new crop cultivars adapted to adverse climate and its variability. CWR have contributed many agronomically beneficial traits in shaping the modern cultivars (Dwivedi et al., 2008), and they will continue to provide useful genetic variation for climate-change adaptation, and also enable crop genetic enhancers select plants that will be well-suited for the future environmental conditions (Jarvis et al., 2008a). Promoting on-farm conservation may allow genes to evolve and respond to new environments that would be of great help to capture new genetic variants that will help mitigate climate-change impacts (Rana and Sharma, 2009).

Climate change is imposing significant stresses upon agriculture at a time when more food is required for an increasing world population. To feed ~9 billion people by the middle of the twenty-first century, the production of high-quality food must increase with reduced inputs. Plant breeding must therefore focus on traits that improve nutritional quality, confer enhanced nutrients- and water-use efficiency (WUE), and those that enhance adaptation to abiotic and biotic stresses to increase yield. New cultivars and breeding populations will need to be continually developed to help withstand climatic extremes and maintain or even increase productivity in the face of increased climatic variability (Ortiz et al., 2008a; Ainsworth and Ort, 2010; Ceccarelli et al., 2010; McClean et al., 2011).

Climate change is altering the availability of resources and the conditions that are crucial to plant performance. Plants respond to these changes through environmentally induced shift in phenotype (phenotypic plasticity). Understanding these responses is crucial to predict and manage the effects of climate change on native species as well as crop plants. The evidence to-date suggests that breeding for phenotypic plasticity in traits other than

yield will potentially afford resilience in increasingly unpredictable environments (Sambatti and Caylor, 2007; Nicotra and Davidson, 2010; Nicotra et al., 2010). Modern tools such as those from applied genomics or transgenics must support conventional breeding to accelerate development of improved open pollinated or inbred cultivars and hybrids in such a way that it increases the available genetic diversity to improve food and nutritional security (Takeda and Matsuoka, 2008; Tester and Langridge, 2010; Dwivedi et al., 2007a, 2010; Fedoroff et al., 2010; Brummer et al., 2011; McClean et al., 2011; Ronald, 2011). However, genetically enhanced seed-embedded technology should be integrated into ecologically sustainable farming systems and evaluated in the light of their environmental, economic and social impacts in order to develop sustainable agricultural systems (Ronald, 2011).

Researchers are currently engaged with identifying climate analog sites across space (between locations) or time (with past or future climates). Once they are identified, these climate analog sites will provide platforms to develop and test various adaptation strategies including genetically enhanced seed-embedded technology to mitigate the adverse effects of global warming on agricultural productivity (Ramirez-Villegas et al., 2011).

This chapter reviews the role of agrobiodiversity in enhancing food and nutritional security, the contribution of plant phenomics for rapid, accurate and cost-effective assays for identifying traits conferring adaptation to stresses; and assesses the progress made in selected crops toward developing climate-ready crop cultivars adapted to climate change and its variability in the twenty-first century. Issues related to lack of food safety and the outbreaks of new pests due to global warming, and approaches to overcome them are also highlighted.

2. MOISTURE STRESS AND RISING CO_2 AND TEMPERATURE IMPACTS ON FOOD QUALITY

Grain quality (excluding grain physical characteristics) refers to the variation in protein and oil contents, protein and oil quality, carbohydrate, minerals (macro- and micronutrients), and vitamins. These characteristics together determine the quality of food and feed crops. The physical characteristics of the grains include grain size and shape, grain color, and grain weight. Drought and heat invariably reduce grain weight (Prasad et al., 2008; Thomas et al., 2009; Balla et al., 2011), while elevated CO_2 increases grain weight (Uprety, 2007; Högy and Fangmeier, 2008). Further, elevated CO_2 also causes variation in seed length and width among wheat species;

being hexaploid wheat more responsive positively to increased seed length and breadth (Uprety et al., 2009).

Extensive literature search revealed that unlike in the case of adverse effects of drought on crops performance (Dwivedi et al., 2010 and references therein), scanty research on the effects of elevated CO_2, drought and heat on grain quality.

2.1. Drought, Heat and Grain Quality

2.1.1. Protein and Protein Quality

The effects of drought or heat on grain quality have been investigated in cereals, pulses and oilseeds (Table 1.1). With few exceptions, drought invariably increased grain protein by ~14–21% in faba bean, peanut, rice and wheat. The pattern of drought also reflected variation in grain protein. For example, the midseason drought stress in pearl millet raised grain protein by 18%, while the terminal drought stress elevated grain protein by 44% (Mahalakshmi et al., 1985). The midseason drought in peanut had no adverse effect on grain protein, while terminal drought increased grain protein by 16% (Dwivedi et al., 1996). Unlike cereals, drought in lupins substantially reduced grain protein by 19–35%, with greatest reduction observed from lupins grains harvested at 75% moisture deficit (Khalil and Ismael, 2010).

Drought stress invariably leads to rise in air and soil temperature, which alone or with drought adversely impact the protein content and its quality. For example, at 32/26 °C, the protein declined by 19.6% in groundnut, at 4 °C increase above the ambient temperature, it declined by 6.7% in rice, while it continued to increase with rise in temperature, but above 40/30 °C, it declined sharply in soybean (Table 1.1). Drought and heat together increased grain protein by 28–34% in wheat. Protein composition, which is the most decisive factor in bread-making quality, is greatly influenced by various types of stresses. The wheat crop exposed to drought and heat (35 °C) at grain filling significantly altered protein composition: gluten either reduced (Ozturk and Aydin, 2004) or increased (Shahryari et al., 2011), while unextractable polymeric protein fraction and glutenin-to-gliadin ratio was reduced (Balla et al., 2010), with drought alone greatly influencing the protein composition than heat (Balla et al., 2011). Thus, reductions in unextractable polymeric protein fraction and glutenin-to-gliadin ratio indicate a poorer grain quality, despite the higher grain protein under drought in wheat (Balla et al., 2010). Temperature above 35 °C also led to loss of dough strength in wheat, and the mechanism involved in dough weakening (if known) should provide breeders with selection tools to assist in the production of cultivars that will tolerate heat (Wrigley, 2006).

Table 1.1 Effect of drought or heat stress on grain protein and protein quality in faba bean, lupins, maize, peanut, pearl millet, rice, soybean, and wheat

Summary of stress effect on grain-protein and protein quality	Reference
Drought stress	
Faba bean	
Drought stress increased protein by 13.7%	Al-Suhaibani, 2009
Peanut	
Terminal drought stress increased protein by 15.8%	Dwivedi et al., 1996
Lupins	
75% water deficit reduced protein by 35% in comparison to control (35% water deficit)	Khalil and Ismael, 2010
Drought stress reduced protein by 19.5%	Carvalho et al., 2004
Maize	
Drought stress reduced protein by 3.9%	Ali et al., 2010
Pearl millet	
Midseason drought increased protein by 18%, while terminal drought 44%	Mahalakshmi et al., 1985
Rice	
Drought stress increased protein by 20.9%	Fofana et al., 2010
Drought stress increased protein by 12.7%	Crusciol et al., 2008
Wheat	
Protein and gluten under drought stress, respectively, reduced by 3% and 6%	Shahryari et al., 2011
Drought stress increased protein by 12.8% continuous drought stress increased protein by 18.1%, while late water stress by 8.3%	Zhao et al., 2009 Ozturk and Aydin, 2004
Heat stress	
Peanut	
Elevated temperature (32/26 °C) significantly decreased protein by 19.6%	Golombek et al., 1995
Rice	
Elevated temperature (ambient + 4 °C) significantly decreased protein by 6.7%	Ziska et al., 1997
Soybean	
Protein increased with rise in temperature but above 40/30 °C it declined sharply	Thomas et al., 2003
Protein remained stable between 18 and 30 °C but significantly increased at 33 °C; most amino acids remained unchanged except methionine that substantially increased at the warmest temperature	Wolf et al., 1982

Table 1.1 Effect of drought or heat stress on grain protein and protein quality in faba bean, lupins, maize, peanut, pearl millet, rice, soybean, and wheat—cont'd

Summary of stress effect on grain-protein and protein quality	Reference
Heat stress	
Wheat	
High temperature and drought stress increased protein by 28.5%	Fernando et al., 2012
Drought and heat stress increased protein by 34.4% but protein quality deteriorated	Balla et al., 2011

2.1.2. Oil and Oil Quality

Drought significantly reduced grain oil, with greatest reductions in lupins (50–55%) and maize (40%). The reduction in oil content was 32% in rapeseed, and 5–10% in peanut and sunflower (Table 1.2). In contrast, heat stress in peanut and soybean increased oil by 20% and 37%, respectively, while oil content was reduced by 23% in heat-stressed kidney bean (Table 1.2).

The nutritional and storage quality depend on the relative proportion of saturated and unsaturated (oleic, linoleic and linolenic) fatty acids in the oil. A high proportion of polyunsaturated fatty acid is desirable as it lowers plasma cholesterol and low-density lipoprotein, which may reduce the risk of coronary heart disease and atherogenesis (Jackson et al., 1978). Further, linoleic and linolenic fatty acids have been associated with oxidation and the development of unfavorable flavors (Dutton et al., 1951; Branch et al., 1990). Drought and heat stress, either independently or together, had significant effects on fatty acid composition in maize, peanut, soybean, and sunflower. An increase in oleic acid in general led to a corresponding decrease in linoleic or linolenic fatty acids (Table 1.2). However, differential response of the test materials to drought and heat (Rennie and Tanner, 1989; Ali et al., 2009, 2010) may provide opportunity to identify genotypes with least adverse effect on oil quality.

2.1.3. Minerals

Globally, over 3 billion people are affected by micronutrient malnutrition (http://www.unscn.org). Malnourishment is often associated with serious physical incapacity, mental impairment, decreased health and parasitic diseases. The micronutrients are also essential for growth and development of crop plants (Dwivedi et al., 2012 and references cited therein). Few studies in barley, lupin, maize, rice and wheat reported the adverse effect of drought and heat on grain minerals (Table 1.3). Drought stress mostly increased zinc (Zn) in barley, lupin,

Table 1.2 Effect of drought or heat stress on grain oil and oil quality in kidney bean, lupins, maize, peanut, rapeseed, soybean, and sunflower

Summary of stress effect on grain oil and fatty acid composition	Reference
Drought stress	
Peanut	
Oil reduced by 5%; oleic acid increased by 9.3% while linoleic acid reduced by 11.5%	Dwivedi et al., 1996
Lupins	
Oil reduced by 50%	Carvalho et al., 2005
Oil reduced by 55%	Carvalho et al., 2004
Maize	
Oil decreased up to 40%; oleic acid increased up to 25.6%; linoleic acid reduced up to 14%; individual (α, γ, β) and total tocopherol increased substantially	Ali et al., 2010
Rapeseed	
Oil reduced by 31.7%	Ahmadi and Bahrani, 2009
Soybean	
Oleic acid increased by 6.5%, while linoleic acid reduced by 3.6%	Kirnak et al., 2010
Sunflower	
Oil decreased by 10.52%; differential response due to water stress in changes in oleic, linoleic and linolenic fatty acids between cultivars; α-, δ- and γ-tocopherol as well total tocopherol increased by several folds 67–251%	Ali et al., 2009
Heat stress	
Peanut	
Oil content increased by 20% as the temperature increased; oleic (O) acid increased by 24% with corresponding decrease in linoleic (L) acid and increase in O/L ratio, a measure of shelf-life of the product	Golombek et al., 1995
Elevated temperature significantly increased oleic and stearic acids by 5% and 9%, respectively, while palmitic and linoleic acids decreased by 3% and 6%	Burkey et al., 2007
Kidney bean	
Elevated temperature (34/24 °C) significantly decreased oil by 22.7%	Thomas et al., 2009

Table 1.2 Effect of drought or heat stress on grain oil and oil quality in kidney bean, lupins, maize, peanut, rapeseed, soybean, and sunflower—cont'd

Soybean

Elevated temperature (40/30 °C) substantially increased oleic acid, while linoleic and linolenic acids correspondingly decreased; however, genotypic differences in response to elevated temperature were noticed	Rennie and Tanner, 1989
Oil increased by 37% as temperature increased; oleic acid increased by 196% with corresponding decrease in linoleic and linolenic acids	Wolf et al., 1982

Table 1.3 Effect of drought or heat stress on grain macro- and micronutrients in barley, lupins, maize, rice, and wheat

Summary of stress effect on grain minerals	Reference
Drought stress	
Barley	
N, Zn, Mn increased by 12, 27 and 7%	Farahani et al., 2011
Lupins	
Drought stress significantly increased both macronutrients (Ca, Na, K, Mg) and micronutrients (Fe, Zn, Mn, Cu) as well as phytate content	Carvalho, 2005
Maize	
Drought combined with soil acidity led to more than twice the accumulation of Zn and 6–9 times accumulation of Mn	Rastija et al., 2010
Rice	
Rainfed rice provided increased grain N (12.7%), P (45.4%), Ca (37.1%), Mg (152.6%), Fe (356.6%) and Zn (73%) compared to sprinkler-irrigated grown rice; however, K reduced by 12.3%, S by 23.1% and Cu by 50%	Crusciol et al., 2008
Wheat	
P increased by 11%, Ca 25%, Mg 8.3% and Zn 20.8%; however, K reduced by 10.5%	Zhao et al., 2009
Heat stress	
Wheat	
Elevated temperature increased Fe by 25%, Zn 24.5%, S 23%, and Ca 6%	Fernando et al., 2012
N and P continued to increase with temperature up to 40/30 °C, then declined	Thomas et al., 2003

maize, rice and wheat; iron (Fe) in lupin and rice; calcium (Ca) in lupin, rice and wheat; phosphorus (P) in rice and wheat; nitrogen (N) in barley and rice; potassium (K) in lupin (but reduced in rice and wheat); magnesium (Mg) in lupin, rice and wheat; and manganese (Mn) in barley and maize. Sodium (Na) in lupin increased, while sulfur (S) and copper (Cu) declined in rice. However, drought brings variable changes in macro- and micronutrients in these crops. For example, produce from rainfed rice showed almost four and half times more grain Fe (51.6 mg kg^{-1}) than those recorded from produce harvested from sprinkler-grown rice crop (11.3 mg kg^{-1}) (Crusciol et al., 2008). Likewise, drought combined with soil acidity led to the excessive accumulation (6–9 times) of Mn in maize grains (Rastija et al., 2010), while maize grains obtained from acidic soils showed much higher Mn and Zn than those from nonacid soils (Rastija et al., 2010). Variation in soil water also impacted grain minerals (P, K, Ca, Mg and Zn), with highest increase of 20.8% detected for Zn in plots receiving 45% of soil water than those that received 85% soil water (45.2 mg kg^{-1} Zn) in winter wheat (Zhao et al., 2009). In sweet corn, 30% water deficit in comparison to no water deficit reduced grain Fe by 57%, Zn by 43% and Cu by 47% (Oktem, 2008). Recent research has shown that postflowering drought stress, in comparison to no stress, significantly increased grain Fe (24–35%) and Zn (15–20%) concentrations in pearl millet (ICRISAT, unpublished data). Minerals in grains were differently affected by drought stress (Peleg et al., 2008), providing an opportunity to select germplasm with least difference between stressed and nonstressed conditions. Wheat grains harvested from heat-stressed plots showed 23–25% greater Fe, Zn and S. However, Ca increased by 6% (Fernando et al., 2012), while in another study, N and P continued to increase with increasing temperature up to 40/30 °C, and then declined (Thomas et al., 2003).

2.1.4. Carbohydrates

Carbohydrates are one of the main dietary components. They are sugars, starches and fibers, classified either as monosaccharide (glucose and fructose), disaccharide (table sugar) or complex (starches) carbohydrates. All of them provide energy to the human body. Up-to-date literature search reveals that lupin and faba bean among the legumes and maize and wheat among the cereals have been investigated for the effects of drought stress on their grain carbohydrate (Table 1.4). Lupin grains harvested from crops with 75% water deficit showed 30% reduction in carbohydrate in comparison to those obtained from the crop that suffered 35% water deficit (Khalil and Ismael, 2010); while earlier reports revealed varying effects of drought stress on grain carbohydrate in lupin (Carvalho et al., 2004, 2005). Drought stress in faba bean caused only marginal increase (4.3%) in carbohydrate, while it increased fiber by 7–21% and sugar by 33–35% in maize (Ali et al., 2010).

Table 1.4 Effect of drought or heat stress on grain carbohydrate in faba bean, lupins, maize, peanut, soybean and wheat

Summary of stress effect on grain carbohydrate, fiber and starch contents	Reference
Drought stress	
Faba bean	
Carbohydrate increased by 4.3%	Al-Suhaibani, 2009
Lupins	
Water deficit (75%) caused 30% reduction in carbohydrate in comparison to 35% water deficit	Khalil and Ismael, 2010
Total carbohydrate, sucrose and sucrose/galactoside ratio increased; however, raffinose reduced	Carvalho et al., 2005
Soluble sugars decreased by 18.25%, crude fiber by 10.6% and starch by 42.6%	Carvalho et al., 2004
Maize	
Fiber increased by 7.3–21.3%, starch by 9.1–9.2%; sugar by 33–35%	Ali et al., 2010
Wheat	
Starch reduced by 20.5%	Zhang et al., 2010
Starch reduced by 3.4%	Zhao et al., 2009
Heat stress	
Peanut	
Elevated temperature (32/26 °C) significantly decreased total sugar by 24.5% and starch by 53%	Golombek et al., 1995
Soybean	
CO_2-induced elevated temperature decreased total nonstructural carbohydrate (TNC), with more reduction in soluble sugars than the starch	Thomas et al., 2003
Glucose, fructose and raffinose remained unaffected while sucrose declined by 56% at 33/28 °C	Wolf et al., 1982

Starch constitutes the major component of the grains, which serves as a multifunctional ingredient for the food industry. The shape, volume and structure determine the starch quality. Drought stress in wheat reduced grain-starch by up to 20% (Zhao et al., 2009; Zhang et al., 2010), while it increased grain-starch by 9% in maize (Ali et al., 2010). Drought also brought changes in the proportion of starch granules: A-type granules increased, while B- and C-type granules decreased, and these effects were cultivar- and stage-dependent in wheat (Singh et al., 2008a; Dai et al., 2009).

Elevated temperature (32/26 °C) substantially decreased total sugar and starch in groundnut, while glucose, fructose, and raffinose remained unaffected in soybean, but sucrose at 33/28 °C temperature regime declined by 56% (Table 1.4). Further, CO_2-induced elevated temperature in soybean increased

total nonstructural carbohydrate, with more reduction in soluble sugars than in starch (Thomas et al., 2003). High temperature from anthesis to maturity reduced the duration of starch accumulation in wheat. Starch accumulation ceased approximately 6 days earlier for grain produced under a 37/17 °C and 21 days earlier under a 37/28 °C than for grain produced under a 24/17 °C. In comparison to 24/17 °C, starch content was approximately 19% lower for mature grain produced under 37/17 °C and 58% less under 37/28 °C. The smaller B-type granules were the predominant class in mature grain produced under 24/17 and 37/17 °C, whereas the larger A-type granules were predominant in grain produced under 37/28 °C (Hurkman et al., 2003).

More recently, Wang et al. (2012) investigated the role of preanthesis high-temperature acclimation in alleviating the negative effects of postanthesis heat stress on stem-stored carbohydrate remobilization and grain-starch accumulation in wheat. Postanthesis heat stress lowered grain-starch content and increased percentages of volume, number and surface area of B-type starch granules in heat at postanthesis as well heat at pre- and postanthesis than in no heat stress situation. However, plants exposed to heat at both stages of development (pre- and postanthesis) had much higher starch content, and caused less modified B-type starch granule size than the plants exposed to high temperature at postanthesis stage, demonstrating that the preanthesis high-temperature acclimation effectively enhanced carbohydrate remobilization from stem to grains, and led to less changed starch content and starch granule size distribution in grains of wheat under postanthesis heat stress.

2.1.5. Tocopherol (Vitamin E)

Tocopherols are well recognized as antioxidants in vegetable oils, and their presence increases the stability of lipids against autoxidation (Goffman and Böhme, 2001). Drought in maize and sunflower increased the individual (α, β, δ) as well as total tocopherol by several folds (67–251%) (Ali et al., 2009, 2010), whereas similar stress in soybean caused two- to threefold increases in α-tocopherol (Steven and Diane, 2002).

2.2. Rising CO_2, Heat and Grain Quality

2.2.1. Protein and Protein Quality

Grain–protein concentration and composition are major determinants of grain nutritional value as well of flour functional properties (Weegels et al., 1996; Feil, 1997; Shewry and Halford, 2002). Wheat flour protein consists of albumins and globulins (~20%) and glutens proteins (~80%). Albumin and

globulin are metabolic proteins while gluten, as storage proteins, influences the baking properties. The gluten proteins based on solubility in aqueous alcohol are further divided into soluble gliadins and insoluble glutenins, with both fractions consisting of numerous, partially closely related protein components (Wieser, 2007). Wheat among the cereals is the most extensively studied crop for the effects of elevated CO_2 on grain–protein and protein quality (Table 1.5). Elevated CO_2 reduced grain protein by 4–15%. Application of varying doses of N fertilizer under elevated CO_2 did not ameliorate the decline in grain protein; however, researchers noted less reduction (14%) at 100 N (Wieser et al., 2008) than when N was either not applied or applied at low rate (up to 27% reduction) (Conroy et al., 1994; Porteaus et al., 2009), suggesting thereby that higher N under elevated CO_2 will have some positive effect, but not enough to arrest the decline of grain protein. Furthermore, elevated CO_2 also brought significant changes in wheat grain–protein composition: gliadins reduced up to 20%, glutenins up to 15%, and glutenin macropolymer up to 19%, while albumins and globulins fractions were not affected. Within gliadins, w5-gliadins and w1,2-gliadins were more affected than α-gliadins and γ-gliadins, while within glutenins, high molecular weight (HMW) subunits were more affected than low molecular weight (LMW) subunits, thus, adversely impacting baking quality (Wieser et al., 2008).

Grain–protein quality is also influenced by variation in amino acids composition, including essential amino acids (leucine, isoleucine, valine, lysine, threonine, tryptophan, methionine, phenylalanine, and histidine), which are not produced by the body, but must be supplied by food. A comprehensive study in rice showed substantial reduction in amino acids under elevated CO_2 conditions, with essential amino acids reduced between 29% and 38% (Xu et al., 1998). Methionine was substantially increased in soybean at the warmest temperature, while the other amino acids remained unchanged (Wolf et al., 1982). More recently, Högy and Fangmeier (2008) detected 8–22% reduction in amino acid composition depending on the exposure system and rooting volume in wheat.

2.2.2. Oil and Oil Quality

Elevated CO_2 is associated with increased global warming. Essentially, high temperature reduced the oil content per se but improved oil quality (as determined by variation in fatty acid composition): oleic acid increased, while linoleic or linolenic acids linearly decreased in oil crops (see Section 2.1).

Omega fatty acids (omega-3 and omega-6), which are not synthesized in the body but obtained through food source or as supplement, are

Table 1.5 Effect of elevated carbon dioxide (CO_2) on grain protein and protein quality in barley, rice, and wheat

Summary of rising CO_2 effect on grain-protein and protein quality	Reference
Barley	
Protein reduced by 11–13%	Erbs et al., 2010
Rice	
Protein reduced by 9%	Ziska et al., 1997
Total amino acids at elevated CO_2 were lowered by 30%; except for cystine (increased by 11%) and arginine (increased by 21.7%), all other 15 amino acids of rice grains were 28–40% lower under elevated CO_2, including essential amino acids, lysine, threonine, methionine, phenylalanine, leucine, and isoleucine	Xu et al., 1998
Wheat	
Protein reduced by 12.7%	Fernando et al., 2012
Protein reduced by 4–13%	Erbs et al., 2010
Protein reduced by 3.5%	Högy et al., 2009a
Protein reduced by 26.8% at elevated CO_2 and low N supply	Porteaus et al., 2009
Protein reduced by 7.4%, changes in amino acid composition with greater reduction in nonessential than essential amino acids	Högy et al., 2009b
Amino acids such as Thr, Val, Ile, Leu, Arg, Tyr, Asp, Ser, Gln, Ala and Phe reduced significantly by 7.7–22.2% depending on exposure system and rooting volume	Högy and Fangmeier, 2008
Protein reduced by 9% at N50 and 14% at N100; substantial effects on protein fractions—gliadins reduced by 13–20%, glutenins by 15%, glutenin macropolymer by 16–19%; diminishing baking quality	Wieser et al., 2008
Protein in grain reduced by 6.25%, while in flour by 12.5%	Ziska et al., 2004
Protein reduced by 15.2% and lysine by 5.8%	Wu et al., 2004
Protein reduced by 13.9%	Bluementhal et al., 1996
Protein reduced by 9–14%, highest reduction under zero N in comparison to limited N application	Conroy et al., 1994

associated with a range of beneficial health effects in humans (Covington, 2004). Fish is a good source of omega-3 fatty acids. However, there is a growing concern about the presence of organic contaminants in seafood (Hites et al., 2004). Hence, we need to find alternative sources of omega-3

fatty acids. Ziska et al. (2007) were probably the first to demonstrate the effects of enriched CO_2 on omega fatty acids in mungbean—omega-6 fatty acids reduced, while omega-3 fatty acids significantly increased in mature grains—which demonstrate that mungbean produced under elevated CO_2 could be an alternative source of omega-3 fatty acids in the diet.

2.2.3. Minerals

Limited studies in barley, rice & wheat have shown that rising CO_2 has major impact on cereal grain micronutrients (Table 1.6). For example, grain Fe and Zn were significantly reduced under elevated CO_2 conditions in rice and wheat. The reductions in Fe ranged between 10% and 29%, while the reduction in Zn varied from 17% to 33%. Some minerals responded differently: K and Ca increased by 12–41% in rice, but decreased from 12% to 23% in wheat. The decline in grain N was in the range of 12–22% in rice and 15–29% in wheat. Soil N also impacted grain minerals. For example, elevated CO_2 and low soil N decreased S by 14% in barley grains, while it increased by 5% in wheat grains. Statistically nonsignificant changes were also noted with respect to other macro- and micronutrients. It is therefore clear that produce harvested from elevated CO_2 conditions will have altered grain mineral contents.

2.2.4. Carbohydrates

Elevated CO_2 also brought changes in grain carbohydrate in rice and wheat (Table 1.7). For example, total sugars and nonstructural carbohydrates substantially increased in rice grains (Uprety et al., 2007). Variation in soil N and enriched CO_2 adversely impacted hemicellulose in wheat—at low N and high CO_2, the hemicellulose reduced by 26%, while at high N and enriched CO_2, the decline in hemicellulose was only 13%. Furthermore, starch content increased by 7–8% under elevated CO_2 irrespective of the variation in soil N, while water-soluble carbohydrates reduced by 7–15% at low/high N supply under elevated CO_2 in wheat (Porteaus et al., 2009). Elevated CO_2 or high temperature also impacted grain carbohydrate in kidney bean: glucose was reduced, while sucrose and raffinose were increased (Thomas et al., 2009).

2.3. Elevated CO_2 and Forage Quality for Ruminants

Ruminants (cattle, sheep or goat) have evolved a four-compartment capacious pregastric stomach where a symbiotic relationship exists with microbes that have an ability to break down complex structural polysaccharides to

Table 1.6 Effect of elevated carbon dioxide (CO_2) on grain macro- and micronutrients in barley, rice, and wheat

Summary of rising CO_2 effect on grain macro- and micronutrients	Reference
Barley	
S reduced on average by 14% under elevated CO_2 and low N supply	Erbs et al., 2010
Rice	
Ca increased by 12.5% and K by 41.2%, while N decreased by 2.5%	Uprety, 2007
P declined by 5%, Zn 28% and Fe 17%; N reduced in the range of 12–22%	Seneweera and Conroy, 1997
Wheat	
Fe reduced by 10.5%, Zn 17%, S 7.5%, and Ca 12%	Fernando et al., 2012
S reduced on average by 5% under elevated CO_2 and low N supply	Erbs et al., 2010
Na, Ca, P, S, Fe, Zn, Cu, Mn and Al decreased, while K, Mg and Mo increased; however, changes were statistically nonsignificant	Högy et al., 2009a
K, Mo, Pb significantly increased, while Mn, Fe, Cd and Si significantly decreased	Högy et al., 2009b
Na, Ca, Mg, S, Fe, Zn and Mn decreased by 3.7–18.3%	Högy and Fangmeier, 2008
N decreased by 15.2%, P 36.6%, K 23.2% and Zn 32.6%	Wu et al., 2004
N, S, Fe and Zn reduced between 21 and 29%, Ca 12–17%, Mg 8–13% and Mn 6–8%	Manderscheid et al., 1995

compounds easily absorbed by the animal. Ruminant digestion is complex as a result of interactions among the diet, the microbial population, and the animal (Owensby et al., 1996; Ehleringer et al., 2002). The plants in CO_2-enriched environments grow faster, produce more biomass and grain yield (Jaggard et al., 2010). However, this rapid growth often leads to poor nutritional quality of the forage (Akin et al., 1995; Cotrufo et al., 1998; Sinclair et al., 2000; Lilley et al., 2001; Pal et al., 2004; Pang et al., 2005). The nutritive value of the forage is highly dependent on leaf N, protein, fiber, nonstructural carbohydrates and minerals. The reduced N and other elements and increased fiber concentrations in plants grown under elevated CO_2 may adversely impact ruminant productivity, unless ruminants are supplemented with additional nutrition in their diets. Besides changes in leaf chemistry, reduction in forage quality may also come from morphological changes associated with

Table 1.7 Effect of rising carbon dioxide (CO_2) and elevated temperature on grain carbohydrate in kidney bean, rice, and wheat

Summary of rising CO_2 effect on grain carbohydrate, fiber and starch	Reference
Elevated CO_2	
Rice	
Total sugar increased by 32.5%, total nonstructural carbohydrate by 29.3% and amylase by 5.2%	Uprety, 2007
Wheat	
Fructose and fructan significantly increased	Högy et al., 2009b
Hemicellulose reduced by 25.9% at elevated CO_2 and low N supply, while under high N supply and elevated CO_2, it reduced only 13%; starch increased by 7–8% under elevated CO_2 and low/high N supply conditions; water-soluble carbohydrate reduced by 7–15% at low/high N supply under elevated CO_2	Porteaus et al., 2009
Elevated CO_2 *and temperature*	
Kidney bean	
Elevated CO_2 (700 μmol mol^{-1}) and temperature (34/24 °C) reduced glucose by 44%, while high temperature alone increased sucrose and raffinose by 32.6% and 116%, respectively	Thomas et al., 2009

elevated CO_2. For example, more waxes and extra layers of epidermal cells in leaves of plants under elevated CO_2 may further reduce forage quality. Likewise, forage cuticles reduce microbial degradation of ingested forages (Owensby et al., 1996 and references therein). The major impact of lowered forage quality is that ruminants will have greater nutritional stress due to reduced intake and consequently lowered productivity (Craine et al., 2009).

Grasses with C_3 photosynthetic pathway are more nutritious host plants than C_4 grasses (Barbehenn et al., 2004 and references therein). However, C_3 types in comparison to C_4 are more adversely impacted by elevated CO_2. The C_3 types under elevated CO_2 environments produce greater amounts of nonstructural carbohydrates, and have greater decline in their N than C_4 types. Barbehenn et al. (2004) raised the issue of whether will C_3 grasses remain superior to C_4 under elevated CO_2 levels. The experiment involving five species each of C_3 and C_4 grasses grown under CO_2-enriched environments clearly demonstrated that a significant increase in sugars, starch and fructan in the C_3 species under elevated CO_2 was associated with a significant reduction in their protein levels, while protein levels in most C_4

species being little affected by an elevated CO_2. However, this differential response of the two types of grasses was insufficient to reduce protein in C_3 to the levels of C_4 grasses. Thus, Barbehenn et al. concluded that C_3 grasses will remain more nutritious than C_4 grasses at elevated CO_2 concentrations, having higher levels of protein, nonstructural carbohydrates, and water, but lower levels of fiber and toughness, and lower total carbohydrate:protein ratios than C_4 grasses.

To sum up, drought, heat and elevated CO_2 will likely impact on forage and grain quality. However, their effects may be variable depending on crop growth stage, the duration and intensity of stresses, and soil N. Produce harvested from drought- or heat-stressed crops will have higher grain protein but of lower protein quality. Likewise, the produce from similar stresses will have reduced grain oil but improved oil quality. Drought and heat will also impact on grain minerals (both macro- and micronutrients); but to a variable extent. The lower nutritive value (reduced leaf N and protein) of the grasses and forages under CO_2-enriched environments will have adverse impact on ruminants unless their diet is supplemented with more nutritive food. More importantly, genotypes responded differentially with respect to grain-quality attributes under drought and heat, which may provide researchers opportunities to identify germplasm or cultivars with least differences in grain quality under stress for direct cultivation or use in crop improvement programs to breed cultivars with no adverse effect of elevated CO_2, drought and heat on grain quality.

3. GLOBAL WARMING AND ALTERED PATHOGENS AND PESTS IMPACTS ON CROP PRODUCTION AND QUALITY

Crop yield depends on growing-season weather, which also influences how pathogens and pests affect crops and their host–plant resistance. Plant pathogens account for 10–16% of global food losses (Chakraborty and Newton, 2011), which amounts to about US$ 220 billion annually (Ghini et al., 2008a). Although there is a paucity of information about the impact of climate change in plant pathogens and pest and their epidemics, its effects—which depend on changes in host distribution and phenology—will be noticed on their geographical spread, crop losses and plant protection options (Chakraborty et al., 2000a,b; Yáñez-López et al., 2012). Shaw (2009) further adds that there will also be changes in plant-associated microflora and direct biological effects on rapidly evolving pathogens.

Insect pests may increase under drought while fungi will benefit from increased rainfall or due to changes in the temperature. Characterizing the dynamic interactions between changes in climate variables and their effects on pathogens and pests will therefore allow assessing their potential impacts on crops, trees and pastures, and to develop sound options for control, e.g. through genetic enhancement.

3.1. Crop Pathogens and Pests in a Changing Climate

Coakley et al. (1999) indicated that temperature seems to be the most important factor affecting insect ecology, epidemiology, and distribution, while plant pathogens are highly responsive to humidity and rainfall, as well as to temperature. CO_2 may further promote the rapid establishment of invasive insect species (Zavala et al., 2008). Climate change will therefore affect the geographical and temporal distribution of pathogens and pests. For example, global warming, rainfall pattern changes, and new crop niches may lead to a significant change of crop health in Scandinavia (Roos et al., 2011).

Plant biomass production may increase as a result of a rise in CO_2 concentration in the atmosphere. Proliferation of shoots, leaves, flowers and fruit provide more tissue that can also be infected by pathogens. Furthermore, sugar-dependent pathogens (e.g. rusts and powdery mildews) may increase as a result of rising carbohydrate content, whereas high canopy density and plant size can promote the growth, sporulation and spread of leaf infecting fungi such as rusts, powdery mildews and leaf necrotrophs, which require high air humidity but not rainfall (Ghini et al., 2008a). Likewise, necrotrophic pathogens will also benefit by an increase in the amount of crop residues. Chakraborty and Pangga (2004) indicated that about 26 plant pathogens may increase their severity in CO_2-enriched environments.

3.2. Plant Pathogen Scenarios under Climate Change

Long-term datasets are important for determining scenarios for pathogens due to variation in CO_2 levels, temperature, rainfall and other climate factors that affect their spread and severity (Jeger and Pautasso, 2008). For example, Evans et al. (2008) used 15 year multilocation data to develop and validate a weather-based forecasting model of phoma stem canker (*Leptosphaeria maculans*) epidemics on oilseed rape across the United Kingdom. They predict that phoma stem canker epidemics will increase in severity and spread northward by the 2020s. However, changes in pathogen ranges due to climate change are not yet well defined due to uncertainty

for predicting the variables affecting them (Shaw and Osborne, 2011). Furthermore, primary data and historical records of many pathogens are often poor or lacking. Hence, Shaw and Osborne (2011) suggest that perhaps monitoring change and retaining the ability to innovate are needed for delineating adaptive responses. Likewise, as indicated by Chakraborty and Newton (2011), there has been limited field research on plant pathogens in the environments that realistically mimic climate change, which therefore reduces the assessment of options to enhance the adaptation of crops to emerging disease epidemics.

Although a lot of knowledge has been gathered about the changes in pathogen life cycle, expression of host-plant resistance, disease epidemiology and severity of disease epidemics, as well as pathogen inoculum production, there has not been enough research regarding the potential changes in pathogen biodiversity (Barbetti et al., 2012). New races or pathotypes will continue evolving and depending on crop husbandry and plant health management, but the changing climate may influence future changes in the distribution of emerging pathogen threat (Gregory et al., 2009). For example, as noted by Lake and Wade (2009), high CO_2 may accelerate plant pathogen evolution, which could lead to changes in virulence. However, an increase in CO_2 may not always affect disease incidence, as shown by experiments in phytotron for powdery mildew on grapevine (Pugliese et al., 2010) because of the increased photosynthetic activity of the host plants in hot environments with elevated CO_2.

Chakraborty et al. (2008) provide a summary of the impacts of climate change (including the influence of elevated CO_2 and O_3) on main pathogens affecting crops and trees worldwide. They acknowledge the scarcity of knowledge in this area, which calls for generating more empirical data on host–pathogen biology under a changing climate. Details are given below for selected pests affecting major crops and trees, and whose severity and incidence may be influenced by climate change.

Irrespective of cereal rusts being an important subject of research, the lack of information on the potential effects of the changing climate on wheat rusts did not allow planning its appropriate forecast and management. Nevertheless, Chakraborty et al. (2011) by extrapolating the results from modeling other crop–pathogen interactions and using available research literature on wheat rusts indicated that the main risks brought by climate change will be increased grain yield loss, new pathotypes evolving faster, and, last but not least, the reduced effectiveness of host-plant resistance to rust pathogens. They further elaborate by indicating that an increase of

biomass—due to high temperatures and elevated CO_2—could lead to an expansion of leaf area available for pathogen attack, which will enhance inoculum pressure. The changing weather may favor the spread of severe rust epidemics elsewhere, and to more conducive environments for increasing pathotypes evolution rates, thereby accelerating new virulence. Moreover, some of the known rust-resistance genes are affected by temperature and crop development, which may also be influenced by climate change. For example, emergence of an *Sr31*-virulent strain of stem rust (*Puccinia graminis* f. sp. *tritici*) in wheat, due to evolution of Ug99, sent an alarm bell to wheat researchers about the damaging effects of this pathotype to wheat production worldwide (Singh et al., 2008b). Likewise, there may likely be an increased prevalence of powdery mildew (*Blumeria graminis* f. sp. *tritici*) and scab (*Fusarium* spp.), and a decrease of stripe rust (*Puccinia striiformis*) affecting wheat production in China due to temperature increase (Yang et al., 1998).

Weather seems to be among the most significant factors affecting incidence, severity and the relative importance of *Fusarium* head blight or scab in wheat (Chakraborty and Newton, 2011). Warm and wet or humid environments at anthesis favor the pathogen, which causes blighting of the head, lowers yield and quality (e.g. shriveled kernels), reduces test weight and bread-making quality, and produces one or more mycotoxins. For example, scab epidemics will be more severe in the United Kingdom (especially in southern England) by 2050s due to climate change, which will also favor early wheat anthesis (Madgwick et al., 2011). Furthermore, scab severity may increase because, as a result of climate change, British farmers will grow more maize, whose debris will remain as a source of inoculum for *Fusarium graminearum*, which causes scab in wheat (West et al., 2012). Increasing rainfall will also favor wheat scab during the spring season in South America's Southern Cone (Ortiz, 2012).

The fungi *Cochliobolus sativus* (spot blotch) and *Pyrenophora tritici-repentis* (inducing tan spot) are the pathogens responsible for leaf blight in humid and hot areas, particularly in the Indo-Gangetic Plain, the major wheat production region in India (Ortiz et al., 2008a). Heat stress enhances their increasing severity with growth stage. Hence, wheat-bred germplasm adapted to heat and with host plant-resistance to leaf blight is needed to avoid grain yield loss in this bread-basket of the world. It can successfully be achieved by crossing cultigen resistance sources or CWR to high-yielding cultivars (Ortiz et al., 2008b).

Wheat head blast (*Magnaporthe grisea*, anamorph, *Pyricularia grisea*) is another emerging threat that may induce grain yield loss >50% in warm

(25–28 °C), humid environments of South America's Southern Cone—one of the major grain baskets worldwide (Ortiz, 2011). Host–plant resistance to this pathogen should also become a priority target for wheat breeding under climate change.

Blast (*Pyricularia oryzae*) and sheath blight (*Rhizoctonia solani*) are among the major pathogens of rice worldwide. Kobayashi et al. (2006) found that rice plants grown under elevated CO_2 concentration were more susceptible to leaf blast and sheath blight. Reduced leaf silicon content under elevated CO_2 concentration leads to more susceptibility to blast disease, and the change in rice canopy structure (due to increased number of tillers under elevated CO_2 concentration) may accelerate spread of sheath blight in the field. Thus, the potential risks to leaf blast infection and epidemics of sheath blight would increase in rice grown under elevated CO_2 concentration (Kobayashi et al., 2006).

There is also potential risk that global warming, drought and high rainfall may lead to changes in pathogen severity and spread, adversely impacting legume production, especially in the developing world. For example, a higher incidence of dry root rot (*Rhizoctonia bataticola*) was noted in chickpea cultivars resistant to fusarium wilt (*Fusarium oxysporum* f. sp. *ciceris*) when temperatures exceeded 33 °C in India, while epidemics of phytophthora blight of pigeonpea (*Phytophthora dreschsleri* f. sp. *cajani*) over the last decade was attributed to high intermittent rainfall (>300 mm) during the crop season in India (Savary et al., 2011 and the references therein). Likewise, Chakraborty et al. (2000a,b) observed an increased aggressiveness and fecundity of *Collitotrichum gloeosporiodes*, the causal agent of anthracnose in tropical legumes such as *Stylosanthes*.

Potato leaf blight (*Phytophthora infestans*) is the most damaging disease of potato worldwide. It is predicted that warming temperature and humidity will increase leaf blight, expanding its range above 3000 m, where it is absent today in the Andes region of South America (Ortiz, 2012). The suitability for producing high-quality coffee, such as the acidic Arabica, will be affected by heat, which also favors some pathogens such as *Hemileia vastatrix* causing coffee rust (Ortiz, 2012).

Climate change may also adversely impact host-plant resistance in banana and plantains. For example, scenario analyses suggest that the favorable period for the development of black leaf streak (or black Sigatoka) (*Mycosphaerella fijiensis*) and other foliar plant pathogens affecting bananas may be reduced (de Jesus Júnior et al., 2008). Black leaf streak of banana and plantain may decrease in producing locations of Central America and

other coastal areas in the American continent because a switch toward unfavorable environments for the pathogen (low relative humidity and rainfall affect adversely its development) despite high temperatures, which are often associated with increased diseases pressure. Nonetheless, there will be extensive areas favoring the occurrence of this disease, especially in environments that favor disease development (Ghini et al., 2007).

Climate change will affect also pathogens affecting forests, as already noted for annual and perennial crops. Europe expects to witness an increased incidence and severity of pathogens, which—due to rising temperature—may move northward in this continent (La Porta et al., 2008). Sturrok et al. (2011) suggest, based on their North American analysis for yellow-cedar and sudden aspen declines, that a strategy for managing emerging pathogen and pest threats due to climate change should include monitoring, forecasting, planning and mitigation. They also highlight that the uncertainty brought by climate change to forests can be reduced by research and risk assessment, and through linking them to policy, planning and decision making.

3.3. Emerging Changes in Pest Dynamics under Climate Change

A range of insect and nematode pests cause substantial damage to crops production and quality. Many of these pests are also carriers of viruses that cause significant losses to production. As for pathogens, the changes in climatic conditions may cause some insect and nematode pests to expand its range into new areas or retract from the areas wherein these pests currently pose a serious threat to crops production or altogether a new virulent biotype may emerge. The present day evidence suggests that the insect pests including lepidopteron respond to warming—from changes in phenology and distribution to undergoing evolutionary changes albeit at the population level in Europe and North America (Menéndez, 2007). Growing plants in elevated CO_2 generally increases the carbon-to-nitrogen (C:N) ratio of plant tissues (Hamilton et al., 2005) and reduces the nutritional quality (Coviella and Trumble, 1999). As a result the insects may increase their food intake to compensate for leaf nitrogen content (Coviella and Trumble, 1999; Holton et al., 2003). Exposure to elevated CO_2 has been reported to increase the loss of leaf area by foliage chewer, western corn rootworm (*Diabrotica virgifera*) and phloem feeder aphids (*Aphis glycines*) in soybean (Dermody et al., 2008). This increased loss of leaf area to these herbivores is associated with increased C:N ratio and leaf surface temperature (Dermody et al., 2008). Further research has shown that increased susceptibility of

soybean to western corn rootworm and Japanese beetle (*Popillia virgifera*) under elevated CO_2 concentrations is associated with the reduced expression of genes related to the jasmonic acid (JA) pathway (Zavala et al., 2008). Global warming may result in the breakdown of resistance to certain pests, for example, sorghum cultivars exhibiting resistance to sorghum midge (*Stenodiplosis sorghicola* (Coq.)) in India become susceptible to this pest under high humidity and moderate temperatures near the equator in Kenya (Sharma et al., 1999).

Insects may also appear in the region(s) where they are not currently known. For example, potato tuber moth (*Phthorimaea operculella*), which at present is restricted to coastal areas and inter-Andean valleys, may expand its range above 3000 m, where it is absent today in the Andes region of South America, due to climate change (Ortiz, 2012).

Root-knot nematode (*Meloidogyne incognita*) infects large number of crops including tomato, causing severe losses in yield. Salicylic acid (SA) and JA are the major signaling pathways of plant response to nematode infection (Vasyukova et al., 2003; Soriano et al., 2004). Sun et al. (2011) studied the effects of elevated CO_2 on nematode-induced defense response in tomato genotypes differing in the JA pathway. Their study revealed that elevated CO_2 reduces the JA-pathway (a JA-defense-dominated genotype) defense against *M. incognita* in the wild type and in a genotype in which defense is dominated by the JA pathway but upregulates the SA-pathway defense in the wild type and in a JA-defense-recessive genotype (jasmonate-deficient mutant), which means that CO_2-induced changes of plant resistance may lead to genotype-specific response of plants to nematode under elevated CO_2.

There will be changes in the spatial distribution of races of root-knot nematodes and leaf miner (*Leucoptera coffeella*) affecting the coffee crop in Brazil (Ghini et al., 2008b; Haggar and Schepp, 2011). An increase in infestation of both pests will likely be due to a higher number of generations per month than before.

3.4. Adapting Crops to Emerging Pathogens and Pests

Adaptation to climate change, which depends on local conditions, will therefore consider integrated plant health management for existing and emerging pathogens and pests. Such an approach will require a set of tools such as models for predicting potential geographical distribution, seasonal phenology, and population dynamics at a range of spatial and temporal scales (Sutherst et al., 2011). Crop–pest models offer the means for designing sound plant health management of pathogens and pests.

Host–plant resistance will continue to be very important for plant health management under climate change because rising temperature and variation in humidity may favor emerging pathogen and pest epidemics. Likewise, plant breeding for host–plant resistance to pathogens and pests leads to fewer pesticide sprays, which also means a reduction in fuel use and lowering CO_2 emissions (Ortiz, 2011), thereby mitigating climate change.

4. MANAGEMENT AND PREVENTION OF AFLATOXIN

At present, over 5 billion people in the developing world are regularly exposed to mycotoxins, especially aflatoxins, through consumption of contaminated staple food. Moreover, the agricultural produce contaminated with mycotoxins drastically limits the access of producers to the global markets, which have set high standards for food safety. Mycotoxins in animal feeds also represent high risk to the growth of livestock and trade of feed (Wu et al., 2011a and references therein). Mycotoxins of worldwide importance include aflatoxins (B_1, B_2, G_1, and G_2), DON, zearalenone, fumonisin B_1, T-2 toxin, and ochratoxin A, produced by fungi on pre- and postharvest foods and feeds.

The fungi *Aspergillus flavus* and *Aspergillus parasiticus* colonize maize and nuts including peanut, with former fungi producing aflatoxin B_1 and B_2 while the latter producing B_1, B_2, G_1, and G_2. Aflatoxin B_1 is the most toxic of aflatoxins. Aflatoxins in food can cause death, impair growth and development of children, suppress the immune system, enhance hepatitis B virus and hepatitis C virus infection, increase risk to certain types of cancer by several fold, and impede the uptake and utilization of micronutrients in humans and livestock (Lewis et al., 2005; Fokunang et al., 2006; Liu and Wu, 2010; Wu et al., 2011a and references therein). It is beyond the scope of this section to discuss in greater detail about the various mycotoxins and associated fungi and their control measures instead we discuss various aspects of aflatoxin research and management options that lead to minimize the risk of aflatoxin contamination under a changing climate in maize and peanut, the two food crops highly susceptible to aflatoxin.

4.1. Modeling Climatic Risks to Aflatoxin Contamination

How does climate change impact food safety, in addition to food security (Battisti and Naylor, 2009; Ronald, 2011; Vermeulen et al., 2011), has recently been dealt elsewhere (Cotty and Jaime-Garcia, 2007; Tirado et al., 2010; Paterson and Lima, 2010, 2011; Magan et al., 2011). Essentially,

the biggest risk with respect to mycotoxins from climate change will be found in the developed world with temperate climates, for example, in areas of Europe and the USA as these regions will become warmer reaching temperature of 33 °C, close to the optimal for aflatoxin production. Currently, in very cold climates aflatoxins may not be of any significantly greater concern than exists where even global warming will not result in optimal temperatures for *Aspergillus* growth. The tropical climates, and if the temperature continue to rise as predicted by various IPCC reports and exceed 40 °C, may become too inhospitable for conventional fungal growth and mycotoxin production. The warmer weather, heat waves, greater precipitation and drought in tropical climates will have greater impact on mycotoxins, and this subject has been discussed in greater detail by Paterson and Lima (2011) for Africa, Europe, Asia, Latin America and North America with respect to the risk of mycotoxins contamination in food and feed crops. They also highlighted that crops introduced to exploit altered climate may be subject to fewer mycotoxins producing fungi. The increased mycotoxins and UV radiation may cause fungi to mutate on crops and produce different mycotoxins. Crops when subjected to drought and high temperature, especially during reproductive phase, are under greater risk to aflatoxin contamination by *A. flavus* and *A. parasiticus*, with even greater risk to aflatoxin during the storage.

Water activity (a_w) and temperature, and their interactions with aflatoxin gene cluster significantly impact fungal growth and biosynthesis of aflatoxin; and gaining knowledge on these interacting factors should facilitate to forecast and develop effective control strategies to minimize the risk of aflatoxin contamination in food and feed crops (see Section 4.5; Miraglia et al., 2009; Magan et al., 2011).

Using agricultural production systems stimulater (APSIM) version 5.1 (aflatoxin risk simulation model; Wright et al., 2005), the weather and the peanut yield data at Kingaroy from 1890 to the present time in South East Queensland, Australia, Chauhan et al. (2008a) showed an 11.7% reduction in peanut pod yield since 1980. The risk of significant aflatoxin contamination, which was one in 11 years until 1979, increased to one in 3 years thereafter. They relate this increase to the changes in climate that indicated that since 1980, when changes in the risk became more noticeable, in-season crop rainfall decreased by 8%, maximum temperature increased by 2.1% (0.6 °C) and minimum temperature by 7.4% (1.1 °C), while radiation remained unchanged. Thus, risk to aflatoxin contamination in peanut could be minimized by growing early maturity cultivars, as well as through a late planting strategy to avoid high temperatures during the pod-filling stage.

Boken et al. (2008) are probably the first to use advanced very high–resolution radiometer satellite data and crop simulation models to predict aflatoxin contamination in peanut in Mali. Using the normalized difference vegetation index (NDVI) averaged for the reproductive phase of peanut, to examine the relationship with annual peanut yield, an indicator of drought and aflatoxin, they found that AVHRR–based NDVI at reproductive phase is moderately correlated ($R^2 = 0.56$) with peanut yield, which could be used to predict drought. The aflatoxin measured in peanut samples collected from various locations across Mali were found to be related to the NDVI, total precipitation, and maximum temperature averaged over the reproductive phase of peanut, which may be considered as potential variables to monitor and predict aflatoxin contamination. Predicting aflatoxin incidence will help identify risk zones to segregate the aflatoxin–contaminated peanuts from distribution among the general public.

High temperatures and end–of–season drought have been found to be associated with increased risk of aflatoxin contamination in peanut (Craufurd et al., 2006; Cotty and Jaime-Garcia, 2007). Chauhan et al. (2010) used APSIM peanut module to investigate the four temperature-response functions at fractional available water <0.20 and the crop in later development of the pod–filling stage to develop aflatoxin risk index (ARI). The ARI explains 95% of the variation in aflatoxin contamination (varied from 0 to 800 µg kg^{-1}) in several Australian tropical and subtropical environments and 96% of the variation in the proportion of aflatoxin-contaminated loads of peanuts in the Kingaroy, Australia during eight seasons of evaluation. The simulation of ARI using climatic data from 1890 to 2007 indicated a three-fold increase in its value since 1980 compared to the entire previous period, which they found to be associated with increases in ambient temperature and decreases in rainfall. The ARI predicted, using this interface for eight growers, correlated significantly with the level of contamination in the crop, suggesting that ARI is a reliable indicator of aflatoxin contamination that can be used in aflatoxin research as well as a decision–support tool to monitor preharvest aflatoxin risk in peanuts.

Maize has been the most extensively studied crop among cereals to predict occurrence of mycotoxins. Chauhan et al. (2008b) developed a similar model to quantify climatic risks of aflatoxin contamination in maize. The model performed well in simulating climatic risk of aflatoxin contamination in maize as it explained 69% and 62% of the variation, respectively for a range of rainfed and irrigated Australian locations. In further evaluations, the risk of aflatoxin contamination in four nonirrigated maize-growing

locations of Queensland using 106 years of climatic data, the model revealed that locations with dry and hot climates had a much higher probability of higher aflatoxin risk compared with locations having dry or hot conditions alone. This finding suggests that under nonirrigated conditions, the risk of aflatoxin contamination could be minimized by adjusting sowing time or selecting an appropriate hybrid to better match the grain filling period to coincide with lower temperature and water-stress conditions. More recently, Wu et al. (2011b) used temperature, rainfall, changes in insect population dynamics, and agronomic factors to predict fungal growth and mycotoxin risks in maize in the USA. The predictions revealed that if the current climate patterns continue in twenty-first century, it is predicted that aflatoxin and fumonisin concentrations in maize will likely increase, whereas DON levels will decrease. However, climate change-induced alterations in cropping patterns or shifts in pathogen populations may create new opportunities for DON risk in the areas where maize is currently not grown or is a minor crop, and where new, more aggressive isolates of *F. graminearum* occur.

Climate databases created under the North American Regional Climate Change Assessment Program (NARCCAP) (www.narcap.ucar.edu), which uses four international global climate models and six regional climate models, provide ranges of climatic conditions until 2070—day and night temperatures, number of days/nights above specific temperatures, precipitation, relative humidity, and soil moisture—that could be used to assess how future climatic scenarios may affect toxin levels in crops around the world.

4.2. Geostatistics and Geographic Information Systems to Monitor Spatial Variability in Aflatoxin

Geographic information systems (GIS) are capable of assembling, storing, manipulating, and displaying data georeferenced using geographic coordinates (latitude and longitude). Geostatistics provides a set of tools useful in characterizing variability in space. Both GIS and geostatistics can be used to describe, analyze and show the spatial distribution of several variables to define the cause–effect relationships of various variables with their geographic position (Nelson et al., 1999). The various reports to-date suggest that there has been increased use of geostatistics, GIS and satellite imagery data in agriculture (Lewis et al., 1998; Boken et al., 2008; Shamseddin and Adeeb, 2012; Kogan et al., 2012). Geostatistics has been used to characterize spatial and temporal variability at regional levels to predict and monitor shifts in community structure of aflatoxin-producing fungi, *A. flavus* (Orum et al., 1999). Such information will be helpful to

develop control strategies directed toward changing the composition of fungal communities.

The two major strains in *A. flavus*, based on sclerotial morphology, are S and L strains, with the former producing sclerotia <400 μm in diameter, and the latter >400 μm in diameter (Cotty, 1989). The S-strain isolates produce high levels of aflatoxin B_1, whereas the L-strain isolates are more variable in aflatoxin production (Cotty, 1997). These two strains also differ in production of the type of aflatoxins, with L isolates producing only aflatoxin B while the S isolates produce both aflatoxin B and G (Cardwell and Cotty, 2002). Populations of both strains comprise numerous subpopulations known as vegetative compatibility groups (VCGs) (Horn and Greene, 1995). Isolates in the same VCG have the same alleles for all compatibility loci, and when paired hyphae fuse to form heterokaryons (Papa, 1986). Isolates in different VCGs differ in sclerotial size, mating type, aflatoxin production, and intra-specific aflatoxin inhibition, while those from the same VCG have similar sclerotial size and mating type and produce the same kinds of mycotoxins (Horn and Greene, 1995; Mehl and Cotty, 2010; Grubisha and Cotty, 2010). Regional differences in aflatoxin contamination of crops may be attributed to climatic conditions and to agricultural practices that increase the suscep-tibility of plants to invasion by *A. flavus*. For example, it is well known that drought stress accompanied by elevated temperatures at seed development promotes *A. flavus* invasion and aflatoxin contamination in cotton, maize and peanut. Surveys of *A. flavus* isolates from various geographic regions have revealed differences in strain composition and in aflatoxin contamina-tion. Using geostatistical analysis, Jaime-Garcia and Cotty (2003) detected recurrent patterns of high and low aflatoxin in commercial cotton seeds produced in South Texas, USA with greatest contamination occurring from the Central Coastal Bend region through the southern Upper Coast region, whereas, the Rio Grande Valley region experiences the least contamination. Likewise, the regional patterns of differences in aflatoxin contamination were also reported in peanut. For example, 62–94% of isolates from western Texas, Georgia and Alabama produced at least 10 μg of aflatoxin B_1 ml^{-1}, while the isolates producing in excess of 10 μg of aflatoxin B_1 ml^{-1} from central Texas, Virginia and North Carolina ranged from 0% to 52%; with other isolates often atoxigenic in nature (Horn and Dorner, 1999).

The Republic of Benin in West Africa has four agroecological zones: the coastal Savanna (CS), the southern Guinea Savanna (SGS), the north-ern Guinea Savanna (NGS) and northernmost Sudan Savanna (NSS). All of them differ in latitude, precipitation and temperature. The latitude

differences among these regions relate to the differences in precipitation and rainfall pattern (bimodal or unimodal) and temperature, with decreasing rains and increasing temperatures noted as the latitude increase—from humid equatorial tropics in the south to the dry savanna near the Sahel in the north. When investigated, the pattern of distribution of *Aspergillus* across the four agroecological zones in Benin, Cardwell and Cotty (2002) found that all soil samples contained *A. flavus*. However, their densities differ from <10 to >200 CFU g^{-1} soil. Furthermore, they detected no yearly variation in CFU counts or significant change with cropping systems within a zone, but differed significantly among zones, with greater number of CFU of L strains in southern latitudes and higher numbers of CFU of S strains in the northern latitudes. These two strains also differ in their distribution and aflatoxin-producing ability. All S strains produce both B and G aflatoxins, while, only 44% of the total L strain detected from the soils produce aflatoxin B. *Aspergillus parasiticus* and *A. tamarii*, though present in <10% of the fields, were not particularly associated with any of the four agroecological zones. More prevalence of S strains is also reported from regions characterized by low rainfall and high temperature in North America (Horn and Dorner, 1999; Orum et al., 1999), which probably is the inherent ability of S strains to produce more small sclerotia as the survival mechanisms to adapt to rapid and extreme fluctuations in moisture and temperature.

Battilani et al. (2008) used aridity index (AI), derived from meteorological data, to estimate the probability of aflatoxin B_1 contamination in maize running a logistic regression. They found that AI as a good index to summarize meteorological conditions in relation to aflatoxin B_1 contamination in maize. In 64% of the cases, it was found that the prediction about aflatoxin B_1 contamination was accurate, while the prediction in 23% cases was overestimated, and underestimated in 13% cases. Such predictions may provide opportunity to maize growers plan maize management pre- and postharvest or segregate the produce either safe or unsafe for human consumptions based on the probability of the occurrence of aflatoxin contamination. Clearly, most of this research using geostatistics and GIS tools is needed to assess the risks of aflatoxin contamination, which in the long run should lead to the identification of regions or cropping systems leading to the production of food free from mycotoxins.

4.3. High-Throughput and Cost-effective Assays to Detect Aflatoxin

A wide variety of aflatoxin-monitoring technologies is available to detect and quantify aflatoxin levels (Pascale, 2009; Margos and Busman, 2010).

Conventional analytical methods for mycotoxin include thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), and gas chromatography (GC). In recent years, significant improvement has been introduced in the analysis of aflatoxins especially using column chromatography technology (Buttinger, 2010). The use of packaging materials with particle sizes below 2 μm, and the use of chromatograph which can support very high pressure, led to dramatic reduction in the analysis time. More recently, Medina and Magan (2012) compared the performance of chromatography columns with particles of 5 and 3 μm with the new 2.7 μm solid core particles for the analysis of aflatoxins using trifluoroacetic acid precolumn derivatization. The results of the comparisons revealed that shorter columns (100 × 4.6 mm) with new solid-core particles in comparison to traditional columns are suitable for the analysis of mycotoxins. This modification reduced the analysis time by 45.5% and 33.3% with respect to columns with particle size 5 μm (150 × 5.6 mm) and 3 μm (150 × 5.6 mm) respectively, without any detrimental effect on performance. This had lead to a reduced analytical cost, and increase in the total number of samples that can be analyzed; i.e. from 57 to 80 samples for columns with particle size of 5 and 3 μm respectively, to up to 111 samples day^{-1}. In spite of all these technological advances and saving in analytical cost and increasing throughput, the use of such assay in the developing countries is still limited due to high cost, difficulties with importation, and the lack of appropriate laboratory facilities and well-trained staff. Immunological methods are preferred over the analytical methods because of their simplicity and cost-effectiveness. However, commercial kits based on immunological methods are expensive, and there may be difficulties in their import. Waliyar et al. (2009) developed a simple and inexpensive competitive enzyme-linked immunosorbent assay (cELISA) that has lower detection limit (1.0 μg kg^{-1}) and costs (about US$ 1 per sample) less than other available methods, with high-throughput efficiency (>100 samples per day). This cELISA test has provided a unique opportunity to researchers in the developing world to select breeding populations possessing resistance to aflatoxin contamination, and to evaluate food, feed and related commodities for aflatoxin contamination. This technology has been successfully transferred and adopted by several laboratories in India, Mozambique, Kenya, Malawi and Mali, and is contributing to the quality certification of the farmers produce, and enhancing the competitiveness of the produce in domestic and international markets. For example, using this technology, Malawi has revived its large-seeded graded peanut export to Europe and South Africa, benefiting her farmers. Likewise, Kumar and Bandyopadhyay (2012) also developed a cost-effective,

high-throughput (up to 200 samples per day), simple and sensitive assay, called Afla-ELISA—for quantitative determination of aflatoxins—with the lowest detection limit of 0.09 ng mL^{-1} and a recovery of 98 $\pm$ 10%. They are advocating its use to monitor aflatoxins in maize in West Africa (Kumar and Bandyopadhyay, 2012).

4.4. Atoxigenic Fungal Strain as Biocontrol Agent to Manage Aflatoxin Contamination in Crops

To date, a number of atoxigenic isolates of *A. flavus* have been reported, of which, few are commercially used as biopesticide to control aflatoxin contamination in cotton, maize and peanut (see below within this section). The *A. flavus* genome has been sequenced (36.3 Mb) and contains 13,091 predicted genes in eight chromosomes (http://www.aspergillus-flavus.org/). Twenty-five genes clustered within a 70 kb DNA region are involved in aflatoxin biosynthesis, and the functions of 19 genes are known (Yu et al., 2004). Knowledge of the molecular mechanisms for the loss of aflatoxin production in atoxigenic *A. flavus* may help develop assays for rapid detection of atoxigenic isolates to plan a better biocontrol strategy by including mixture of genetically different atoxigenic isolates to provide effective control of aflatoxin contamination in food crops. Using aflatoxin-gene-specific primers, Chang et al. (2005) grouped the 38 atoxigenic *A. flavus* isolates from the southern USA into eight deletion patterns, which appear to be diverse but not rare. They found genetic drift as the probably driving force for the loss of the entire aflatoxin gene cluster in atoxigenic isolates. A detailed analysis of two atoxigenic isolates commercially used as biopesticide further reveals that AF36 (used as biocontrol agent in cotton in the USA) has a defective polyketide synthase gene required for aflatoxin biosynthesis (Ehrlich and Cotty, 2004), while the NRRL 21882 (used as biocontrol agent in peanut and maize in the USA) lacks the entire aflatoxin biosynthesis gene cluster (Chang et al., 2005). Chang and Hua (2007) also found a defective polyketide synthase gene in TX9-8, which competitively prevented aflatoxin accumulation by *A. flavus* isolates producing varying levels (low to high) of aflatoxin. More recently, Chang et al. (2012) found that a lack of production of aflatoxin in *A. flavus* isolate K49 (NRRL30797) relates to single nucleotide mutations in the polyketide synthase and hybrid polyketide-nonribosomal peptide synthase genes.

Molecular analysis of the 35 atoxigenic *A. flavus* isolates collected from peanut fields in China reveals that 24 of these isolates containing no detectable aflatoxin had the entire aflatoxin gene cluster, while 11 had five

different deletion patterns in aflatoxin gene cluster (Yin et al., 2009). Likewise, Criseo et al. (2008) investigated deletions in aflatoxin gene cluster among 134 natural atoxigenic *A. flavus* isolates, and found deletion as a cause of loss of aflatoxin-producing ability in 84 isolates. Jiang et al. (2009) detected 89.59 kb deletion in the aflatoxin gene cluster in atoxigenic strain A051, which is replaced by a 3.83 kb insert located at 300 bp upstream *ver 1* gene and 2594 bp downstream a putative gluconolactone oxidase gene. Donner et al. (2010) detected deletion of varying size among six of the 18 atoxigenic *A. flavus* isolates from Nigeria, while the remaining 12 isolates had all the aflatoxin-pathway genes, but with defects. These findings suggest that deletions within the aflatoxin gene cluster in some atoxigenic *A. flavus* isolates are very common, but diverse; and the analysis of deletion pattern within aflatoxin gene cluster would be an effective method for the rapid identification of atoxigenic isolates for developing biocontrol agents. The exact mechanisms for the loss of aflatoxin production in some atoxigenic *A. flavus* isolates with no deletion in their aflatoxin gene cluster indicate that there must be other mechanisms responsible for the loss of aflatoxin production in these isolates, which is the subject for further investigation.

Recently, Degola et al. (2011) developed a simple and an inexpensive procedure to scale up the screening process employing a 96-well microplate (or with 380-well microplates based on preliminary work) for evaluating high numbers of pairings of afla$^-$/afla$^+$ strains. A visual observation of fluorescence in the wells allowed them to detect a positive signal down to 250 ppb; and the processing of the digital image of the microplate has been effective in detecting aflatoxin concentrations as low as 50–100 ppb, which correlates well with those obtained using a microplate fluorescence reader or using an ELISA immunoassay. This assay requires small volumes (200 μL well^{-1}) of inexpensive medium and 4 days of incubation at 28 °C is sufficient to detect aflatoxin production even with "slow" mycotoxin producer inoculated with 10^4 conidia (Degola et al., 2009).

Competitive exclusion of aflatoxin producers by atoxigenic strains of *A. flavus* is a viable option for aflatoxin management. Biological control is based on this idea of competitive exclusion whereby a large population of atoxigenic strains of *A. flavus* is established in the soil that outcompetes toxigenic strain, which results in reduced concentration of aflatoxin. Essentially, the introduced atoxigenic strains have the ability to compete and overtake native populations because of their competitive ability to occupy ecological niches and deprive the native populations for space and essential nutrients (Abbas et al., 2011a,b and references therein).

Huang et al. (2011) investigated how the competition suppresses the fungus' ability to infect or produce aflatoxin when challenged. They found that an unknown signaling pathway is initiated in the toxigenic strain by physical interaction with an appropriate atoxigenic strain in the first 24 h, which prevents or downregulates normal expression of aflatoxin. These authors termed thigmo-downregulation of aflatoxin synthesis as the mechanistic basis of intraspecific aflatoxin inhibition and the major contributor to biological control of aflatoxin contamination. Furthermore, the timing of host contact (i.e. atoxigenic strain making host contact prior to aflatoxin producers) is as important for the competition during disease cycles as is the innate competitive ability (Mehl and Cotty, 2011).

The two major atoxigenic strains of *A. flavus* used as biopesticide in biological control of aflatoxin in the USA are AF36 (NRRL 18543) in cotton (U. S. Environmental Protection Agency, 2003) and NRRL 21882 (Afla-Guard®), which was initially used in peanut and later on in maize as well (U. S. Environmental Protection Agency, 2004; Dorner, 2009a,b; Abbas et al., 2011a,b). Few more atoxigenic *A. flavus* strains reported in the literature are K49 (NRRL 30797) (Abbas et al., 2006), TX9-8 (Chang and Hua, 2007), AF 051 (Jiang et al., 2009), TOφ (Degola et al., 2011), and isolate 51 (Huang et al., 2011). A recent in vitro study using toxigenic and atoxigenic strains, in pairs, led researchers to identify additional atoxigenic *A. flavus* strains such as NRRL 50427, NRRL 50428, NRRL 50429, NRRL 50430, and NRRL 50431, which are more effective than NRRL 21882 for the biocontrol of aflatoxin in peanut (Horn and Dorner, 2011). However, large-scale field trials have been suggested to evaluate the effectiveness of these strains under field conditions as the in vitro aflatoxin inhibition does not always reflect the true effectiveness of the strain in controlling aflatoxin contamination in developing crops (Cotty and Bhatnagar, 1994). Moreover, the inoculum rate, the carrier of inoculum used, the method (delivery) and timing of inoculum application significantly impact the success of the biological control of aflatoxin in the field (Dorner, 2009a,b; Lyn et al., 2009; Abbas et al., 2011a). For example, a direct spray application of atoxigenic *A. flavus* strain is better than soil inoculation in controlling maize aflatoxin contamination, and that a water-dispersible granule is a viable delivery system for maintaining viability and efficacy of the biological control agent K49 (Lyn et al., 2009). In the case of peanut, it is recommended that the inoculum be applied at 22.5 kg ha^{-1} in the field at between 60 and 80 days after planting when the foliage canopy is well developed and at a time when enough soil moisture is available (Dorner, 2009a).

AF36 has so far been applied in over 50,000 ha to control aflatoxin contamination in cotton in the US southwestern (Das et al., 2008). Afla-Guard® is now commercially used to prevent aflatoxin contamination in peanut in the USA. The Afla-Guard® has so far been applied approximately in 2000 ha in the states of Alabama and Georgia to test its efficacy in controlling the aflatoxin in peanut, which resulted in mean reduction in aflatoxin concentration by 85% in the treated peanuts (Dorner, 2009a). Similar package has also been developed, and is in use to control aflatoxin contamination in peanut in Australia (Pit and Hocking, 2006).

Of late, there has been systematic effort to identify atoxigenic strain among the native *A. flavus* populations to control aflatoxin contamination in maize in West Africa. Atehnkeng et al. (2008) investigated 11 naturally occurring atoxigenic isolates of *A. flavus* to reduce aflatoxin contamination in maize in Nigeria. They detected two strains from Lafia, La 3279 and La 3303, as the most effective in reducing aflatoxin concentrations in both laboratory and field trials. In a 2 year field study, La 3279 was the most effective isolate that reduces aflatoxin contamination by 99%, while La 3303 could provide up to 92% reduction in aflatoxin contamination in maize. These two endemic strains from the region are well adapted to West African environments and thus, will not have any regulatory issue over their use as biopesticide for the biocontrol of aflatoxin contamination in maize throughout the region. Furthermore, Donner et al. (2009) found significant differences in *A. flavus* distribution in maize fields, sampled across three agroecological zones in Nigeria. They detected significantly greater proportions of atoxigenic *A. flavus* strains in the Northern Guinea Savanna than in the Southern Guinea Savanna or from Derived Savanna zones of Nigeria. This finding suggests that it is possible through systematic surveys to identify regions within a country that are predominated by atoxigenic *A. flavus* strains as safe regions to minimize aflatoxin contamination in food crops. Atehnkeng et al. (2010) evaluated the efficacy of a mixture of four atoxigenic strains in the field for reducing aflatoxin contamination through displacement of aflatoxin producers in maize, using sterile sorghum grains as carriers of atoxigenic strains, in Nigeria. In 2 years of evaluation, they found that 67–95% reduction in aflatoxin, which was associated with 74–80% displacement of aflatoxin producers. These results demonstrate that effective atoxigenic strains native to West Africa can be selected from fungal communities associated with maize production, and successfully utilized to minimize the risk of aflatoxin exposure in human populations. Another study in the central Republic of Benin also revealed the natural occurrence of

atoxigenic strain BN030D. BN030D release greatly increases its population, leading to its spread in both time and space, thus, it is a promising candidate for the development of competitive displacement strategies for the control of aflatoxin in maize in the Republic of Benin (Klueken et al., 2009). More recently, Probst et al. (2011) evaluated the aflatoxin-producing potential of the 290 *A. flavus* strains from Kenya; and they identified 12 most effective strains that could reduce aflatoxin levels above 80% in maize in Kenya. The reduction in aflatoxin levels by these selected atoxigenic strains are comparable to those reported with a strain from USA (NRRL-21882), which is used commercially for aflatoxin management in maize and peanut. Hence, these atoxigenic strains have potential to biologically control within highly toxigenic *A. flavus* communities associated with maize production in Kenya. Probst et al. (2011) further noted that these strains belong to different VCGs, which may provide opportunity to utilize VCG mixtures that may compete effectively in a greater diversity of environmental niches than individual strains, similar to those reported for West Africa (Atehnkeng et al. 2010).

Abbas et al. (2011b) assessed the competitiveness of three atoxigenic strains (K 49, NRRL 21882 and AF 36) to displace toxigenic strains (K 54 and F3W4) in maize. Their study revealed that K 49 and NRRL 21882 are superior to AF 36 in reducing total aflatoxin contamination. Neither K 49 nor NRRL 21882 produce cyclopiazonic acid (CPA), another form of mycotoxin, however, when challenged with toxigenic strains, both had similar effect on reducing the CPA and aflatoxins (84–97% with K 49 and 83–98% with NRRL 21882). In contrast, AF 36 reduces aflatoxins by 20% using F3W4 and 93% with K 54, but there was no reduction in CPA by F3W4 and only a 62% reduction in CPA with K 54. These results indicate that K 49 is as effective as NRRL 21882 is in reducing both aflatoxins and CPA in maize.

For biological control to remain effective, it is essential that atoxigenic isolates should belong to VCG that do not have toxigenic members to ensure that atoxigenic and toxigenic strain within a VCG do not exchange genetic material and generate progenies that produce aflatoxin (Ehrlich et al., 2007), which makes the use of these strains for biological control agent ineffective in minimizing aflatoxin contamination in crops. The recent discovery of sexual cycle in atoxigenic fungi may alter the population dynamics of the fungus, given the high heritability of aflatoxin and the relative ease of gaining or losing toxicity via cross-overs and independent assortment, which has significant implications for managing aflatoxin contamination of crops, and for effective biocontrol using atoxigenic *A. flavus* strains (Olarte et al., 2011). However,

it should be noted that biological control alone may not be sufficient to completely eliminate the risk of aflatoxin contamination. It must be integrated with other pre- and postharvest crop management strategies including deployment of resistant cultivars (Menkir et al., 2006; Ortiz et al., 2007; Abbas et al., 2009; Hell et al., 2010; Hell and Mutegi, 2011). Furthermore, it is suggested that a network approach involving all stakeholders both from public and private sectors, advanced research institutes, development investors and state or national governments, and active participation by farmers' organizations must be in force to share responsibility for developing crop management strategies that allow farmers produce aflatoxin-free food and feed crops.

Integrated management of aflatoxin provides farmers with natural, safe, and cost-effective solution to prevent aflatoxin contamination of maize and peanut in Africa. This integrated management of aflatoxin uses the biocontrol product "Aflasafe", which is now available for commercialization in Nigeria. Researchers are assessing various strains for further selection of the most effective ones for the development of a biocontrol product in Kenya (http://r4dreview.org/2011/11/initiative-tackles-killer-aflatoxin/). More such integrated projects are needed to provide effective control of aflatoxin contamination in food crops, especially in the developing countries around the world.

Biocontrol together with the use of resistant cultivars is probably the most cost-effective strategy to reduce the risk of aflatoxin contamination in maize and peanut. However, the cost of biocontrol varies depending on the product and the locale. For example, the cost in the use of AF36 and Afla-Guard^{TM} to control aflatoxin in cotton and peanut in the USA, respectively, ranges from US$ 6 to 16 and US$17–32 acre^{-1}, while the cost of biocontrol using local atoxigenic strains of *A. flavus* in Nigerian maize is about US$10–12 ha^{-1} (Khlangwiset and Wu, 2010 and references therein).

4.5. A System-Based Approach to Control Aflatoxin Contamination

Biosynthesis of mycotoxin is strongly dependent on substrate composition, pH, water activity, temperature or modified environments (Sanchis and Magan, 2004; Ribeiro et al., 2006; Giorni et al., 2008). Combinations of these ecophysiological factors either completely inhibit or fully activate the biosynthesis of aflatoxin. Knowledge of such relationships enables to establish as to which parameter combinations can control aflatoxin biosynthesis, and which are conducive to aflatoxin contamination; and this in the long run is beneficial to develop a system-based approach to predict and control mycotoxin contamination in food and feed crops.

Schmidt–Heydt and Geisen (2007) developed a mycotoxin biosynthesis genes–specific microarray carrying oligonucleotides of the fumonisin, aflatoxin, ochratoxin, trichothecene and patulin biosynthesis pathways, with in-built option to add any newly identified pathway genes. The initial application of this microarray demonstrates that it is specific in detecting the signals of gene expression under conditions conducive for mycotoxin biosynthesis, with insignificant cross-hybridizations. Recently, Lezar and Barros (2010) reported a diagnostic oligonucleotide microarray to identify the most common food-borne fungi, as well as the genes, leading to toxin production. By using this array, they could identify 32 fungi, and determined their potential to produce mycotoxins. These technological innovations offer opportunity to identify food-born fungi and associated most common mycotoxins, and to investigate the influence of environmental parameters on the activation of the mycotoxin biosynthesis genes and thereby on mycotoxin biosynthesis.

The optimum temperature for the production of aflatoxin is between 28 and 30 °C, and the toxin decreases as temperatures approach 37 °C, which is the optimum temperature for fungal growth. O'Brian et al. (2007) studied the effect of temperature on aflatoxin production and the expression of more than 5000 *A. flavus* genes at 28 and 37 °C. A total of 144 genes were differentially expressed between the two temperatures, with all aflatoxin biosynthetic genes highly expressed at 28 °C relative to 37 °C, and the transfer of an aflatoxin-producing culture from 28 °C to 37 °C quickly turns of aflatoxin biosynthesis. Schmidt–Heydt et al. (2009) investigated the effects of varying combinations of water activity, a_w, and temperature effects on the activation of aflatoxin biosynthesis genes in *A. flavus*. They found that certain combinations of a_w and temperature, especially combinations that imposed stress on the fungus, resulted in a significant reduction of the growth rate and at these conditions the induction of the whole aflatoxin biosynthesis gene cluster occurred, however, aflatoxin B_1 produced was low. In all other combinations (25 °C/0.95 and 0.99; 30 °C/0.95 and 0.99; 35 °C/0.95 and 0.99) they detected a reduced basal level of cluster gene expression, but a high fungal growth and high aflatoxin production. At single gene comparison, two groups with different expression profiles in relation to a_w and temperature combinations occurred, which were coordinately localized within the aflatoxin gene cluster. The ratio of *aflR/aflJ* expression correlated with increased aflatoxin biosynthesis. Schmidt–Heydt et al. (2010) further studied the influence of a_w (0.90–0.99) × temperature (17–42 °C) interactions on growth and the biosynthesis of aflatoxin in *A. parasiticus*. Optimum growth

was at 35 °C, but marginal growth occurred at 17 and 42 °C. The optimum conditions for the biosynthesis of aflatoxins B_1 and G_1 in *A. parasiticus* differed, which did not coincide with the growth optimum (35 °C), but was either below (aflatoxin G_1, 20–30 °C) or above the optimum temperature (aflatoxin B_1, 37 °C). More importantly, aflatoxin B_1 synthesis was independent of a_w at certain temperatures as long as a_w was above 0.90, whereas the optimum production of aflatoxin G_1 was more dependent on a_w than on temperature in the range 20–30 °C. Such shift between optimum toxin production and optimum fungal growth has also been reported for ochratoxin production by *Penicillium verrucosum*, trichothecene production by *Fusarium culmorum* (Schmidt-Hedyt et al., 2008), fumonisin production by *Fusarium verticillioides* (Jurado et al., 2008) or aflatoxin B_1 production by *A. flavus* in peanut (Abdel-Hadi et al., 2010). Furthermore, the expression profiles of two regulatory genes at the different combinations of a_w and temperature showed a relationship with the production profiles of two different aflatoxins produced by *A. parasiticus*. The *aflR* gene expressed at higher levels at above 30 °C, whereas the most prominent expression of the *aflS* gene was at below 30 °C, which corresponded with the production profile of either aflatoxin B_1 or G_1. Schmidt-Heydt et al. (2009) in an earlier study reported that the ratio between *aflS* and *aflR* expression seems an indicator for the activation of aflatoxin B_1 biosynthesis in *A. flavus*.

More recently, Abdel-Hadi et al. (2011) investigated the relative expression of 10 key genes involved in aflatoxin biosynthesis pathway with respect to environmental factors (a_w, 0.995–0.90; temperature, 20–42 °C) on production of aflatoxin B_1 by *A. flavus*. The optimum conditions for the growth of *A. flavus* were 30–35 °C and 0.99 a_w, with marginal conditions at 15 and 40 °C at 0.99 a_w. For aflatoxin B_1 production, the optimum conditions were between 25 and 30 °C at 0.99 a_w and this changed to between 30 and 35 °C at 0.95 a_w. They used mixed growth model to relate the relative expression of these genes under different interacting environmental factors to growth and aflatoxin B_1 production, and developed a predictive model that gave a good relationship between the observed and predicted aflatoxin B_1 production. Schmidt-Heydt et al. (2011) also reported that changes in a_w and temperature significantly affect fungal (*Fusarium culmorun* and *F. graminearum*) growth and expression of six transcription genes (*TRI4*, *TRI5*, *TRI6*, *TRI10*, *TRI12* and *TRI13*) from trichothecene gene cluster significantly associated with toxin production, DON, in wheat. These results suggest complex interactions between gene expression, environmental factors and mycotoxin production. This is a powerful tool for understanding the role of genes in relation to

environmental factors for the development of effective targeted control strategies for mycotoxins. However, an effective integration of molecular, ecophysiological and mycotoxin data are critical in predicting the relative risk of mycotoxin contamination under different stress scenarios that have an impact on both food quality and food security (Magan et al., 2011).

To sum up, it is reasonable to believe that food and feed crops, in years to come due to climate change and variability, will be subjected to more frequent precipitation, drought and high temperature or altered pest–disease scenario, which will result into increased threat to food and feed contamination by mycotoxin-producing fungi. Researchers are now using crop simulation models to predict fungal growth and aflatoxin production. New tools such as GIS, geostatistics and satellite (imagery) data are being employed to monitor shift in community structure of aflatoxin-producing fungi and predict geographic regions with low risk to aflatoxin production. Simple and inexpensive assays such as *cELISA* or *Afla-ELISA* are available which are adopted to monitor aflatoxin contamination in crops such as maize and peanut. With the decoding of *A. flavus* genome, it became clear that 25 genes clustered within a 70-kb DNA region in chromosome are involved in the biosynthesis of aflatoxins, of which, the function of 19 genes are known. Several patterns of deletion of aflatoxin genes have been found associated with the loss of aflatoxin-producing ability in some *Aspergillus* isolates, while others had all the aflatoxin biosynthesis pathway genes, but with defects. With the development of mycotoxin biosynthesis genes-specific microarray, the researchers have been able to study the effects of nutrition, water activity (a_w), temperature and modified atmosphere and their interactions to predict and identify aflatoxin biosynthesis genes associated with fungal growth and mycotoxin production, which is guiding researchers to adapt a system-based approach to control mycotoxin contamination. Several management options, both pre- and postharvest including the use of resistant cultivars and biocontrol agents, are available to reduce the risk of aflatoxin contamination.

5. AGROBIODIVERSITY TO ENHANCE NUTRITIONAL QUALITY OF FOOD CROPS

5.1. Global Warming Changes Plant and Soil Biodiversity

5.1.1. Plant Biodiversity

Today, 150 plant species (out of 250,000 known plant species) dominate the world's agricultural landscapes, and only 12 crop species provide 80% of the world's food (Motley et al., 2006). Agricultural biodiversity is crucial

to coping with climate change as the entire diversity of genes, species and ecosystems in agriculture represents the resource base for food (Kotschi, 2007; Ortiz, 2011). Gene banks around the world, including those of the CGIAR Consortium, maintain about 7.4 million accessions of plant genetic resources, with cereals and legumes constituting 52% of the accessions (SWPGRFA, 2009). The CGIAR Consortium holds about ¾ million accessions of 3446 species from 612 genera. In spite of these large collections maintained ex situ, there are still important collection gaps that must be addressed before these priceless genetic resources are lost as a result of climate change or other driving forces leading to the genetic erosion and loss of biodiversity (SWPGRFA, 2009). These ex situ collections are to a large extent safe from the adverse impact of climate change. Unlike cultivated germplasm, there are difficulties associated with ex situ conservation of CWR due to their specific crop husbandry, tendency for natural pod dehiscence, seed shattering and seed dormancy, high variability in flowering and seed production, and rhizomatous nature of some of the species. There is a growing interest that CWR should be preserved in situ in protected areas to ensure evolutionary processes of wild species contributing new variants, which as and when captured by plant explorers, should be able to contribute to addressing new challenges to agricultural production (Ortiz, 2011). Worldwide, there are 76,000 protected areas, spread in ~17 million km^2, and various countries have taken initiatives for establishing CWR in situ conservation (Meilleur and Hodgkin, 2004; Maxted et al., 2008; Maxted and Kell, 2009; SWPGRFA, 2009).

The Intergovernmental Panel on Climate Change (IPCC) predicts that by 2100 the temperature will rise in the range of 1.1–6.4 °C due to global warming, which will have serious consequences for global agriculture and food production (IPCC, 2007; Lobell et al., 2008). There may be a great loss of biodiversity due to global warming, because organisms are no longer adapted to this changed environment (McLaughlin et al., 2002; Thomas et al., 2004; Biggs et al., 2008). For example, the sub-Saharan Africa and the Caribbean regions are projected to suffer a decline in land area suitable for cultivation of the crops currently grown; while there will be increased opportunities to expand the crop acreage in Europe and North America (Fischer et al., 2002; Lane and Jarvis, 2007). However, the projected gains in area will occur in the regions where these crops are currently not an integral component of food security. This situation may lead to a significant threat to agrobiodiversity, increasing genetic erosion of landraces and threatening wild species, including CWR (Jarvis et al., 2008b).

Kelly and Goulden (2008) predict that changes in climate could rapidly shift species distribution—some species may expand to newly favorable areas while others may decline in increasingly adverse locations. Using the current and projected future climate data for ~2055 and a climate envelope species distribution model, Jarvis et al. (2008a) predicted the impact of climate change on the wild relatives of cowpea (*Vigna* spp.), peanut (*Arachis* spp.) and potato (*Solanum* spp.), which revealed that climate change affect them. An estimated 16–22% (depending on migration scenario) of these CWR are predicted to go extinct and most species losing over 50% of their geographic range, and becoming highly fragmented. The CWR of peanut will be the most affected, while those of cowpea may be the least affected. Similarly, today the Western Ghats in southwestern India are very rich in *Vigna* and *Cajanus* species. However, with changes in temperature and photoperiod coupled with other factors such as habitat destruction, their CWR populations are becoming alarmingly less, which calls for a strategy for collecting and conserving CWR in this region of India (Upadhyaya et al., 2011e).

Global warming is also impacting changes in plant phenology. For example, some species show advancement in timing of phenological events such as breeding or flowering, of 2.3 days per decade (Parmesan and Yohe, 2003), while others exhibit an evolution of multiple life-history traits (Franks and Weis, 2008), new variants for phenological traits (Vigouroux et al., 2011), or shrinking body sizes (Sheridan and Bickford, 2011). All these events could negatively impact both crop plants and protein sources, which are important to human nutrition (Sheridan and Bickford, 2011).

Franks and Weis (2008) investigated the effects of 5 years of prolonged drought on life-history traits in an annual plant *Brassica rapa*. By comparing the ancestors with their descendants, they found that drought caused many changes in life-history traits, including a shift to earlier flowering, reduced peak flowering, and greater skew of the flowering schedule. Descendants had thinner stems and fewer leaf nodes at the time of flowering than ancestors, indicating that the drought selected for plants that flowered at a smaller size and earlier ontogenetic stage rather than selecting for plants to develop more rapidly. The Sahel will be the most adversely affected in sub-Saharan Africa due to extreme variation in climate change as a result of frequent droughts of varying intensity and duration. Pearl millet in Niger is the main staple crop, and farmers grow this crop in 65% of the cultivated area for producing more than 80% of the entire caloric intake in the country. Vigouroux et al. (2011) analyzed the impact of drought on variation in phenological and morphological differences in pearl millet landraces collected

from the same villages in Niger in 1976 and 2003. Their study revealed no major changes in the main cultivars or in their genetic diversity. However, these authors observed a significant shift in the adaptive traits. Samples collected in 2003 displayed a shorter life cycle, increased frequency of early flowering allele at the *PHYC* locus, and a reduction in plant and spike size in comparison to the sample collected in 1973. This clearly indicates that recurring drought can lead to selection of earlier flowering in major crops in the Sahelian region.

The increased CO_2 and N supply often drive plant stoichiometry in opposite directions (Ainsworth and Long, 2005; Novotny et al., 2007), but the productivity in the same direction (Ainsworth and Long, 2005; Luo et al., 2006). Several studies have shown that increases in N availability decrease plant diversity (Stevens et al., 2004; Suding et al., 2005; Harpole and Tilman, 2007; Clark and Tilman, 2008; Hautier et al., 2009). Likewise, elevated CO_2 (hereafter eCO_2) reduces plant diversity (Oren et al., 2001; Ainsworth and Long, 2005; Reich et al., 2006a,b). However, no consensus has emerged so far as to how rising CO_2 concentrations interact with N to influence plant biodiversity (Zavaleta et al., 2003; Niklaus and Kröner, 2004). In a long-term (10 years) open-air experiment involving 16 grassland species grown under all combinations of ambient CO_2 (aCO_2) and eCO_2 and ambient N (aN) and elevated N (eN), Reich (2009) found that eN reduces species richness by 16% at aCO_2 but by just 8% at eCO_2. This resulted from multiple effects of CO_2 and N on plant traits and the soil that altered competitive interactions among species. Hence, eCO_2 ameliorated the negative effects of N enrichment on species richness.

5.1.2. Soil Biodiversity

Soil biodiversity exceeds the aboveground systems biodiversity, and is crucial for the sustainability of agroecosystems (Wardle, 2002). It consists of macrofauna or soil engineers (earthworms and termites), mesofauna (microarthropods such as mites and springtails), microfauna (nematodes and protozoans), and microflora (bacteria and fungi). The soil organisms perform a number of vital functions such as decomposition and degradation of plant litter and cycling of nutrients; converting atmospheric nitrogen into organic forms (immobilization) and remineralization of mineral nitrogen, leading to the formation of gaseous nitrogen; suppression of soil pathogens through antagonism; regulating microclimate and local hydrological processes; synthesizing enzymes, vitamins, hormones, vital chelators and allelochemicals that regulate population and processes; and altering soil structure and other soil

physical, chemical and biological characteristics (Paoletti et al., 1994; Altieri, 1999). In addition to the effects on soil desertification, land use pattern and soil pollution—including those resulting from N enrichment—alter soil biodiversity (Wall et al., 2010; Sylvain and Wall, 2011; Prichard, 2011). The changes in soil biodiversity are observed through effects on soil organisms as a result of the changes in temperature and precipitation and through climate-driven changes (rising atmospheric CO_2 and warming) in plant productivity and species composition, as discussed in the following.

Accumulated evidence so far reveals that soil biota is vulnerable to global changes and soil disturbance. A metaanalysis—involving data from over 75 experiments—revealed that soil biota response to global change are predictable and unique for each global change factor. For example, positive-effect size of eCO_2 on abundance of soil biota diminished with time, whereas the negative-effect size of warming and positive-effect size of precipitation intensified with time. Likewise, the abundance of microflora and microfauna increased with eCO_2. The effects of CO_2 varied between field and greenhouse studies, less in the former while more pronounced in the latter (Blankinship et al., 2011 and references therein). In a 5-year open-top chamber experiment involving single and combined effects of aCO_2 and eCO_2, ambient and elevated temperature and changes in precipitation, Kardol et al. (2011) concluded that changes in soil moisture content, either as a direct effect of the changes in precipitation or as an indirect effect of warming or eCO_2, had a larger impact on microarthropods communities than did the direct effects of warming and eCO_2. In a multifactor climate change experiment, Castro et al. (2009) reported increased fungal abundance in warmed treatments, increased bacterial abundance in warmed plots with eCO_2 but decreased in warmed plots under aCO_2, changes in precipitation altered the relative abundance of proteobacteria and acidobacteria where acidobacteria decreased with a concomitant increase in the proteobacteria in wet relative to dry treatments, altered fungal community composition due to the changes in precipitation, and differences in relative abundance of bacterial and fungal clones varied among treatments. All these observations led the authors to conclude that climate change drivers and their interactions among them may cause changes in the bacterial and fungal abundance, with precipitation having greater effect on the community composition.

Dominique et al. (2006) exposed different sets (5, 12, or 31) of plant species (grasses) to aCO_2 or eCO_2 for 5 years to analyze how plant diversity affects below ground diversity. The results revealed that increased levels of CO_2 had no significant influence on both soil bacterial community

composition and bacterial richness. In contrast, the variability in plant diversity level had significant effects on bacterial composition but no influence on bacterial richness. This research therefore suggests that the soil microbial composition is mainly related to plant diversity, assuming that different plant species might harbor specific rhizospheric microbial populations, rather than altered soil carbon fluxes induced by eCO_2 that lead to increased photosynthesis. Bardgett (2011) points out that there is sufficient evidence to show that the transfer of carbon through plant roots to the soil plays a primary role in regulating ecosystem responses to climate change and its mitigation. Research to answer this question, however, continues, e.g. what is the mechanism involved in transfer of plant carbon to soil, its consequences for ecosystem carbon cycling, and the potential to exploit plant–root traits and soil microbial processes that favor soil carbon sequestration are issues that need further research?

Very little is known about the influence of eCO_2 on the structure and functioning of below ground microbial community. In a 10-year field exposure of a grassland ecosystem to eCO2, Zhili et al. (2010) detected dramatic alteration in the structure and functional properties of soil microbial communities. They found that the total microbial and bacterial biomass significantly increased under eCO_2, while the fungal biomass remained unaffected. Furthermore, the structure of microbial communities was markedly different between aCO_2 and eCO_2. More recently, using tag-encoded pyrosequencing of 16S rRNA genes, Deng et al. (2012) also found that the soil microbial community composition and structure were significantly altered under eCO_2. In both the studies, the changes in microbial structure was significantly correlated to soil moisture, soil status relative to C and N contents, and plant productivity.

5.2. High-Throughput Assays for Monitoring Nutritional Traits

There is greater emphasis in plant-breeding programs worldwide to select and or develop cultivars that are high yielding as well as their grains or stover are also more nutritious to meet both total calorie and nutritional demands of the growing populations, and for improving livestock productivity. However, one essential requirement for progress in this area of research is the timely and economic determination of quality traits of a large number of samples, as often required in breeding programs, which is laborious, time consuming, and expensive. Obviously, there is a need to develop analytical assays that are nondestructive, high throughput, fast, accurate, cost-effective, requiring relatively small samples, and allowing a simultaneous profiling of multinutrient elements. Assays

based on the use of wet chemistry are the most accepted to measure the levels of seed components. However, they require a relatively large sample size, and are destructive, time consuming, and relatively slow when a large numbers of samples need to be screened in a short period to allow breeders make a decision about the breeding lines for generation advance in the following crop season.

5.2.1. Minerals from the Soil Samples

Numerous methods have been developed for extracting fractions of plant nutrients, which are available to plants in the field. A universal problem with all these methods is relative to the selection of a ubiquitous extractant due to the fact that these extractants suffer from shortcomings to accurately mimic the field conditions during the growing season, which generally leads to poor prediction, accuracy and lack of robustness (Sinclair and Edwards, 2008 and references therein). Salts of Ethylenediamine tetra acitic acid (EDTA) and Diethylenetriamine pentaacetic acid (DTPA) are the most commonly used for predicting the availability of micronutrients to the plants in the field (Sahrawat et al., 2002, 2011; Sinclair and Edwards, 2008).

The technique diffusive gradient in thin–films (DGT) measures the diffusive supply of elements, thereby mimicking plant roots' role in accessing and uptake of nutrient elements. This technique differs from the other extraction techniques by responding to kinetics of release from soil rather than pseudoequilibrium between the extractant and soil nutrients. It was earlier used to access bioavailable trace elements in waters, heavy metals in contaminated soils and P in waters and soils (Zhang et al., 1998, 2001; Menzies et al., 2005; McBeath et al., 2007). Tandy et al. (2011) tested the ability of DGT technique to assess plant-available P, Zn and Cu in a wide range of Scandinavian soils and compared the results with those obtained using conventional laboratory methods (EDTA) and DTPA for Cu and Zn; $NaHCO_3$ for P and soil solution concentrations). The results of the study showed that the soil test values obtained by the DGT method better predicted the concentrations of various nutrients in the youngest fully developed leaf of pot-grown barley plants. They also reported that the DGT method could predict accurately plant uptake of Zn and P, while the conventional soil test methods based on the extraction of nutrients using chemical extractants performed poorly. The results with the DGT technique were also more accurate in predicting Cu concentration in the leaves. Thus, Tandy et al. (2011) concluded that the DGT-based method is more accurate at predicting plant-available P, Zn and Cu than commonly used methods for analyzing plant-available nutrients in soils. However, while analyzing a large

number of samples on a routine basis, simplicity, rapidity and cost involved need to be considered in addition to of course the precision in the analyses of samples (Sahrawat et al., 2011; Sahrawat and Wani, 2013).

5.2.2. Minerals from Plant Tissues or Grains Samples

Inductively coupled plasma–mass spectroscopy (ICP-MS) is the state-of-the-art technique used for plant analysis for multinutrients with a high sensitivity. However, fast and thorough sample digestion of plant materials is a time-consuming task, and is a major bottleneck in modern multielemental analysis. Additional limitation may be the sample size or too low concentration of elements in the sample. Hansen et al. (2009) developed a high-throughput microscaled method that enables digestion of small quantity of plant sample for elemental profiling and distribution of trace elements between and within plant organs by ICP-MS. In comparison to existing vial–in–vial systems, it represents a significant methodological advancement in terms of higher capacity, reduced labor and material costs, less contamination and improved accuracy following the introduction of microscaled digestion of plant samples. Furthermore, the results from this method have also shown to be in good correspondence with the single grain concentrations and those obtained by analysis of bulked samples of milled rice grain (Hansen et al., 2009). Thus, the use of single grains for analysis of elements in cereals provides a valuable tool for high-throughput screening of a large number of lines in plant-breeding programs. More recently, Husted et al. (2011) have developed a novel hyphenated technique, which is based on liquid chromatography and ICP-MS (LC-ICP-MS) and has been extensively complemented by molecular mass spectrometry for structural information of biologically relevant plant species. Moreover, Wheal et al. (2011) reported another novel closed-tube nitric acid/hydrogen peroxide digestion method for inductively coupled plasma optical emission spectrometry (ICP-OES) for analysis of plant tissue, which they tested on six botanical reference materials. This method they found superior over to open–tube digestion of reference materials, as it showed less values of blanks as a result of probably less contamination than that in the case of open–tube digestion method. The closed–tube method is as efficient as the open–tube digestion of reference materials recorded 94–113% recovery of published concentrations for most essential elements in comparison to 93–115% recovery by the open–tube digestion method, with recovery standard deviation comparable in the two methods (2–6% for the closed-tube and 1–8% for the open-tube method). Because of its fixed digest time (2.5 h), it is short enough to conduct three digest runs per

working day per digestion block, while in the open–tube digestion method, it takes relatively longer time for the digestion of plant materials, especially with dense materials, reducing the daily throughput considerably.

Near infrared (NIR) reflectance—a promising technique discovered by Friedrich Herschel in 1800 (Davies, 2000)—has found wide application in estimating various chemical components in seed, plants and food in many crops. It covers the range of the electromagnetic spectrum from 780 to 2500 nm. Essentially, the product (including the seed) is exposed to NIR irradiation, and the reflected or transmitted radiation is measured. While the radiation penetrates the product, its spectral characteristics change through wavelength-dependent scattering and absorption. This change depends on the chemical composition of the product, as well as on its light scattering properties, which are related to the plant microstructure. Multivariate statistical techniques such as partial least squares regression are then applied to extract the required information from the usually convoluted spectra (Lee et al., 2011). The NIR technology has been applied to predict oil per se and its quality in rapeseed, safflower, and soybean (Tajuddin et al., 2002; Kim et al., 2007; Patil et al., 2010; Rudolphi et al., 2012; Wittkop et al., 2012); fiber fractions in rapeseed (Wittkop et al., 2012); starch, protein, oil, amino acid composition and weight of individual grains in maize (Spielbauer et al., 2009; Tallada et al., 2009; Rosales et al., 2011); stover quality in maize (Melchinger et al., 1986); amino acid composition in soybean (Kovalenko et al., 2006); protein, starch and seed weight in intact seed in common bean (Hacisalihoglu et al., 2010); and for sensing moisture content of in–shell peanut (Sundaram et al., 2012).

Baianu et al. (2012) developed a high-resolution nuclear magnetic resonance and NIR models that they validated by estimating amino acid profiles of proteins from a large number of single and bulked soybean seeds, without protein extraction from the seed. Using partial least squares regression technique to analyze the NIR spectral data, they found that single soybean seed NIR spectra are broadly similar to those of bulk whole soybeans, with the exception of minor peaks, 950–1000 nm, in single soybean NIR spectra. The highly resolved NIR chemical images that they obtained for selected regions of mature soybean embryos allowed for the quantitation of oil and protein components, extending the NIR sensitivity range to the *picogram* level, with submicron spatial resolution in the component distribution throughout intact soybean seeds and embryos. These technological innovations are potentially important for the application of biotechnology that requires rapid and ultrasensitive analyses, such as those concerned with high-content microarrays in genomics and proteomics research.

Several spectroscopy methods are now available for studying the accumulation and distribution of essential nutrients in the seed. Proton-induced X-ray emission targets the embryo region. Scanning and transmission electron microscopy (STEM) in combination with energy-dispersive X-ray (EDX) microanalysis, or STEM-EDX, focuses on aleurone and scutellum cells for providing subcellular information. Nano-secondary ion mass spectrometry visualizes the subcellular distribution but limited to regions of only a few square micrometers. X-ray fluorescence (μ-XRF) provides elemental maps for various elements in whole grain sections. Maia captures intricate detail in natural material. These assays differ in terms of resolution and sensitivity, depth of analysis, and in their capacity to provide mass resolution or molecular information (Dwivedi et al., 2012 and references therein). Paltridge et al. (2012) investigated energy-dispersive X-ray fluorescence spectrometry (EDXRF) for measurement of Zn and Fe in whole grains of rice and pearl millet. The results obtained by EDXRF were variable, but highly correlated ($r^2 = 0.79$–0.98) with those determined using ICP-OES values for both Zn and Fe in both the species, with predicted values for Zn and Fe in rice to within 1.9 and 1.6 mg kg^{-1} of ICP-OES values, and for Zn and Fe in pearl millet to within 7.6 and 12.5 mg kg^{-1} of ICP-OES values, at 95% confidence levels. Hence, this assay offers a convenient, economical tool for screening a large number of samples for Zn and Fe in rice and pearl millet, which can also be used on grains of other species, or for other mineral elements, with some modification including crop-specific calibrations.

Lorenz et al. (2007) developed a rapid and an inexpensive method for measuring phytate and inorganic phosphorus (Pi) concentrations in maize, which provides adequate precision and simplicity to deal with a large number of breeder's samples for estimating phytate and Pi levels simultaneously. Estimates obtained from this technique match closely with those obtained from ion exchange methods, and the repeatability of the values across fields suggests that the protocol can be used to make heritable measurements for both phytate and Pi.

Hulshof et al. (2007) developed a fast screening method for estimating β-carotene in maize seeds. This method is based on the principle of semi-quantitative analysis that allows distinction between lines—without the need of a full HPLC analysis (Rodriguez-Amaya and Kimura, 2004)—the low, medium and high levels of provitamin A carotenoids, thereby reducing analysis costs. It is an appropriate assay to initially discard low β-carotene lines; and to carry out the HPLC analysis for β-carotene with select group of lines.

5.3. Profiling Genetic Variation for Nutritional Traits

Genes for desirable traits are embedded in biodiversity. The genetic variation in crops genepool including wild relatives, when systematically characterized, evaluated and documented, and dissected through applied genomic tools provides crop genetic enhancers for the agronomically important gene(s) and their allelic forms to develop crops cultivars that are more productive and nutritious. For example, a key regulating gene that regulate oil content and oil composition in maize, a gene responsible for increasing the flux of β-carotene in maize, or a wild-species allele (*Brix9-2-5*) for increasing the sugar yield of tomato (Lippman et al. 2007; Zheng et al., 2008; Harjes et al., 2008). These few examples demonstrate the usefulness of agrobiodiversity (both cultivated germplasm including landraces and wild and weedy relatives) to discover new sources of variation and to mine alleles associated with such variation for improving the quality of food crops (Zamir, 2008). In recent years, there has been increased emphasis to enhance the nutritional value of the food crops that constitute the bulk of the calories, particularly in the developing world (Dwivedi et al., 2012 and references cited therein). The CGIAR Consortium gene bank holdings include about 0.422 million accessions of cereals and legumes genetic resources (www.singer.cgiar.org, assessed on 17th April 2012), and in all these crops, the reduced subsets in the form of core (Frankel, 1984) or mini core (Upadhyaya and Ortiz, 2001) collections, representing diversity in the entire collection of a given species, are available (Dwivedi et al., 2005, 2007a and references therein) for use in crops improvement programs. Research to-date suggests that these subsets are ideal resource to find new sources of variation and mine alleles associated with agronomically beneficial traits (Upadhyaya et al., 2009). The CGIAR Consortium gene bank holdings have been extensively characterized for morphoagronomic traits including resistance to abiotic and biotic stresses, whereas very limited information on these genetic resources is available and documented about their chemical attributes (SWPGRFA, 2009). Hence, the need to screen these reduced subsets for seed chemistry to identify germplasm with improved seed composition, for use in breeding programs.

5.3.1. Variation for Fe, Zn, Phytate and Carotenoids

The CGIAR Challenge Program HarvestPlus (www.harvestplus.org) provided opportunity to researchers to screen these genetic resources for grain iron (Fe) and zinc (Zn), the two most important micronutrients, and β-carotene, the precursor of vitamin A (Pfeiffer and McClafferty,

2007; Bouis and Welch, 2010). The evidence accumulated so far suggests substantial genetic variation for Fe and Zn in the grains of both cereals and legumes, e.g. high Fe in common bean from Chile, Colombia, Peru, Rwanda and Tanzania; high Fe and Zn in maize from southern Africa; high Fe and Zn in pearl millet from West Africa; high Fe in sorghum from Benin; traditional rice cultivars from India having more Fe and Zn; and high Fe and Zn in finger millet and foxtail millet from China and India (Upadhyaya et al., 2011a,b; Dwivedi et al., 2012 and references therein). Few studies involving core or mini core collections have led to identifying accessions with high grain Fe and Zn in common bean, finger millet, foxtail millet and sorghum (Islam et al., 2002; Kumar et al., 2009; Upadhyaya et al., 2011a, b). The CGIAR Consortium continues evaluating chickpea, peanut, pigeon-pea, pearl millet, and sorghum mini core collections for variation in grain Fe and Zn concentrations; and the preliminary evidence from these ongoing evaluations suggests substantial variation for these traits (H. D. Upadhyaya, unpub.), which clearly suggest the usefulness of the core and mini core sub-sets for identifying variation for grain-quality traits as well.

CWR have been the source of resistance to many pests and diseases both in cereals and legumes (Dwivedi et al., 2008). Likewise, researchers detected abundant genetic variation in the grain for Fe and Zn among wild and weedy relatives in common bean and wheat (Guzmán-Maldonado et al., 2000; Chhnneja et al., 2006; Acosta-Gallegos et al., 2007; Xie and Nevo, 2008). Some introgression progenies showed exceptionally high Fe or Zn in the grain than cultivars of common bean and wheat (Acosta-Gallegos et al., 2007; Neelam et al., 2010; Tiwari et al., 2010).

Phytic acid is the major form of phosphorus storage in the grains. However, its high concentration limits micronutrients bioavailability as it binds with these minerals (Fe and Zn) to form mixed salts (phytin), largely excreted by humans and nonruminant animals, with a potential to significantly impact water pollution (Lott et al., 2000). However, phytic acid is vital for seed development, seedling growth and development and may have a positive role as antioxidant and anticancer agent (Oatway et al., 2001). Research to-date suggests availability of low–phytic acid germplasm or mutants in barley, common bean, lentil, maize, rice, sorghum, soybean and wheat (Dwivedi et al., 2012 and references therein) for use in crops breeding. Bioavailability of micronutrients, particularly Fe and Zn, merits research about the usefulness of mineral–dense cultivars in human nutrition. Limited studies in common bean, maize and wheat have shown large differences in Fe and Zn bioavailability, with a few mineral-dense germplasm

having increased amount of bioavailable Fe/Zn (Dwivedi et al., 2012 and references therein).

Some maize germplasm could be used as source of pro-vitamin A, β-carotene, α-carotene, and β-cryptoxanthin and the non-provitamin A including lutein and zexanthin. The metabolite profiling of germplasm collection identified a subset of 10 genetically diverse germplasm representing biochemical extremes for maize kernel carotenoids (Chander et al., 2008a). *Hydroxylase 3* alleles contributed to 78% of the variation and approximately 11-fold differences in β-carotene relative to β-cryptoxanthin and 36% of the variation and fourfold difference in absolute levels of β-carotene (Harjes et al., 2008). More importantly, the discovery of a rare genetic variation increases β-carotene substantially in maize (Yan et al., 2010). Likewise, some of the elite maize inbreds representing major heterotic groups in China were reported to contain high protein (up to 14.7%), starch (up to 70.2%), oil (up to 5.2%), α-tocopherol (up to 74.8 μg^{-1}), γ-tocopherol (up to 78.0 μg^{-1}), δ-tocopherol (up to 5.3 μg^{-1}) and total tocopherols (120.7 μg^{-1}), and carotenoids (Chander et al., 2008b). Sorghum landraces, particularly those with yellow endosperm from Niger and Nigeria, have shown significant variation for carotenoids, with lutein, zeaxanthin and β-carotene, the predominant carotenoids (Salas Fernandez et al., 2008, 2009). Natural variations for β-carotene have been reported in banana (30–2780 μg 100 g^{-1}) and giant swamp taro (*Cyrtosperma chamissonis*) (50–2040 μg 100 g^{-1}) (Englberger et al., 2003), cassava (up to 4 mg kg^{-1} in the cassava landrace UnB 400 from Brazil) (Nassar et al., 2009), and sweet potato (up to 226 μg fruit weight^{-1} in a breeding line 11–20 from sweet potato breeding program at North Carolina State University) (Teow et al., 2007).

5.3.2. Variation for Protein and Oil Concentrations and Their Quality in Maize

The maize grain protein is deficient in lysine and tryptophan. The discovery in Peru's maize of two mutant alleles, *opaque 2* (*o2*) and *floury 2* (*fl2*), which alter amino acid profile and composition of maize endosperm protein, opened up exciting opportunities to improve maize endosperm protein quality (Mertz et al., 1964; Nelson et al., 1965). However, the mutants adversely affected agronomic performance, and had soft and chalky kernels, not liked by maize growers in developing countries. The researchers used *o2* and genetic modifiers (Paez et al., 1969) to remove these defects, and developed a number of agronomically superior maize germplasm or hybrids with better protein quality profile, branded as "quality protein maize", and

many version of this are currently grown by farmers in sub–Saharan Africa, Brazil, China and South Africa (Vasal, 1999; Krivanek et al., 2007). Another pioneering work in maize relates to Illinois long–term selection (100 cycles of selection) experiment that developed populations exhibiting phenotypic extremes for grain composition and correlated traits. The selection responses of both protein and oil are greater than 20 standard deviations from the original population mean (Dudley, 2008) in the positive direction and four standard deviations in the negative direction. These lines at the present time are being used as a source of favorable alleles associated with oil, protein and starch accumulation (Moose et al., 2004).

5.3.3. *Variation for Improving Oil Quality in Peanut*

Peanut oil quality is determined by the ratio of oleic (O) and linoleic (L) fatty acids. A higher ratio results in a better storage quality of oil, and longer shelf–life of the products (Branch et al. 1990). Norden et al. (1987) were the first to report exceptionally high oleic trait, O/L ratio (40), in the Florida breeding line, F 435. A few elite germplasm with high O/L ratio were registered and released as high–oleic (O/L ratio >10) peanut cultivars (Olin, SunOleic 95R, SunOleic 97R, and Tamrun OL11) in USA from this source (Gorbet and Knauft, 1997, 2000; Simpson et al., 2003b; Baring et al., 2012). However, F 435 is not easily available to peanut researchers. Recently Upadhyaya et al. (2011c) evaluated a peanut mini core collection (Upadhyaya et al., 2002) for variation in fatty acid profiles, and identified ICG 2381—a subsp. *hypogaea* accession from Brazil—with O/L ratio ~7, which is 2–3 times more than normal range reported in cultivated peanut germplasm. It provides a unique source for peanut breeders to improve oil quality of new cultivars.

5.4. Sustaining Food Quality by Manipulating Soil Microbial Diversity

The imbalance in demand and supply of food is mainly the result of inherent low soil fertility and the degradation of the natural resources, such as soil and water due to unsustainable farming practices without adequate investment. Such practices lead to the depletion of groundwater, soil organic matter and plant nutrient reserves of not only major but also secondary and micronutrients (Sanchez, 2002; Kijne, 2004; Kibblewhite et al., 2008; Lal, 2009; St. Clair and Lynch, 2010; Sahrawat et al., 2011; Graham et al., 2012). Equally important is that the nutrient-impoverished soils contribute to human malnutrition at least in two ways. First, by reducing crop yields,

thereby causing food scarcity that results in protein–energy malnutrition. Second, food produced on nutrient-deficient soils contain low concentrations of minerals in plant tissue including critical micronutrients such as Fe and Zn (Welch, 2002; St. Clair and Lynch, 2010; Sahrawat et al., 2011).

The deficiency of vitamin A, widely deficient in humans and the cause of anemia (Bloem et al., 1989; Graham et al., 2012) is however, not a nutrient for plants; and plants biosynthesize the carotenes that human body converts into vitamin A. Thus, for vitamin A deficiency, there is no fertilizer or nutrient management strategy. However, a viable strategy to provide vitamin A in food is to introduce carotene-rich secondary staples as supplement in the food system (Welch, 2002; Graham et al., 2012) unlike for nutrients such as iron and zinc, which is based in exploiting the synergy between biofortification and nutrient management via enhanced crop husbandry (Graham et al., 2012).

The evidence suggests that soils deficient in multinutrients including major and micronutrients result in poor grain and fodder quality; and balanced nutrient management not only increases food production but also enhances crop quality relative to protein, oil, and Fe and Zn (Sahrawat et al., 2011 and references therein). The mineral composition of plant foods, which depends on soil nutrients status, has direct implications for human health (Welch, 2002). To enhance crop productivity and quality of food, there is need to simultaneously overcome the constraints posed by low soil fertility, and also addresses the limitations imposed by the impact of climate change on food production and quality through soil fertility improvement in general and nutrient acquisition and utilization by crops in particular (Gavito et al., 2001; Taub et al., 2008).

Microbes are the unseen majority in the soil; and form a large portion of life's genetic diversity. Soil microbes play key roles in agroecosystem; for example, heterotrophs decompose organic matter applied to the soil and release nutrients such as nitrogen and phosphorus in the mineral available form for acquisition by plants, which eventually impact food production and food quality (van der Heijden et al., 2008; St. Clair and Lynch, 2010). Although of great importance, the effects of climate change via soil fertility and microbial diversity on nutrient acquisition and utilization by crops are poorly understood and have not received the attention they deserve (Lynch and St. Clair, 2004). Indeed, soil biota and their diversity are the main drivers of mineral nutrient cycling (Kibblewhite et al., 2008), and play equally important role in causing and suppressing pathogenesis in plants and foods (Garbeva et al., 2004). The microbial activity in the soil affects

both biotic and biological soil properties that influence plant growth, food production and quality (Bardgett et al., 2005; Barrios, 2007; Cortois and De Deyn, 2012). For example, altering the rhizosphere microflora by seed or root inoculation with specific organisms (i.e. biofertilizers) has long been recognized as a practical strategy with potential to promote plant growth by enhancing the availability of nutrients such as phosphorus or the release of growth promoting substances. There is a large body of literature on the effects of various bacterial cultures on plant growth of a range of crops including both cereals and legumes under controlled conditions, their role in practical agriculture needs to be established by future research (Zahir et al., 2004 and references therein). Nevertheless, the general thinking is that the climate change effects via rising temperature, drought and increase in intense rainfall events are most likely to influence nutrient availability in the soil, and their subsequent acquisition and utilization by crop plants (Sinclair, 1992; Lynch and St. Clair, 2004; St. Clair and Lynch, 2010).

To sustain a positive response to increased CO_2 concentration requires an increase in plant uptake of the total amount of plant nutrients, otherwise the crop quality in terms of mineral composition is likely to decline (Gavito et al., 2001; Erbs et al., 2010). However, it is very difficult to predict the response to climate change on nutrient acquisition and utilization by crop plants because of the large uncertainty about the availability of nutrients in the soils as a result of climate change (Sinclair, 1992). For example, while increased CO_2 concentration would stimulate biological nitrogen fixation by free-living organisms and symbiotic systems (Sinclair, 1992), whereas increased temperature and altered rainfall pattern and intensity may result in enhanced losses of minerals by leaching and soil erosion (Meadows, 2003; Tang et al., 2008; Zougmore et al., 2009). Such a situation may adversely affect the amount of minerals or nutrients available in the soil for acquisition and utilization by crops. Furthermore, the acquisition, uptake and utilization of minerals by crop plants is further affected by drought, exacerbated by climate change, through the availability and transport of both mass flow and diffusion of plant nutrients in the soil (Barber, 1995; Bassirirad, 2000; Biggs et al., 2008; Brouder and Volenec, 2008). For example, the reduction of root growth, alteration in root architecture and impairment in root functions under drought reduce the mineral acquisition capacity of plant root system (Mackay and Barber, 1985; Barber, 1995). In addition to the effects of drought on nutrient availability, accessibility and acquisition by roots, drought also adversely influences root–microbe associations, which are the principal strategy for mineral capture and acquisition by crop plants.

For example, it has been observed that due to reduced carbon and oxygen fluxes and nitrogen accumulation in root nodules retard or at times inhibit biological nitrogen fixation in leguminous plants (Dakora and Keya, 1997; González et al., 2001; Ladrera et al., 2007). Equally importantly, drought also alters both composition and activity of soil microbial community that control carbon and plant nutrient transformations, availability and cycling (Schimel et al., 2007; St. Clair and Lynch, 2010).

The soil microbial organisms are the foundation of soil fertility maintenance and efficient plant nutrition in food production systems. Moreover, the widespread presence of the *Arbuscular mycorrhiza* (AM) symbiosis in nodulated legumes and the role of AM fungi in improving nodulation and biological nitrogen fixation are universally recognized. The synergy between biological nitrogen fixation and AM fungi leads to improved availability and accessibility of phosphorus and mitigation of water stress by the action of AM fungi. At times, the AM fungi may also be involved in the biological control of root pathogens. The role of the AM fungi in an overall improvement of soil quality is yet another factor involved (Barea et al., 2005).

Studies have shown that the root–mycorrhizal symbiosis in plants is not very sensitive to soil moisture stress of moderate intensity (Entry et al., 2002; Garcia et al., 2008; St. Clair and Lynch, 2010). In fact, there is a large body of literature documenting the beneficial effects of mycorrhizal fungi in plants under varying degrees of drought or soil moisture stress conditions (Entry et al., 2002; Jones et al., 2004; Garcia et al., 2008); and indeed a part of the benefit provided by mycorrhizae under drought stress is associated with increases in mineral nutrient transfer to plant roots (Goicoechea et al., 1997; Jones et al., 2004; St. Clair and Lynch, 2010; Cavagnaro, 2008; Cavagnaro et al., 2010).

The information on the effects of soil warming suggest that nutrient uptake by crop plants could increase from 100% to 300% by enlarging the root surface area that will promote increased rates of mineral diffusion and water influx (Ching and Barber, 1979; Mackay and Barber, 1985). Likewise, warm temperature increases rates of transpiration and as a consequence plants tend to acquire water–soluble nutrients at enhanced rates as temperature increases (Barber, 1995). In addition, temperature increase can also stimulate nutrient acquisition by plants via both faster ion diffusion rates and increased root metabolism (Bassirirad, 2000). However, such positive effects of warm temperature on mineral acquisition by plants are dependent on the availability of adequate soil moisture; as under dry conditions, the extreme vapor pressure deficit (VPD) may trigger stomatal closure, leading

to decreased nutrient acquisition mediated by mass flow (Abbate et al., 2004; Cramer et al., 2009).

Clearly, more research needs to be undertaken to better elucidate the role of microbes especially their diversity on food production and quality (Sinclair, 1992; St. Clair and Lynch, 2010). An integrated and holistic approach including controlled environment and field research is needed to investigate the long-term effects of manipulated soil biota communities on plant growth and mineral composition across soils with diverse abiotic characteristics. The emphasis should be to enhance our understanding of the plant–microbe interactions specific biota in their natural complex biotic and abiotic environment (Ladygina et al., 2010; Cortois and De Deyn, 2012).

In summary, agrobiodiversity (both plant and soil biota) is crucial to coping with climate change. The accumulated evidence reveals that there is a looming threat to biodiversity due to global warming as the organisms have to adapt to the changed environments to play their roles effectively in respective agroecosystems. The wild relatives among the plant species are at added risk due to genetic erosion and biodiversity loss. They need to be preserved in situ in protected areas to ensure evolution of new genetic variants, which may contribute to addressing new challenges to agricultural production and crop quality. The below ground biodiversity (soil biota) should also be explored, preserved (ex situ or in situ), and their role in coping with adverse effects of global warming on agriculture assessed, documented and knowledge disseminated. With the available modern instrumentation, it has become feasible to conduct accurate and cost-effective analysis of a large numbers of grain samples using high-throughput assays, allowing researchers to identify germplasm and breeding lines with desirable seed composition for subsequent use in crop breeding. These advances have led to the selection of various germplasm sources and new cultivars with desirable seed chemistry. However, very limited information is available globally on seed chemistry of germplasm collections. Hence, the need to screen core or mini core collections for seed chemistry to identify germplasm with improved seed composition for use in plant breeding programs. Soil fertility will be greatly impacted by both increase in atmospheric CO_2 concentration and temperature. The effects of climate change via soil fertility and microbial diversity should be investigated on nutrient acquisition and utilization by crops, and their effects on food production and quality. The emphasis should be to enhance our understanding of plant–microbe interactions specifically of biota in their natural complex biotic and abiotic environments.

6. CLIMATE CHANGE ANALOG LOCATIONS REPRESENTING FUTURE CLIMATE

Climates are changing rapidly elsewhere. This trend is likely to continue or even accelerate in the future, with likely serious, but variable consequences for agricultural productivity and quality around the world (IPCC, 2007; Challinor and Wheeler, 2008; Challinor et al., 2009; Boote et al., 2010; Moss et al., 2010; Palm et al., 2010; Reynolds, 2010). With climate change already posing a threat to food production around the globe, scientists are developing a form of virtual time travel that may offer farmers in various countries a glimpse of their future by the identification of regions and sites therein where the growing conditions at the present time match those that will exist in 2030 (Hallegatte et al., 2007; Kopf et al., 2008; Ramirez-Villegas et al., 2011). According to a recent report from the CGIAR Research Program on Climate Change, Agriculture and Food Security (CCAFS), the climate change analogs are today a view of tomorrow's agriculture, and will play a crucial role in adaptation research under climate change (Ramirez-Villegas et al., 2011).

The analogs approach in climate change research is a relatively novel way of supporting modeled recommendations with on-the-ground practical, empirical testing. The underlying principle is that the analogs tool connects sites with statistically similar or "analogous" climates across space in other geographic locations and/or time with historical or projected future climates. This approach helps to answer the key questions regarding climate change analogs: where sites can be found with similar climate at present or in the past or projected to be in the future analogous to the selected sites. In essence, a spatial analog is a location or site whose climate at present appears as a likely analog to the projected future climate of another site or location; and thus these two sites represent promising areas for conducting comparative research on adaptation plans in the face of climate change. On the other hand, temporal analogs are constructed using historical data that allow us to identify historical events that potentially can provide insights into the possible future consequences or fall-outs as a result of climate change. In addition, historical events provide an opportunity to learn as to how farmers adapted to climate shifts in the past. They equip farmers to deal with the changes that follow climate change at a site or in region. Although the current version of the analogs tool does not yet allow effective historical searches, the scientists involved in CCAFS are hopeful that this important

function will be added in the future (Ramirez-Villegas et al., 2011). This tool is indeed an important development in facilitating adaptation through learning from analogous sites in both space and time.

The identification of analog sites coupled with relevant information collected from local field site studies or available databases can be employed and compared for developing further research studies including the analysis of the results obtained using simulating modeling (Hallegatte et al., 2007; Kopf et al., 2008) or for proposing high-potential adaptation pathways in a region (Ramirez-Villegas et al., 2011). Kopf et al. (2008) described a method to analyze the results of climate simulation models, which is an improvement on the method reported by Hallegatte et al. (2007). The proposed method is based on the concept of climate analogs, which in practical terms means finding a site (in this study a city) B whose present climate statistically corresponds to the simulated future climate of an evaluated site A. This exercise provides an intuitive visualization of the climate change effects on areas, by replacing the change of climate in time with a change of a site's location in space. Through the use of several models and scenarios, this methodology also helps to clarify the extent of the uncertainty in climate change prediction, and their impacts on targeted areas. The proposed method characterizes climate using three annual indicators—AI, heating degree days and cooling degree days—which are computed from monthly rainfall and temperature datasets. According to these authors, the methodology provides a better and strong basis for the visualization of climate change. Equally importantly, the proposed approach allows socioeconomic adaptation to different climates to enter the mental model for visualization (for details see Kopf et al., 2008).

By identifying and connecting analog sites in a network, research output can enable farmers to better envision as to how their site-specific agricultural future might look. Equally importantly, they can facilitate creating knowledge chain through which strategies or approaches and information regarding farming can be passed on or shared to facilitate more effective adaptation to climate change via interactions and learning. The analog tool also permits targeted field evaluation and testing of the climate resilience of crop production systems or specific technologies, and to identify effective technologies that can be implemented at analogous sites in the future. Furthermore, the analogous methodology allows the sharing and interpretation of historical data, and to learn lessons from case studies in an effort to building understanding of the best ways to improve climate resilience and enable adaptation to climatic changes (Ramirez-Villegas et al., 2011). Clearly, the use of climate analogs for locating future climates at the present time helps to

ground models in field-based realities, significantly increasing our knowledge of adaptation capacity and identifying appropriate site-specific interventions. Although there is need for region-specific tweaks, validation processes, and to generate additional data for more robust results, the CCAFS analogs tools indeed offer important platform for future research and decision making relative to climate change adaptations (Ramirez-Villegas et al., 2011).

Haywood et al. (2011) discussed thoroughly the usefulness to benefit the society, of identifying a true prequaternary geological analog for future climate change. According to them, the identification of such sites provides a natural laboratory for evaluating numerical models of climate and the Earth system that are used to produce better predictions of future climate change. Indeed Earth history provides examples of how the planet responded to variations in atmospheric greenhouse gas concentrations in the longer term (several centuries). This methodology based on Earth history and using paleoclimate and environmental reconstruction and modeling are facilitating the assessment and calculation of the response of global temperatures to increasing carbon dioxide in multiple centuries' time span, also referred to as *Earth system sensitivity*, which derives climate change. Thus prequaternary geological analogs provide insight into future greenhouse warming and climate change with better precision (Crowley, 1990; Covey, 1995; Pagani et al., 2005; Haywood et al., 2011).

Despite the progress made in the use of climate analogs for adaptation to climate change, considerable uncertainty remains especially regarding projections of future climates and their impact on diverse farming systems at the local level. For example, the intrinsic adaptive and adaptation capacity of diverse rural communities is not taken into account in the global or regional-level models, which are used by policy makers for planning and implementation. Notwithstanding these limitations, the use of climate analogs for locating future climates at the present time can ground models in field-based realities. It is also important to note that the recently proposed CCAFS analogs tool offers an important platform for future research and decision making in the overall adaptation strategy to climate change (Ramirez-Villegas et al., 2011).

7. PLANT PHENOMICS TO SCREEN TRAITS FOR ADAPTING TO STRESSES

Precise phenotyping is the key to finding gene(s) and its allelic variants, analyze their expression, and thereafter for introducing these agronomically

beneficial alleles into crop cultivars to enhance adaptation and meet new challenges to agricultural production. In the past, researchers have made significant progress toward developing conventional phenotypic screens for identifying novel sources of genetic variation, dissecting physiological and molecular basis of the response and adaptation to stresses, and finding alleles associated with beneficial traits. It is true that by using these phenotypic screens, crop genetic enhancers contributed substantially toward developing new cultivars in most of the agriculturally important crops. However, these screens involve large phenotyping costs, and are laborious, time-consuming, and are prone to errors due to variation in environmental conditions, with potential to severely limit the genetic gains.

Plant phenomics is the study of plant growth, performance and composition. It is a rapidly emerging area of research, offering a suit of new technologies to accelerate progress in understanding gene function and environmental responses, which will bridge the gap between genomics, plant function and agricultural traits, enabling breeders to develop improved germplasm or new cultivars with specific attributes (Furbank and Tester, 2011; Cabrera-Bosquet et al., 2012). High-throughput plant phenomics will also be beneficial to do research on crop water needs, fertilizer-induced deficiencies or other stresses including biotic and abiotic stresses, which will further help growers to take precautionary measures for intensifying sustainable crop production in commercial agriculture. Phenomics include both forward and reverse approaches, in which the former uses phenotyping tools to screen germplasm for valuable traits, while the latter dissects traits to discover their mechanistic understanding and allow exploitation of this mechanism to enhance the trait value into new germplasm. For this purpose various automatic high-throughput plant growth and phenotyping platforms have been established. They are known by the following names: PHENOSIS (Granier et al., 2006), PHENODYN (Sadok et al., 2007), GROWSCREEN (Walter et al., 2007), TraitMill™ platform (Reuzeau et al., 2005), LemnaTec (http://www.lemnatec.com), and HTPheno (Hartmann et al., 2011), and are being progressively used in plants research and development, as detailed below.

7.1. Root System Architecture

Roots act as the interface between plants and soil. The root system architecture (RSA) refers to the temporal and spatial distribution and configuration of a root system in their natural undisturbed state within soil. RSA is a complex and difficult-to-quantify trait, and may differ between species.

Knowledge of the root development and architecture therefore holds potential for manipulation of root traits to improve the productivity and sustainability of agricultural systems; and to better understand and manage natural ecosystems (De Smet et al., 2012). In the last 10 years, there has been considerable research done on understanding RSA in model plant *Arabidopsis* and a few agriculturally important crops such as common bean, maize, rice and wheat, which resulted in the development and application of a few phenotyping platforms and associated software for assessing RSA, including Shovelomics (Trachsel et al., 2010), RootChip (Grossmann et al., 2011), or nondestructive image-analysis toolbox for automatic phenotyping of RSA (Iyer-Pascuzzi et al., 2010), or X-ray computed tomography (CT) scanners for studying three-dimensional RSA (Fang et al., 2009; Clark et al., 2011; Flavel et al., 2012; Mooney et al., 2012).

Shovelomics is a high-throughput, low-cost, and easy to learn platform for phenotyping root architecture in maze in the field, which virtually scores 10 root architectural traits of the crown root of an adult maize plant in a few minutes. Trachsel et al. (2010) visually scored the number, angles and branching density of crown and base roots in three recombinant inbred lines (RILs) populations of maize, which provided reliable data as indicated by high correlations between measured and visually scored trait values for numbers ($r^2 = 0.46$–0.97), angles ($r^2 = 0.66$–0.76), and branching ($r^2 = 0.54$–0.88) of base and crown roots. Further, using these visual scores, Trachsel et al. were able to discriminate between populations. For example, RILs derived from the cross between NY821 × H99 generally had the greatest number of roots, the highest branching density and the most shallow root angles, while inbred lines from the cross between OH43 × W64a generally had the steepest root angles. Moreover, the genotypes ranking remained the same across environments, emphasizing the suitability of the method to evaluate genotypes across environments. This platform should be directly applicable to other cereal crops such as sorghum or millet, while scoring system for nongraminaceous crops would have to account for a root architecture that is completely different than the one observed in maize. Thus, the visual evaluation of root architecture will be a valuable tool in tailoring crop root systems for specific environments (Trachsel et al., 2010).

Grossmann et al. (2011) reported a generic microfluidic chip platform, RootChip, which integrates live-cell imaging of growth and metabolism of *Arabidopsis* roots with rapid modulation of environmental conditions. It has separate chambers for individual regulation of the microenvironment of multiple roots from multiple seedlings in parallel, which can be modified for

use with roots from other plant species, by adapting the chamber geometry to facilitate the systematic analysis of root growth and metabolism from multiple seedlings, paving the way for a large-scale phenotyping of root metabolism and signaling. This high-throughput root phenotyping assay provides a major advance for studying root biology with possibility of continuous imaging at the cellular and subcellular resolution for several days and under conditions that allow for root growth and root hair development. RootChip will greatly facilitate the ability to investigate nutrient uptake in different root zones, cell-type-dependent metabolite flux, and response of individual cells to different environmental stimuli.

The ability to nondestructively image and automatically phenotype complex root systems is fundamental to identifying genes underlying RSA. Iyer-Pascuzzi et al. (2010) reported a nondestructive image and analysis system for automated phenotyping and trait ranking of RSA in rice. Using 16 automatically acquired phenotypic traits for 2297 images from 118 individuals; Iyer-Pascuzzi et al. (2010) detected wide variation in phenotypes among genotypes and greater intergenotype than intragenotype variance of RSA, with option to integrate these data into computational pipeline that utilizes supervised learning methods to determine which traits best separate two genotypes. It also ranks the traits according to their contribution; and can assist to identifying candidate traits for automatic phenotyping of RSA in mapping populations. Further, Lobet et al. (2011) developed novel, semiautomated image-analysis software—SmartRoot—to facilitate the quantitative analysis of root growth and architecture of complex root systems, which combines a vectorial representation of root objects with a powerful tracing algorithm that accommodates a wide range of image source and quality. It also supports a sampling-based analysis of root system images. SmartRoot is an operating system–independent freeware based on ImageJ and relies on cross-platform standards for communicating with data-analysis software. Lobet et al. (2011) demonstrated the utility of this software to conduct time-lapse analysis of cluster root formation in lupin and maize root systems. Furthermore, Clark et al. (2011) developed a novel imaging and software platform, RootReader3D, for high-throughput phenotyping of three-dimensional root traits, and characterized 27 phenotypic root traits in rice. After validating these observations with two-dimensional measurements, they found them highly correlated. This highly flexible platform provides a capacity to measure root traits with a high degree of spatial and temporal resolution, which can facilitate novel investigations into the development of entire root systems or selected components of root systems, thus

a powerful resource to explore the molecular and genetic determinants of RSA in crops.

X-ray CT, a nondestructive and noninvasive technique, offers new opportunity to monitor root growth and development in time and space in undisturbed environments (Mooney et al., 2012 and references therein). More recently, Flavel et al. (2012) reported high correlations between micro-CT observed roots and roots observed by standard methodology (soil core washing and WinRhizo analysis) in wheat. The potential drawbacks of this technology relate to the software to digitally segment roots from soil and air, which will improve significantly as automated segmentation algorithms are developed. A combination of rapid scans and automated segmentation will allow CT methodology to realize its potential as a high-throughput technique for the quantification of roots in soils.

7.2. High-Throughput Imaging to Diagnose and Quantify Plant Response

Phenomics has also advanced to develop other high-throughput phenomics technologies, a few of which include plant response to biotic and abiotic stresses (Matouš et al., 2006; Chaerle et al., 2007; Jones et al., 2009; Sirault et al., 2009; Berger et al., 2010; Munns et al., 2010; Furbank and Tester, 2011), dissecting dynamic changes in plant structure and functions (Jahnke et al., 2009), multisensor stress catalog (Chaerle et al., 2009), or module for handling large-scale phenotyping and genotyping data (Jung et al., 2011), which is beyond the scope of this chapter to provide procedural and technical details (Lenk et al., 2007). Nonetheless, we highlight their applications in agriculture including in plant breeding to developing cultivars with better adaptation to stress-prone environments.

Thermal and chlorophyll fluorescence imaging are powerful tools for the study of spatial and temporal heterogeneity of leaf transpiration and photosynthetic performance. Chlorophyll fluorescence imaging provides a powerful, noninvasive tool for investigating photosynthesis and its heterogeneity, and the variations in fluorescence transients could be used for early detection of biotic and abiotic stresses. Matouš et al. (2006) developed a new technique of combinatorial fluorescence imaging that significantly enhances our capacity to reveal dynamics of the plant–pathogen interaction. These images yield the highest contrast throughout the progression of infection in plants, which can be divided into segments that show tissue in different infection phases, as Matouš et al. demonstrated in case of *Pseudomonas syringae* infection in *Arabidposis*. The thermal and chlorophyll

fluorescence imaging, when combined, provide specific signatures for diagnosis of distinct diseases and abiotic stresses, for example, rapid screening for stomatal responses can be achieved by thermal imaging, while, combined with fluorescence imaging to study photosynthesis, it can potentially be used to derive leaf WUE as a screening parameter (Chaerle et al., 2007). These images allow continuous automated monitoring of dynamic spatial variation. Moreover, such dual-imaging systems could be extended with complementary techniques such as hyperspectral and blue-green fluorescence imaging, which will increase the power of stress diagnosis and the potential for screening of stress–tolerant germplasm (Chaerle et al., 2007). Likewise, using hyperspectral and chlorophyll fluorescence imaging, Bauriegel et al. (2011) were able to detect wheat plots infected with head blight (*F. culmorum*). The disease severity was highly correlated with photosynthetic efficiency and above the infection limit of 5% severity of disease, chlorophyll fluorescence imaging reliably detected infected ears.

Salinity is another major abiotic stress affecting crops production worldwide, which can be exacerbated by the changing climate. Sirault et al. (2009) developed a high-throughput automated image protocol that captures, identifies and analyzes thermal images acquired from a long-wave infrared (IR) camera to quantify the osmotic stress response of wheat and barley to salts. This technology in comparison to porometry (James et al., 2008) measures stomatal conductance through a precise, noninvasive and quick method, and needs only few seconds to acquire a thermograph comprising more than 3000 individual measurements for each object. It also takes into account spatial heterogeneity. IR thermal imaging could be therefore used to screen a large numbers of germplasm accessions, breeding lines and cultivars varying in the stomatal traits related to salt tolerance.

Drought is a complex stress that elicits a wide variety of plant responses. The nondestructive imaging techniques allow a temporal resolution and monitoring of the same plants throughout the experiment, which provides vital information on the physiological changes in response to drought over time to identify and characterize drought-response mechanisms into a series of component traits (Berger et al., 2010). A number of nondestructive imaging methods are now available to study physiological changes occurring in plants under drought, which include thermal IR imaging or IR thermography to assess transpiration rates (Trs), NIR to assess growth and water stress of plants, or fluorescence imaging to complement reflectance imaging. However, researchers should be aware of technological challenges that should be properly addressed to avoid erroneous results. It is thus clear that

trait-dissection affected by high-throughput phenotyping could provide a significant new opportunity to enhancing our knowledge of plant response to drought, including elucidating the genetic basis of these responses and integrating such traits into appropriate combinations to improve crops performance under varying drought conditions (Berger et al., 2010; references therein; Furbank and Tester, 2011).

Agricultural production is limited by various abiotic and biotic stresses. Imaging technology has advanced to provide valuable information on plant stresses; and when supplemented by conventional video imagery (for study of growth), they provide an efficient early warning system to discriminate between different stressors to develop matrix that identifies specific signatures for multiple stress types, which allow agriculturists to reduce production losses in stress-prone environments (Chaerle et al., 2009).

Maize researchers took the lead to adapt some of the above high-throughput phenotyping tools to study variation in maize hybrids in response to drought under field conditions. Using high-throughput sensors, Winterhalter et al. (2011) demonstrated that canopy water mass (CWM, amount of water kg m^{-2}) correlates well ($r^2 > 0.70$) with spectral indices and IR temperature with varying drought–stress levels, which enabled them to differentiate hybrids into three groups (above, below or average performer) under control and stressed environments. Their finding demonstrates that it is indeed possible to both detect CWM and discriminate between groups of maize hybrids using nondestructive high-throughput phenotyping, thus, potentially a useful technique for crop breeding. Resilience to soil water deficit and its genetics are a priority research area for maize breeding. More recently, Chapuis et al. (2012) tested and compared three methods of determining resilience to water deficit: the ability of hybrids to maintain leaf growth in a range of soil water potentials in a phenotyping platform, a direct estimator of resilience of seed number to water deficit in a network of field experiments, and classical methods involving adaptation to drought indices and variance analysis. They evaluated 19 maize hybrids by growing in 14 environments. Using the slope of regression line between drought index and seed number (which was taken as an estimate of the resilience to soil water deficit for each hybrid), Chapuis et al. (2012) detected twofold differences that correlated with resilience of leaf growth to soil water deficit in the phenotyping platform. Resilience estimated via genotype × watering treatment was nonsignificant due to large differences in drought indices between genotypes in a given watering treatment. This result led them to propose that the direct estimation of

resilience to water deficit is feasible in the field with a minimum amount of environmental measurements.

O'Shaughnessy et al. (2011) used canopy temperature data to investigate whether an empirical crop water stress index (CWSIe) could be used to monitor spatial and temporal crop water stress in soybean and cotton. Using the benchmark relationships between CWSIe and leaf water potential (ΨL), they detected significant negative linear correlation between mid-day ΨL measurements and CWSIe at well-established soil water differences. Average seasonal CWSIe values were inversely related to crop water use (r^2 values > 0.89 for soybean and 0.55 for cotton). O'Shaughnessy et al. (2011) also detected a significant inverse relationship between the CWSIe and soybean (2-year average r^2 = 0.85) and cotton (r^2 = 0.78 in 1 year) yield, which enabled them to conclude that contour plots of CWSIe may be used as maps to indicate the spatial variability of within-field crop water stress; and thus may be useful for scheduling irrigation or identifying areas within a field where water stress may impact crop water use and yield.

7.3. Sensor-Based Phenotyping Platform for Assessing Biomass

High-throughput nondestructive biomass determination assay has a great potential to unravel the genetical, physiological and biochemical basis of plant growth. The traditional approach to phenotype biomass in the field is destructive, laborious, time consuming, and expensive. Tackenberg (2007) reported digital image analysis as nondestructive method to measure growth rate, fresh- and oven-dried biomass and its vertical distribution, and dry matter content, which is time saving and cost-effective. Recently, Montes et al. (2011) designed a high-throughput phenotyping platform that employs light curtains (LC) and spectral reflectance (SR) sensors mounted on a tractor to measure biomass in a field-grown experiment, spread over five environments, consisting of 20 maize hybrids. Using sensors (SR and LC + SR) and partial least square regression and support vector machine regression, Montes et al. (2011) found that biomass obtained by a combination of LC + SR gave the lowest mean relative error of prediction Mean relative error (MRE) (0.11) and the highest R_v^2 (coefficient of determination of validation = 0.97), with high repeatability. Their research provided a proof-of-concept that this high-throughput, nondestructive phenotyping platform based on LC and SR sensors has a great potential for early biomass determination in field trials of maize and other space planted row-crops. Furthermore, Golzarian et al. (2011) proposed a method for accurate

estimation of plant shoot dry weight that employs information obtained from the images of plants and their age. It also provides an accurate and practical model for the estimation of shoot dry weight as a substitute for conventional destructive methods of biomass measurement in cereals.

Rascher et al. (2011) presented state-of-the-art phenotyping approaches to address aspects of resource use efficiency in plants, below ground roots, aboveground shoots and transport/allocation processes, using magnetic resonance imaging and automated fluorescence imaging (Pulse amplitude modulated (PAM) fluorometry) in combination with automated shape detection that allowed them doing high-throughput screening of photosynthetic traits. They are of the opinion that these phenotyping techniques together with mechanistic knowledge on the plant structure–function relationships will open new opportunities in whole-plant ecophysiology, which may assist in developing new germplasm with enhanced resources use efficiency.

7.4. Developing Modules to Store, Retrieve, Add or Modify Large Datasets

Technological development in high-throughput assays, mostly in genotyping but of late in phenotyping as well, allows researcher's phenotype and genotype large sets of individuals, which generate large datasets. Linking phenotypic variation and genotypic diversity is a major requirement for basic and applied genome–centric biological research. The mechanisms to be established should efficiently store, add, retrieve, or modify, query it, and analyze these datasets to derive meaningful interpretation between genotypic and phenotypic data. Currently, Chado, a generic, modular, community-based database is widely used in the biological community to store information associated with genome sequence data (http://gmod.org/wiki/GMOD_Users). Its ontology-driven module allows researchers use the same schema in projects with widely different metadata, which can be modified or added, as new data types become available. Its modular design allows researchers select those modules needed to manage their datasets, and add to new modules as and when new dataset is generated. Chado is an open source, consists of 18 modules, and any user can contribute to schema (http://sourceforge.net/projects/gomd/), provided these contributions are consistent with the Chado generic design principle.

Recently, Jung et al. (2011) reported a new module, "ChadoNatural Diversity" module for storing large-scale phenotyping, genotyping and breeding data. This module strictly adheres to the Chado remit of being generic and ontology driven, and allows the storage of data from each

experimental line that are scored for a large number of phenotypic traits, and genotyped with a set of genetic markers. It consists of tables for data storage from various experiments, and tracks the relationships of the experiments with data stored in other modules (http://gmod.org/wiki/Chado_Natural_Diversity_Module). This module can store any type of experiment that either uses or generates specimens or stock organisms, which may be grouped or structured hierarchically, whereas any kind of biological entity can be stored as the observed unit, from a specimen to be used in genotyping or phenotyping experiments, to a group of species collected in the field that will undergo thorough laboratory analysis.

In spite of these recent developments, high-throughput phenomics research faces technological challenges to generate novel tools in computation and informatics. Its success will allow researchers to amass, access, integrate, organize, and manage phenotypic databases across species and conduct genome-wide analysis to associated phenotypic information for its applied value in agriculture (Lussier and Liu, 2007).

To sum up, it is clear that recent technological advances to developing high-throughput phenotyping platforms have taken a leap forward, and it is expected that more new innovations to refine these assays for multiple tasks, which in turn, will be useful in monitoring, diagnosing and taking corrective measures to plant growth and development to address yield-reducing constraints at the crop, community, regions or global scale. It is visualized that there will be increased interactions among the various stakeholders to use these resources, and tailor their germplasm with specific attributes to stress-prone environments.

8. PLANT TRAITS TO ACCELERATE ADAPTATION TO CLIMATE CHANGE

8.1. Genetic Enhancement for Adaptation to Abiotic Stress

Plant breeders are often reluctant to use exotic germplasm including landraces, wild and weedy crop relatives, as these germplasm often show poor adaptation and low yield compared to modern cultivars. These germplasm may however possess desirable traits, e.g. host-plant resistance or adaptation to abiotic stresses. The use of exotic germplasm in breeding is a long-drawn process, often associated with undesirable traits; and shedding these negative linkage drags requires careful planning and execution of germplasm enhancement steps in such a way that the coadapted gene complexes,

while introgressing new genes into improved genetic background, are not lost. Germplasm enhancement (also referred to as prebreeding by other authors) is a step before practical breeding for cultivar development. The products of germplasm enhancement are the intermediate (or semifinished) genetic materials that plant breeders can use to transfer desirable traits into an adapted genetic background. This process may take several years and following this plant breeders still have to develop commercial cultivars. Germplasm enhancement contributes to broadening the total genetic diversity in crops and provides specific traits to plant breeders. It is a vital link between conservation of plant genetic resources in gene bank collection and utilization of these resources in crops improvement.

CWR, which offer a rich source of genetic variation, are often grown in their natural habitats in conditions of climate and soil that may not be suitable for cultivation of modern crops. Evidence to-date suggests that CWR have provided many agronomically beneficial alleles which when brought into cultivated genetic background not only broaden the genetic base of cultigens and provided protection against biotic and abiotic stresses but also in some cases enhanced grain yield and quality attributes of crop cultigens. For example, *Oryza officinalis*, a source for early morning flowering trait, has been used to change the time of flowering of cultivated rice (*Oryza sativa*), which can mitigate yield loss due to global warming by escaping high-temperature stress at anthesis during the day time (Ishimaru et al., 2010), Moreover, CWR contribute to host-plant resistance to many pests and pathogens in both cereals and legumes (Dwivedi et al., 2005, 2008 and references therein). Furthermore, hybrid rice in China became reality when male sterility was discovered in a wild-rice *O. sativa* f. *spontanea* (Yuan, 1993). Similarly, nuclear genes from *Cajanus acutifolius* interact with pigeonpea (*Cajanus cajan*) to produce cytoplasmic nuclear male sterility system in pigeonpea (Dwivedi et al., 2008 and references therein). Likewise, wild species contribute yield and quality enhancing alleles in rice and tomato (Dwivedi et al., 2008 and references therein).

Hexaploid bread wheat resynthesis can be done by crossing tetraploid durum cultivars with diploid wild relatives (including nonprogenitor wheat species), enabling a whole new gene pool to be introduced that did not come from the original wild crosses from which the first ancient wheat ensued. These resynthesized wheats provided rich source of diversity for adaptation to stresses to the wheat cultigen pool (Dwivedi et al., 2008 and references therein).

Simpson et al. (1993) were probably the first to produce fertile amphiploid, peanut (TxAG-6) by colchicine treatment of the sterile triploid obtained from crossing the AA-genome donor hybrid (*Arachis cardenasii* × *A. diogoi*) with the BB-genome species, *Arachis batizocoi*. It is thus a useful resource for introducing genetic variability into the peanut cultigens. Using this approach, two peanut cultivars Coan and NemaTAM carrying genes for root-knot nematode (*Meloidogyne arenaria*) resistance from *A. cardenasii* have been released for cultivation in the USA (Simpson and Starr, 2001; Simpson et al., 2003a). Likewise, researchers in Brazil and India have produced a range of new amphidiploids, that when doubled the chromosomes, produced fertile synthetics (Mallikarjuna et al., 2011; Fonceka et al., 2012). They are expected to release new source of variation for use in genetic improvement of peanut. For example, Fonceka et al. (2012) detected many wild alleles that contributed positive variation to several traits (pod and seed size, pod maturity and biomass production) involved in peanut productivity and adaptation. Likewise, breeding lines with higher 100-seed weight (up to 95 g) compared to 32 g of cultivated parent TMV 2, and with 23–68% more pod yields than TMV 2 (3343 kg ha^{-1}) were selected in a segregating population involving TMV 2 and TxAG-6 (H. D. Upadhyaya, ICRISAT, unpub). TxAG-6 yielded 2–5 g plant^{-1} with very low 100-seed weight (~10 g). These results demonstrated that the novel alleles of CWR, which were considered to be lost in evolution to cultivated types, could be used to enhance important agronomic traits in cultivar (Upadhyaya, 2008).

Developing exotic genetic libraries, also known as *introgression lines*, consisting of marker-defined genomic regions taken from wild species and introgressed onto the background of elite crop cultivars is another approach suggested to enhance trait variability for use in crop improvement (Eshed and Zamir, 1994). Such an approach has been widely employed in tomato genetics and breeding for transferring desirable traits in cultivated tomato. For example, pyramided tomato introgressed lines containing three independent yield-promoting genomic regions produced more than 50% greater yields compared to controls under both wet and dry field conditions (Gur and Zamir, 2004). Likewise, introgressed progenies containing yield-enhancing alleles from wild rice produced higher grain yield in rice (Xiao et al., 1996).

Clearly, CWR are excellent source of genetic variation for agronomically beneficial alleles that when brought together into cultivated genetic backgrounds have the potential to enhance trait value(s). However, useful traits from interspecific crosses will often carry negative linkage drag, thereby

making the use of CWR a long-term process in breeding programs because of the lengthy process in which relevant traits are gradually transferred to an elite background. A long-term consortium-based approach involving conservationists, geneticists, plant breeders, and molecular biologists may be able to harness the wealth of CWR to address agriculturally important problems, more so in this time of climate change.

8.2. Integrating Trait Diversity to Develop Climate-Proof Nutritious Crops

8.2.1. Drought Adaptation in Cereals

Breeding for adaptation to drought-prone environments is extremely challenging due to the complexities associated with various stress–adaptive mechanisms, uncertainties in onset of stress, and large genotype × environment interaction. A detailed review of progress toward understanding the mechanisms of adaptation to drought, the component traits associated with it, their genetic control, and the genomic regions quantitative trait locus (QTL) associated with such adaptation in barley, maize, pearl millet, rice, sorghum and wheat has been made elsewhere (Dwivedi et al., 2010). Here, only briefly report on current progress to further enhance our understanding of physiological and molecular basis of adaptation to drought and how this knowledge is being employed to develop crops targeting drought-prone areas using conventional and applied genomic tools.

8.2.1.1. Rice

Bernier et al. (2007) detected a major QTL, *qtl12.1* on chromosome 12 in Vandana/Way Rarem mapping population, which contributed 51% phenotypic variance for grain yield under drought over two dry seasons. The drought-susceptible parent, Way Rarem, contributed alleles that confer advantage to improved yield under drought-stressed conditions. Subsequent work on large-scale evaluation of a subset of 100 lines, 50 homozygous for the positive (Way Rarem-derived allele) allele and 50 for the negative (Vandana-derived allele) allele, across locations in the eastern India and the Philippines for 2 years, revealed that relative effect of the QTL on grain yield increased with increasing intensity of drought stress, from having no effect under well-watered conditions to having an additive effect of more than 40% of the trial mean under severe stressed conditions (Bernier et al., 2009).

Guan et al. (2010) characterized a set of 48 rice-pyramiding lines (PLs), obtained through QTL pyramiding (Li and Xu, 2007), and their recurrent parent, IR64, under drought-stress and nonstress conditions. In comparison

to IR64, all PLs had significantly improved adaptation to drought at reproductive stage, while 36 PLs also had significantly improved adaptation to drought at vegetative stage. Seventeen PLs had higher yield than IR64 under drought stress, while the remaining 31 PLs yielded at par with IR64 under irrigated conditions. The characterization of these PLs further revealed that dehydration avoidance, efficient partitioning high harvest index (HI) and drought escape (early flowering) together contributed to their improved adaptation to drought. These results indicate that the selection for yield together with some secondary traits under appropriate type(s) of stress and nonstress conditions similar to the target environments are critically important for improving adaptation to drought without any yield penalty in rice. Adaptation to drought has also been found related to deep root growth and water uptake ability in both upland and lowland rice agroecologies (Gowda et al., 2011). More recently, Gowda et al. (2012) evaluated a set of 20 diverse rice genotypes, identified as Oryza single-nucleotide polymorphism (SNP) panel, for root water uptake ability as a candidate trait for response to drought in greenhouse lysimeter experiments. They detected large genotypic differences in water uptake and plant growth in response to drought. The total water uptake and water uptake rates correlate with relative root length density, especially at depth below 30 cm, which reveals that response to drought by deep root growth, rather than a conservative soil water pattern, is important for lowland rice, with aus rice genotypes showing greatest values for water uptake and root growth. It will be interesting to investigate possible association between root traits and grain yield under drought stress.

Rice cultivars such as Sahbhagi Dhan, Sahod Ulan 1, and Tarharra 1, developed through conventional breeding and selections, have been recently released because of their enhanced adaptation to drought-prone environments of India, the Philippines and Nepal, respectively. They are being disseminated to farmers in drought-prone areas in these countries. These cultivars have shown yield advantage of ~1 t ha^{-1} under stress (Mackill et al., 2012). Recent study on the new rice for Africa (NERICA) rice cultivars, developed by crossing Asian rice (*O. sativa* L.) and African rice (*Oryza glaberrima* Steud.) and released for cultivations in sub-Saharan Africa, show that these NERICA rices do not consistently provide the expected combination of superior yield potential with strong weed-suppressive ability and overall adaptation to low soil fertility conditions usually found in the target regions. The yielding ability of these upland NERICA cultivars tested to date is similar to one of their *O. sativa* parents, WAB56-104, whereas they have inferior ability to suppress weeds to their *Oryza glabarata* parent; and they

also have lower adaptability to low soil fertility than aerobic rice and *aus* cultivars, thus a clear need to further improve the NERICAs (Saito et al., 2012 and references therein). Saito et al. (2012) also identified some aerobic rice and *aus* cultivars with high-yielding ability, strong weed competitiveness, and superior adaptation to low soil fertility, and thus would seem ideal resource for the development of upland interspecific cultivars adapted to sub-Saharan African conditions.

Grain yield under drought is a complex trait, and grain yield heritability in drought-prone environments is relatively low compared to irrigated control (Venuprasad et al., 2007). Gouda et al. (2012) evaluated *O. sativa* IR58025B × *Oryza meridionalis* BC_2F_3 derivatives under water stress to estimate heritability for grain yield and identify physiomorphological traits associated with enhanced adaptation to drought. The broad-sense heritability for grain yield under water stress was 33% compared to 59% under irrigated control. From this evaluation, they identified 20 high-yielding lines that together with 40 randomly selected lines they further evaluated under control (irrigated) and stress conditions to estimate selection response. In addition, they also evaluated 10 lines (five each from high- and low-yielding groups) for root morphological traits. This study revealed that high-yielding lines had recorded maximum root length and root volume compared with checks and low-yielding lines, indicating positive influence of drought avoidance root traits contributing to stress tolerance, and selection for grain yield under stress is an effective strategy in deriving rice lines with enhanced adaptation to drought.

8.2.1.2. Wheat

A technique based on spectral reflectance indices (SRI) has been proposed as a potential methodology to assess canopy biomass, leaf area index, light absorption, photosynthetic activity, and for predicting yield by periodic measurements of reflectance during the plant development in crops (Rudorff and Batista, 1990; Wiegand et al., 1991; Peñuelas, 1998; Reynolds et al., 1999). The most commonly known index for analyzing vegetation is the NDVI (Araus et al., 2001; Osborne et al., 2002; Royo et al., 2003; Gutierrez-Rodriguez et al., 2004; Prasad et al., 2007a,b). Gutierrez-Rodriguez et al. (2010) determined the relationship between the SRI and yield of the spring wheat in irrigated, water-stressed, and high-temperature environments using three and five SRI, respectively; and for this purpose data on vegetative and water indices were collected at booting, heading, and grain-filling stages of the crop in advanced lines in three trials. This study revealed

that two water indices (NWI-1 and NWI-3) consistently provided high correlations with grain yield when the data on the indices at heading and grain-filling stages were combined in all the three trials. Vegetative indices showed inconsistency in their relationship with grain yield. The water indices gave higher genetic correlations and correlated response with grain yield than the vegetative indices. Likewise, there was a strong relationship between grain yield and canopy temperature determined at grain filling. The high-temperature environment showed the strongest association of the SRI with grain yield. It thus is clear that water indices can be used for plant breeding purposes for selecting high-yielding lines in well-irrigated, water-stressed and hot environments; and the canopy temperature could complement this selection.

8.2.1.3. Maize

Farmers' fields in southern Africa are rarely characterized by only one abiotic stress. An open pollinated or hybrid cultivar must combine adaptation to drought and low-N stress, with high yield potential under optimal conditions to become popular among farmers (Bänziger et al., 1999; Kumar et al., 2008). Selection under abiotic stress, optimal, and/or low-N conditions allows identification of broadly adapted genotypes. CGIAR Consortium researchers and partners evaluated large number of elite hybrids under optimal, low-N, and manage drought conditions during the 2001 and 2009 crop seasons to predict the performance under abiotic stress and low-N fertility usually faced by African farmers. This study reveals high genetic correlations between abiotic stress and low-N or optimal conditions. The indirect selection under low-N and optimal conditions is therefore more efficient than direct selection under abiotic stress or indirect selection under managed drought, especially for early maturing genotypes. Direct selection is however most efficient for predicting performance under low N. Elite hybrids tolerant to abiotic stress can be most efficiently selected under optimal or low-N conditions while low-N-tolerant genotypes should be selected directly under low N (Weber et al., 2012).

Conservative water use early in the growing season particularly under high atmospheric VPD is an important adaptive strategy to save water for the later growing season to support grain development. The partial closure of stomata under high VPD decreases the effective average daily VPD for transpiration allowing more efficient use of available water in the later growing season (Sinclair et al., 2005). Yang et al. (2012) studied the response of Tr to changes in atmospheric VPD at two different temperatures in maize

hybrids in a growth chamber. Some hybrids in this study showed limitation of increase in Tr at high VPD above a break point (BP), which could operate through anatomical or physiological features; thus seems an important adaptive strategy to improve adaptation to drought in maize. Further research to better understand the mechanism of transpiration regulation that will assist maize breeding to improve adaptation to drought by incorporating the transcription restriction trait is suggested.

8.2.1.4. Sorghum

The stay-green trait (i.e. reduced leaf senescence) improves tolerance to postflowering moisture stress in sorghum. Various QTL associated with stay-green have been reported, but only a few have a major effect on trait expression in sorghum. The QTL-introgressed lines produced significantly higher grain yield under moderate water stress than irrigated control (Kassahun et al., 2010; Dwivedi et al., 2010 and references therein), with majority of the introgression lines having higher leaf chlorophyll levels at flowering and a greater percentage green-leaf area during the latter part of grain filling (Kassahun et al., 2010). Recently, Jordan et al. (2012) investigated the relationship of the stay-green with grain yield using data from sorghum breeding trials that sampled 1668 unique hybrids and 23 environments in which the mean grain yield varied from 2.3 to 10.5 t ha^{-1} in Australia. The strength and direction of the associations between stay-green and grain yield varied with both environment and genetic background (male tester). The majority of the associations were positive, particularly in environments with yields below 6 t ha^{-1}. As the trial mean increased above 6 t ha^{-1} there was a trend toward an increased number of negative associations. However, the number and magnitude of the positive associations were larger. These results indicate that selection for stay-green in elite sorghum hybrids may be broadly beneficial for increasing yield in a wide range of environments.

8.2.1.5. Pearl Millet

The research to-date suggests that genotypes that flower early possess fewer but effective basal tillers, are low in biomass, and have high HI perform better under terminal drought stress. A major QTL on linkage group 2 is associated with postflowering adaptation to drought in pearl millet (Dwivedi et al., 2010 and references therein). The physiological basis of this QTL for adaptation to drought reveals that water conserving mechanisms, mostly expressed under nonstressed conditions allow water saving in the soil profile during the early crop stage, which is available to the plant during

postanthesis period (Kholová et al., 2010a,b). Recently, Kholová et al. (2012) evaluated RILs involving H77/833-1 (sensitive to terminal drought) and PRLT2/89-33 (tolerant to terminal drought) to map Tr and associated physiological traits. Two alleles each from H77/833-1 and PRLT2/89-33 on linkage group 2 increased Tr, whose importance depended on the VPD. More importantly, the two H77/833-1 and one PRLT2/89-33 alleles in this study comapped to a previously identified major terminal drought-tolerant QTL, while the other *Tr* allele from H77/833-1 enhances biomass dry weight and colocated with a previously identified stover and tillering QTL (Yadav et al., 2002, 2004). Leaf traits were linked to two loci on linkage group 7. Kholová et al. (2012) also detected variable plant water use depending on allelic combinations for Tr, tillering and leaf characteristics, whose importance depended on the environmental conditions. These authors therefore concluded that different alleles influence plant water use, and have close interactions with one another and with the environment, which may be used to design plant ideotypes from specific allele combinations, conferring particular physiological traits for specific adaptation to a range of terminal drought conditions.

8.2.2. Submergence and Phosphorus Deficiency Tolerance in Rice

A large part of the rainfed lowland rice acreage in South and Southeast Asia is affected by submergence of varying degree and duration, resulting into substantial loss to rice production in these regions. FR13A and a few other landraces from India and Sri Lanka have been identified as sources of complete submergence, with FR13A an extremely submergence tolerant. A major QTL on chromosome 9 in FR13A, *Sub1*, accounting for 69% of variation, provides tolerance to complete submergence for up to 2 weeks (Xu and Mackill, 1996). Subsequent work on fine mapping of this QTL delineated *Sub1* to a genomic region of approx. 0.06 cM (Xu et al., 2006). The sequencing of *Sub1* region in an FR13A-derived line revealed the presence of three genes encoding putative ethylene responsive factors, *Sub1A*, *Sub1B* and *Sub1C*, with *Sub1A* identified as the major determinant of submergence tolerance in rice (Xu et al., 2006). Using flanking markers and marker-assisted breeding, researchers have introgressed *Sub1A* into popular high-yielding cultivars from Bangladesh, India, Laos and the Philippines. To-date, a number of submergence-tolerant mega cultivars such as Swarna-Sub1, SambaMahsuri-Sub1, Thadokkam1-Sub1, BR11-Sub1 and IR64-Sub1 have been released in Bangladesh, India, Indonesia, the Philippines and Sri Lanka (Neeraja et al., 2007; Sarkar et al., 2009;

Singh et al., 2009; Septiningsih et al., 2009; Bailey-Serres et al., 2010; Iftekharuddaula et al., 2011). These cultivars with *Sub1* tolerate complete submergence for 2 weeks (Das et al., 2009), whereas a few other germ-plasm could withstand 3 weeks of complete submergence with greater variation in plant height and elongation ability under submergence (Sarkar and Bhattacharjee, 2012). Encouraged with these successes, rice breeders are now using *Sub1* locus to develop submergence-tolerant cultivars for submergence-prone areas in Africa and Asia.

Phosphorus is the second most important plant nutrient only after nitro-gen; and phosphorus availability is limited because applied soluble phos-phorus is converted into insoluble form due to phosphate sorption by soil minerals and or due to reactions to form iron- and aluminum–phosphorus compounds in acid soils, and calcium- and magnesium-phosphorus com-pounds in alkaline soils. The availability of phosphorus is optimum around a neutral pH in soils (Sahrawat et al., 2001; Sparks, 2003). Phosphorus fer-tilizers are manufactured from phosphate rock, a nonrenewable resource, and thus likely to deplete in 50–100 years. The quality of phosphate rock is declining and production cost is increasing. About ~60% of rainfed rice in Asia is grown on soils that are affected by multiple stresses, including P defi-ciency (Haefele and Hijmans, 2007; Sahrawat, 2009). Genotypic differences in rice have been reported for tolerance to phosphorus deficiency (Wissuwa and Ae, 2001a). A major QTL, *Phosphate uptake 1* (*Pup1*) on chromosome 12, confers tolerance to phosphorus (P) deficiency in soil (Ni et al., 1998; Wissuwa et al., 1998, 2002). The evaluation of near isogenic lines (NILs) with and without the *Pup1* QTL showed that *Pup1* increases P uptake (Wissuwa and Ae, 2001b; Wissuwa et al., 2002), confers a significant yield advantage in P-deficient soils and mostly conserved in germplasm or culti-vars adapted to drought-prone environments (Chin et al., 2010). Recently, *Pup1* has been the target for development of tolerant rice cultivars. Chin et al. (2011) introgressed *Pup1* into five cultivars, adapted in irrigated or rainfed agroecology. The phenotypic evaluation of the introgression lines suggest that *Pup1* is effective in different genetic backgrounds and envi-ronments and that it has the potential to significantly enhance grain yield under phosphorus deficient soils. Ismail et al. (2007) indicated that ongoing rice genetic resources and breeding research aims identifying germplasm tolerant to problem soils, dissect the genetics of component traits confer-ring advantage of tolerant germplasm on these soils, fine map the genomic region(s) using gene-based markers, and transfer these beneficial alleles con-ferring tolerance to nutrient deficiency into improved genetic backgrounds.

8.2.3. Adaptation to Drought in Legumes

8.2.3.1. Soybean

Studies in cereals have clearly demonstrated that a limitation on Tr at high VPD allow soil water conservation during vegetative growth stage that the plants uses during the postanthesis period to maximize yield under moisture stress conditions (see Section 8.2.1). Using a simple experimental system in which whole plant Tr could be measured while subjecting plants to a wide range of VPD conditions, Fletcher et al. (2007) identified a slow-wilting soybean germplasm PI 416937 with a two-segment Tr response to VPD; i.e. above a BP of ~2 kPa, Tr remains constant. Further study on this unique response of PI 416937 to increasing VPD revealed that there is a BP in this genotype at about 2 kPa and that the limitation on Tr at higher VPD is linked to low hydraulic conductance at the leaf level, which is absent in genotypes that had no BP in Tr in response to VPD (Sinclair et al., 2008). Sadok and Sinclair (2009) assessed Tr response in soybean genotypes having wide genetic base and identified additional two genotypes (N73-1102 and NTCPR94-5157) as expressing a BP in Tr response to VPD. More importantly, these two genotypes displaying the two-segment Tr response to VPD are not derived from PI 416937; however, these showed a similar BP value (approximately 2 kPa) as of PI 416937. Recently, Sinclair et al. (2010) used a relatively simple, mechanistic soybean growth and yield model (Sinclair, 1986) and 50 years of weather data for 2655 US grid locations of 30 km by 30 km size to assess the yield response to modification of root depth, rate of leaf area development, decreased stomata conductance at high soil water content, reduced maximum Tr, and drought-tolerant nitrogen fixation. The simulation results reveal that both water conservation during early growth decreases stomata conductance with soil drying and by reducing the maximum Tr: i.e. slow-wilting phenotype, resulted in increased yield, with high probability of yield gains for much of the soybean production areas in 70% or more of the years. The genetic material carrying slow-wilting trait and early stomata closure thus have the potential to improve germplasm for yield beyond the current levels observed in breeding programs for much of the soybean production. Further, the drought-tolerant nitrogen fixation had the greatest benefit of all traits with yield gain in more than 85% of the years at almost all locations in soybean, which should also be incorporated along with slow wilting and early stomata closure into soybean improvement programs. Clearly, identification of these germplasm with water saving adaptive trait is an ideal genetic resource for enhancing adaptation to drought in soybean.

8.2.3.2. Peanut

Several sources of adaptation to mid- and/or end-of-season terminal drought, both in subspecies *hypogaea* and *fastigiata*, have been reported in peanut, which showed variation for physiological traits such as specific leaf area, chlorophyll content, amount of water transpired (T), transpiration efficiency (TE), WUE, and HI under drought-stress conditions (Upadhyaya, 2005; Dwivedi et al., 2007b; references therein; Jyostna Devi et al., 2009a). Most of the peanut breeding programs follow an empirical approach, which was largely based on pod yield as selection criterion for adaptation to drought, resulting thereby in slow progress. Nigam et al. (2005) were the first to evaluate the relative efficiency of physiological trait-based selection vis-à-vis empirical selection for adaptation to drought in peanut. The physiological trait-based method did not show a consistent superiority over the empirical method of breeding for drought adaptation for producing high kernel yield in peanut. Nonetheless, the integration of physiological traits (or their surrogates) in the selection scheme would be advantageous in selecting genotypes that utilize water more efficiently and partition the photosynthates more effectively into economic yield. When evaluated for variation in TE under progressive soil drying conditions, Jyostna Devi et al. (2009a) detected no differences in TE among 17 genotypes under well-watered conditions. However, under soil drying conditions there were substantial differences among genotypes. This indicates that TE with drying soil might interact with traits associated with water loss on drying soils. The genotypes showed large variability for fraction transpirable soil water (FTSW) content (0.22–0.71), and a decline in transpiration with soil drying. This difference in FTSW in peanut is much greater than those reported for other plant species (Jyostna Devi et al., 2009a and the references cited therein). The existence of a break point in the Tr at the elevated VPD offers the opportunity to directly influence crop transpiration use efficiency under field conditions. Jyostna Devi et al. (2010) evaluated 17 tolerant germplasm with large variation in TE and detected genotypic variation in Tr in response to high VPD. Nine of these genotypes showed a BP of about 2.2 kPa, above which there was little or no further BP increase in Tr, which reveals that these genotypes with a BP have the potential to soil water conservation when VPD exceeded 2.2 kPa. This trait will be useful in terminal drought conditions as genotypes with a BP will be able to make use of conserved water to generate a greater yield than genotypes without the BP. It should be noted that decrease in stomata closure associated with decreased Tr would adversely affect photosynthesis. However, this loss may be offset by water savings in the soil due to rising VPD for use later

in the season, resulting greater yield. Furthermore, the identification of few major, many minor and epistatic QTL reveals that the adaptation to drought is complex and multigenic in peanut (Ravi et al., 2011).

8.2.3.3. Chickpea

Chickpea is among the most studied legume crop for response to drought. Adaptation to drought has been found associated with variation in drought avoidance root traits (root length density, root to total dry plant weight ratio, root depth, and root to shoot length density), TE, carbon isotope discrimination ($\delta^{13}C$), Soil plant analysis development (SPAD) chlorophyll meter reading (SCMR), and canopy temperature (Kashiwagi et al., 2005, 2006, 2008, 2010). Drought escape as a result of early maturity is another important adaptive mechanism, which has opened up new possibilities for growing chickpea in semiarid and arid regions globally (Kumar and Abbo, 2001). Conservative use of water at vegetative stage saves water for use by the crop during the reproductive stage. Zaman-Allah et al. (2011a) evaluated 20 chickpea genotypes with similar phenology but differing in response to terminal drought stress. The pattern of water extraction in their study clearly discriminated tolerant and sensitive genotypes. Tolerant genotypes had a lower water uptake and a lower index of stomatal conductance at vegetative stage than the sensitive ones, while genotypes with enhanced adaptation to drought extracted more water than sensitive genotypes after flowering. Further, the magnitude of the variation in root traits did not distinguish the tolerant from the sensitive genotypes. The seed yield was not significantly correlated with root traits, whereas seed yield was negatively related to water uptake between 23 and 38 days after sowing (DAS), and positively related to water uptake between 48 and 61 DAS. Zaman-Allah et al. (2011a) therefore concluded that under terminal drought conditions, conservative use of water early in the cropping cycle is the most critical component of adaptation in chickpea, partly as a result of a lower canopy conductance, which resulted in more water available in the soil profile during reproduction leading to higher reproductive success. Zaman-Allah et al. (2011b) further tested whether plant water use at the vegetative stage and under no-limiting water conditions could relate to the degree of sensitivity of chickpea to terminal drought. They found that the genotypes with enhanced adaptation to drought had a lower canopy conductance under fully irrigated conditions at vegetative stage, while the trend reversed at the early pod-filling stage. The sensitive genotypes had high early growth vigor and leaf development, while the genotypes adapted to drought had

lower growth under progressive soil drying. The genotypes with enhanced adaptation to drought also exhibited decreased transpiration in wetter soil compared to sensitive genotypes. These results suggest that some traits contribute to water saving when water does not limit plant growth and development in chickpea with enhanced adaptation to drought. It is encouraging to know that chickpea breeders are now using these traits to enhance performance in drought-prone environments. Of recent, a large number of chickpea germplasm with enhanced adaptation to terminal drought and heat stress have been reported in literature (Kashiwagi et al., 2005, 2006, 2008, 2010; Krishnamurthy et al., 2010; Upadhyaya et al., 2011d; Zaman-Allah et al., 2011a,b). Chickpea genomics research has advanced considerably with the availability of large number of microsatellites and SNP (Varshney et al., 2007, 2009a; Nayak et al., 2010), fairly covered genetic maps (Millan et al., 2010; Nayak et al., 2010), and a large number of Expressed sequence tag (EST) resources involved in responses to drought and salinity (Varsheny et al., 2009b). Molecular biologists are working with crop physiologists and plant breeders to identify genomic regions associated with enhanced adaptation to both drought and salinity. Their research success will speed up chickpea breeding for stressful environments.

8.2.3.4. Cowpea

The high productivity of some of the cowpea landraces such as DWDCC 015, DWDCC 006 and DWDCC 001 reflects the ability of these landraces to tolerate and respond to a wide range of temperature- and moisture-stress conditions (Hegde and Mishra, 2009). These and other germplasm with enhanced adaptation to drought and heat produce more pods and seeds plant^{-1}, grain yield plant^{-1} and show high HI (Ehlers and Hall, 1998; Hall, 1992, 2004; Hegde and Mishra, 2009). Breeding efforts have been successful toward developing several cultivars that have been released globally (Cisse et al., 1995, 1998; Ehlers et al., 2000; Ismail et al., 2000; Elawad and Hall, 2002; Hall et al., 2003; Hall, 2004). More recently, genotypic differences in water-saving traits: i.e. lower Tr under no stress and restricted Tr under high VPD offer opportunities to enhance drought adaptation in cowpea (Belko et al., 2012). Delayed leaf senescence in IT93K–503-1 and IT98K–499-39 is associated with adaptation to drought (Muchero et al., 2008). Muchero et al. (2009) evaluated RIL populations, derived from IT93K503-1 (showing enhanced adaptation to drought) and CB 46 (susceptible), in the greenhouse and fields. They detected 10 QTL associated with seedling drought-induced senescence, mapped on linkage groups

1, 2, 3, 5, 6, 7, 9, 10, and with each contributing between 5% and 12% to the phenotypic variance. The stability of QTL expression across time and environment in their study further suggests that the genomic regions harboring the identified QTL carry genes that are of major importance in determining cowpea response to drought. The drought-induced senescence QTL (except *Dro-8*) was independent from eight QTL for maturity on linkage groups 7 and 8. This finding suggests that it should be possible to pyramid early maturity with delayed drought-induced senescence to manage both early and late-season drought in cowpea. Interestingly, several RILs in this study expressed combined traits of early maturity with delayed drought-induced senescence.

8.2.3.5.   Common Bean

Common bean has two major gene pools—Andean and Mesoamerican—each with three races: Chile, Nueva Grenada and Peru in the former and Durango, Jalisco and Mesoamerica in the latter (Beebe et al., 2000). Sources and plant traits associated with enhanced adaptation to drought in common bean have been identified in both the Mesoamerican and Andean gene pools (Acosta-Gallegos and Kohashi-Sibata, 1989; Acosta-Gallegos and Adams, 1991; Acosta-Gallegos and White, 1995; Singh, 1995; Ramirez-Vallejo and Kelly, 1998; Terán and Singh, 2002; Muñoz-Perea et al., 2006). Traits that confer enhanced adaptation to drought in common bean include a deep rooting system with appropriate architecture that increases extraction of soil moisture from a greater soil depth; maximization of WUE for photosynthesis, growth and development; greater photosynthates transport to seed under stress through efficient remobilization; early maturity; and recovery from drought. Several germplasm and breeding lines possessing a combination of these traits have been either identified from gene pools or developed by crossing and selection in many bean breeding programs around the world (Frahm et al., 2004; Micklas et al., 2004; references therein; Beebe et al., 2008; Acosta-Diaz et al., 2009; Asfaw et al., 2012a). As is the case with peanut (Nigam et al., 2005), seed yield (empirical selection) shows greater genotype × environment interaction than functional traits such as the transpiration, WUE, and HI. However, the negative correlation between these traits may limit genetic progress using trait-based selection (Dowkiw et al., 2000). Some recently bred lines from the CGIAR Consortium outyielded the commercial checks by 15–25% under favorable environments or up to 36% under drought stress. Some of these lines also produced more grains in phosphorus-limited environments, which indicate

the potential to combining traits enhancing adaptation to drought and tolerance to phosphorus deficiency in common bean (Beebe et al., 2008). Enhanced adaptation to drought is multigenic in common bean and adoption of recurrent selection is an effective breeding strategy (Beebe et al., 2008). Few QTL associated with enhanced adaptation to drought has been identified (Schneider et al., 1997; Blair et al., 2012). Improved photosynthate acquisition, accumulation, and remobilization have been observed as important mechanisms for adaptation to drought stress. Asfaw et al. (2012b) evaluated RILs, derived from Mesoamerican intragene pool cross, under eight environments differing in drought stress in Africa and South America. They mapped nine QTL for 10 drought-tolerant traits on six of the 11 linkage groups, with six QTL showing significant QTL × environment interaction. The QTL for SCMR and pod partitioning traits the most stable across environments, which may be used to enhance drought adaptation in common bean. The common bean genome sequence, when it becomes available, will provide a major resource to discover genomic regions differentiating stress-responsive and non–stress-responsive genotypes, and for highlighting specific molecular-based stress responses, which will assist on the selection of parental lines to improve the efficiency of common bean improvement programs (McClean et al., 2011).

8.2.4. Salinity Tolerance in Cereals and Legumes

Rice, wheat and barley, among the cereal crops, have been most extensively studied to dissect the physiological and genetic basis of salt tolerance and the knowledge gained from such studies may help to develop salt-tolerant cultivars. Essentially, the salt tolerance in these crops is sodium exclusion, which limits the entry of sodium into the plants and its transport to the leaves, resulting in relatively higher K^+/Na^+ ratio in the plant. Although genetic variation for salt tolerance has been reported in almost all major cereal crops, its genetic basis and genomic regions associated with salinity tolerance have been discovered only in rice, wheat and barley. Further, a major salt-tolerance QTL (*SKC1*) on rice chromosome 1 has been cloned, which expresses in the parenchyma cells surrounding the xylem vessels. *SKC1* encodes a sodium transporter involved in regulating K^+/Na^+ homoeostasis under salt stress (Dwivedi et al., 2010 and references therein). *Saltol* (located on chromosome 1) has been the major QTL for marker-assisted selection in rice (Thomson et al., 2010). Targeted breeding for salt tolerance led to the release of few salt-tolerant rice and wheat cultivars in salt-affected areas of Egypt, India, Indonesia, Pakistan,

the Philippines, Thailand, the United Kingdom, and Vietnam (Dwivedi et al., 2010 and references therein).

Legumes unlike cereals have received less attention to identifying and understanding the physiological and genetic basis of salinity tolerance. Soybean is probably the most extensively studied legume crop for salinity tolerance. Sources of resistance both in cultivated soybean and its wild progenitors have been identified (Parker et al., 1983; Xu et al., 1999; Li et al., 2000; Hamwieh and Xu, 2008), and the physiological basis of salt tolerance is fairly known (Luo et al., 2005; Phang et al., 2008). Essentially, salt tolerance includes maintaining ion homeostasis by withholding toxic ions from sensitive aerial parts, adjusting osmotic potential in cell by accumulating metabolites, and restoring oxidative balance to prevent further damage due to excess accumulation of reactive oxygen species (ROS). Furthermore, in some genotypes, as is the case with soybean WF 7, multiple mechanisms in part operate (Abscisic acid (ABA)–dependent pathway, involvement of ROS and withholding toxic Cl^- ions from leaves) to confer salt tolerance (Ren et al., 2012). The salt tolerance in *Glycine soja* (soybean progenitor) is primarily from exclusion of sodium ions preventing accumulation at the toxic concentrations in stems and leaves. This difference in tolerance mechanisms in *Glycine max* and *G. soja* indicates that interspecific crosses between these two species offer the possibility to improve salt tolerance in soybean cultivars. Furthermore, the major QTL for salt tolerance have been mapped on linkage group N and validated across environments and genetic populations (Lee et al., 2004, 2009; Hamwieh et al., 2011). More importantly, recent research indicates that the salt-tolerant QTL is conserved in both wild and cultivated soybeans (Hamwieh and Xu, 2008). The strong relationship between the microsatellite alleles and salt tolerance suggests that these markers could be used for marker-assisted selection in soybean breeding. Very recently, salinity-tolerant germplasm in chickpea, pigeonpea and peanut were identified and they provide means for researching the physiological basis of salt tolerance in these crops. For example, reproductive stage in chickpea is most sensitive to salinity and tolerance is not related to the shoot Na^+ or K^+ but ability to maintain a large number of filled pods under saline conditions (Vadez et al., 2007a; Krishnamurthy et al., 2011). Clearly, more such studies are needed to better understand the component traits and the genomic regions associated with these traits conferring salt tolerance in major legume crops.

8.2.5. *Biofortification to Enhancing Nutritional Quality of Food Crops*

Micronutrient malnutrition arising from Fe, Zn and vitamin A deficiencies affects billions of people around the world (http://www.unscn.org).

Widespread micronutrient malnutrition results in an enormous negative socioeconomic impact at the individual, community, and national levels (Darnton-Hill et al., 2005; Stein, 2010). More importantly, the rise in atmospheric CO_2 and temperature, as a result of climate change and variability, has been found associated with declining nutritional value of food crops (Section 2). Crop biofortification is a sustainable and cost-effective strategy to address malnutrition in developing countries. Several reviews on crop biofortification as a strategy have been published (see Dwivedi et al. 2012 for the most recent one). Plant breeders have used available natural variations for nutritional traits for developing nutrient-dense cultivars for some cereal and legume crops. To date, a few nutrient-dense cultivars have already been released for cultivation in some regions, e.g. seed iron-dense common bean and rice in Latin America; seed iron-dense common bean in eastern and southern Africa; and seed iron-dense rice in the Philippines. There are more nutritionally enhanced lines in the release pipeline. Temperate maize germplasm have shown exceptionally large variations in β-carotene, which have been transferred into tropical maize hybrids. They are being evaluated prior to their release in Mexico and in some countries in Africa. The high β-carotene trait in Golden Rice 2 is being introgressed into several Asian rice cultivars. Molecular markers for *LycE* and *HydB* linked to increased β-carotene have been fully implemented in some maize breeding programs, which has accelerated breeding by one season and substantially enhance efficiency and effectiveness of high-provitamin A maize breeding. Marker-assisted selection has been successfully employed to transfer low phytate into improved soybean cultivars. Enhancing nutritional quality of food crops through transgene(s) is also underway for select crops and nutrients. Biofortified crops are also being investigated for efficacy with human and animal systems. Biofortification has been included as core breeding activity in some countries in Latin America to ensure that newly developed crop cultivars meet nutritional needs of humans (Dwivedi et al., 2012 and references therein). Clearly, more efforts are needed to strengthen crop biofortification as a strategy to develop nutritionally dense cultivars for the major food crops worldwide.

8.3. Genetically Modified Crops Tolerant to Abiotic Stresses

Recently, Dwivedi et al. (2010) provided a detailed account of the progress realized through genetic modification for developing putative transgenic plants in barley, maize, rice and wheat, which showed an improved adaptation to drought, salinity or extreme temperature. A number of genes including

DREB and its homolog, *HVA1*, *ZmPLC1*, *Sod1*, *HARDY*, *OsNAC6*, *CMO*, *SsNHX1*, and *AtAVP1*, to name a few, have been used to develop transgenic events with improved adaptation to drought, heat or salinity stress (Dwivedi et al., 2010). Isopentenyltransferase (IPT) is a critical enzyme in the cytokinin biosynthetic pathway. The expression of *IPT* under the control of a maturation- and stress-induced promoter delays stress-induced plant senescence, which results in an enhanced adaptation to drought in both monocot and dicot plants (Rivero et al., 2007, 2009, 2010). The transgenic rice plants expressing P_{SARK}::*IPT* gene showed enhanced drought adaptation and significantly higher grain yield with improved quality (nutrients and starch content) when compared to wild types (Peleg et al., 2011).

Pellegrineschi et al. (2004) were probably the first to generate and evaluate in a greenhouse *DREB1A*–wheat transgenic events with enhanced survival to severe drought stress at the 4–5 leaf stage. The selected transgenic wheat lines showed water stress symptoms later than the control. More recently, Saint Pierre et al. (2012) evaluated 14 of these selected transgenic lines (five with high WUE, WUE-11 to WUE-14, and nine that survived severe water-stress conditions in previous evaluation) for biomass and WUE under greenhouse conditions and for survival to severe water deficit in the field conditions. Their results revealed that *AtDREB1A* driven by the stress-inducible promoter rd29A increased the survival rate of transgenic plants without growth retardation. They also note a positive association between WUE and biomass, which suggests that an increase in grain yield may be possible by increasing WUE in transgenic plants (assuming that the HI is maintained). The selected events, due to their WUE in greenhouse screens, were found to combine an acceptable yield—and even higher yield for WUE11 under well-watered condition—and stable performance, without any pleiotropic effects, across the environments in their field experiment. Taken together, it is encouraging to be optimistic that the goal of high-yielding wheat transgenic lines would be achievable in near future if adequate transformation and screening protocols are implemented.

Bhatnagar–Mathur et al. (2007, 2009a,b) introduced *P5CSF129A* in chickpea and *DREB1A* in peanut. Both are driven by the stress–inducible promoter *rd29A*. The transgenic plants overexpressing *P5CSF129A* or *DREB1A* showed substantial increase in TE in the greenhouse (Bhatnagar–Mathur et al. 2009a,b). Root traits are an important source of variation and provide mechanisms to enhance adaptation to drought in plants. Vadez et al. (2008) found that transgenic plants overexpressing *DREB1A* had enhanced root growth under water-deficit conditions,

particularly in the deep soil layers. Some transgenic events had enhanced water uptake under water-deficit conditions up to 20–30% compared with wild types, and they were highly correlated ($r^2 = 0.91$) with root dry weight below the 40 cm soil depth in the profile (Vadez et al., 2007b). Jyostna Devi et al. (2009b) noted that high TE in some transgenic events, containing *rd29A*:*DREB1A* construct, under drought-stressed conditions were significantly correlated with SCMR. Furthermore, they detected a significant negative correlation between TE and FTSW threshold values where transpiration declines upon soil drying, which led to higher TE.

The regulation of P_{SARK}::*IPT* in peanut significantly improved drought tolerance under both laboratory and field conditions. The transgenic plants did not only maintain higher photosynthetic rates, higher stomatal conductance, and higher transpiration than wild-type (WT) plants under reduced irrigation conditions, but also produced significantly higher yield (58% more seeds on average based on 2 years data) than WT plants in the field, indicating a great potential for the development of crops with improved performance and yield in the water-limited areas of the world (Qin et al., 2011).

Tissue tolerance (to accumulate Na^+ or Cl^-) is an important adaptive mechanism in plants to salinity (Munns and Tester, 2008). Membrane proteins, such as vacuole Na^+/H^+ antiporters (NHX), play key roles in tissue tolerance to Na^+ in vacuoles in plants (Apse et al., 1999; Blumwald et al., 2000). For example, overexpression of *OsNHX1* and *TaNHX 2* in rice and wheat, respectively, confers high salt tolerance to transgenic plants (Chen et al., 2007; Jian et al., 2009). Recently, Cao et al. (2011) transformed a *TaNHX 2* gene into soybean and the homozygous transgenic lines overexpressing *TaNHX 2* showed enhanced salt tolerance, as measured by plant biomass and flowers plant^{-1}, compared to WT plants grown on sand culture containing 150 mM NaCl. The transgenic line, C12-11 showed longer survival, less growth inhibition and greater number of flowers than wild types, indicating that overexpression of *TaNHX 2* could enhance salt tolerance in soybean.

In summary, crop germplasm enhancement supports applied plant breeding in the form of providing intermediate genetic materials with beneficial traits for cultivar development. CWR contributed many beneficial alleles to broaden the genetic base of food crops. Resynthesized lines contributed rich source of variation for enhancing resistance to abiotic and biotic stresses in wheat. These amphiploids are expected to release new source of variation for use in genetic improvement of peanut. There is increasing emphasis to develop marker-aided introgression of novel alleles from exotic germplasm onto the

background of elite crop cultivars to enhance the trait value for use in crop improvement. Sources of resistance and genomic regions (QTLs) associated with abiotic stresses have been identified, both in cereal and legume crops. Few QTLs with major effect for drought, salinity and submergence tolerance have been mapped or cloned. The physiological basis of drought tolerance has been dissected into component traits such as drought escape (early maturity), drought avoidance root traits, stay-green QTL, and conservative use of water under high VPD as an important adaptive strategy for adaptation to drought environments; all these traits have potential to design plant ideotypes from specific allele combinations conferring particular physiological traits for specific adaptation to a range of terminal drought conditions. A major QTL for submergence (*Sub1*) tolerance has been introgressed and the products of such introgressions have been recently released for cultivation in some Asian countries. The major QTL for phosphorus deficiency (*Pup1*) has been the target for the development of tolerant rice cultivars. The NILs containing *Pup1*, mostly conserved in rice germplasm and cultivars adapted to drought-prone environments, conferred significant yield advantage in P-deficient soils. Likewise, a major QTL for salt tolerance (*Saltol*) is being introgressed into rice cultivars. Targeted breeding for drought and salinity tolerance has led to the development and release of several cultivars resistance to these stresses in chickpea, common bean, cowpea, maize, peanut, pearl millet, rice, sorghum, and soybean in some African and Asian countries. A number of genes including *DREB* and its homologs have been used to develop transgenic events with improved adaptation to drought, heat or salinity stress in chickpea, maize, peanut, rice, and wheat, with many events showing no adverse impact on agronomic traits. Global warming is also associated with declining nutritional quality of food crops (see Section 2). Plant breeders have also succeeded in developing nutrient-dense cultivars in common bean, maize, and, rice that have been released in some countries, while many nutritionally enhanced lines in the release pipeline. The high β-carotene trait in "Golden Rice 2" is being introgressed into several Asian rice cultivars. Enhancing nutritional quality of food crops through transgene(s) is also underway, particularly for Fe and β-carotene in rice grains.

9. OUTLOOK

The information available especially from the IPCC reports, and climate models predict that some regions of the world, especially in developing world, will be greatly adversely affected due to global warming, thereby

poising of threat to both food production and quality for human nutrition and feed for livestock. The crop produce could be likely less nutritious, thereby spreading more malnutrition in the developing world. Research to-date suggests that drought, heat and elevated CO_2 reduce food and feed quality. However, genotypic differences with respect to grain-quality attribute under drought and heat stress do provide researchers opportunities to identify crop germplasm or cultivars with lowest differences in grain quality under stress. Such germplasm are the ideal sources for use in crop improvement programs, and to breed cultivars with no adverse effect of global warming on grain quality. To support the development of nutritious crops, a number of novel high-throughput assays are now available to facilitate screening of a large numbers of grain samples for seed composition and quality. The feasibility for breeding seed-nutrient-dense biofortified crops has been shown in crops such as bean, maize, pearl millet, rice, and wheat (Dwivedi et al., 2012). However, a paradigm shift is needed to include biofortification in core breeding programs to assure that no crop cultivars that do not meet the minimum quality attributes are released for cultivation. Another important research area will be to assess how global warming may affect the bioavailability of plant nutrients?

The threat to altered pest and disease dynamics will adversely impact agricultural production, while increased risk of mycotoxin contamination will make the produce unsafe for human and livestock consumption. The current knowledge about the impact of global warming on pests and pathogens affecting agricultural crops is highly limited. We call upon researchers to generate more empirical data on the host–pathogen or host–insect biology under emerging climates as the emergence of more aggressive pathotypes (or biotypes) may reduce effectiveness of host-plant resistances, leading to substantial loss to production. There is need to develop crop–pest (pathogen) models that predict potential geographic distribution, seasonal phenology and population dynamics at a range of spatial and temporal scale, which will provide researchers opportunity to design sound plant health management practices for pathogens and pests.

The use of geostatistics, GIS and satellite imagery data are adding to our knowledge to predict and monitor shifts in community structure of the aflatoxin-producing fungi *A. flavus*. Limited studies on use of long-term weather and crop yields data together with crop models have made it possible to predict the risk of aflatoxin contamination in agricultural produce. The NARCCAP database (www.narcap.ucar.edu), which provides ranges of climatic conditions, offers opportunities to assess how future climate

scenarios may affect aflatoxin levels around the world. Cost-effective assays such as cELISA and Afla-ELISA are providing unique opportunity to evaluate food, feed and related commodities for aflatoxin contamination. The water activity (a_w), temperature, and their interactions with aflatoxin gene cluster significantly impact the fungal growth and toxin production. The mycotoxin biosynthesis gene-specific microarray provides researchers opportunities to identify food-borne fungi, the most common mycotoxins, and investigate the influence of environmental parameters and their inter-actions to predict and identify mycotoxin biosynthesis genes, and adopt a system-based approach to control mycotoxin contamination in food crops. Atoxigenic strains of *A. flavus* can be used as biopesticide because they provide an effective control and minimize risk of aflatoxin contamination in cotton, maize and peanut. Efforts should be directed toward identifying locally adapted atoxigenic strains competitive enough to replace naturally occurring toxigenic strains. The use of the biocontrol agent "Aflasafe" (based on an atoxigenic strain) is gaining momentum and large-scale adoption tri-als are underway to assess its efficacy to minimize aflatoxin contamination in maize in Nigeria. This approach is also being experimented using locally adapted atoxigenic strains to control aflatoxin contamination in maize in Kenya and the Republic of Benin. We envisage that this biocontrol approach will find wider acceptance and together with resistant cultivars and other management options will provide effective measures to minimize the risk of aflatoxin from agricultural produce in developing world.

Agrobiodiversity is crucial to coping with adverse impact of global warm-ing on food production and quality. The most threatened plants are CWR, and every effort should be made for their in situ conservation in protected areas to ensure in situ evolutionary process of wild species contributing new variants, which contribute to addressing new challenges to agricultural production. Taping new variants for phenological traits as a result of global warming is another window of opportunity to address for life-history traits for changes in frequency of alleles favoring adaptation to global warming, as evidenced for shift in early flowering alleles at *PHYC* locus and reduction in plant and spike size in pearl millet in Sahel (Vigouroux et al., 2011). It is understood that soil fertility will be greatly impacted by increase in both atmospheric CO_2 concentration and temperature. While increase in CO_2 concentration will mean opportunity for enhanced photosynthetic rate, the growing plants will not be able to take advantage of this unless the photo-synthesis-driven increased nutrient requirement is met through balanced and integrated nutrient management strategy. Soil microbial population,

especially microbial structure diversity will play a critical role in this effort. Increased temperature will influence soil fertility and nutrient availability and uptake by its influence on soil water (would decrease soil water content and duration) and organic matter decomposition (decomposition rate would increase provided soil moisture is available).

The genetic base of many of our food crops is narrow because of the bottlenecks associated with domestication of these species. Germplasm enhancement contributes to broadening the total genetic diversity in crops, and provides specific traits to plant breeders. The evidence to-date in peanut, rice, tomato and wheat suggests that CWR (that could be the main source of genetic variation for some crops) often provide alleles that enhance the trait value in crop cultigens. It is suggested that CWR and other exotic germplasm should be integrated in breeding programs to develop intermediate breeding products such as introgression lines with specific characteristics, not present in cultigens gene pool, to support crops breeding. The crop genetic enhancers to-date have made considerable progress toward identifying sources of resistance and genomic regions (QTLs) associated with abiotic stresses, that when transferred into improved genetic background contributed to enhance adaptation of newly developed germplasm to these stresses. Targeted breeding (including use of applied genomic tools) for enhanced adaptation has led to the development and release of cultivars of chickpea, common bean, cowpea, maize, peanut, pearl millet, rice, sorghum, and soybean with enhanced adaptation to drought, salinity and flooding in some African and Asian countries. Furthermore, plant response to these abiotic stresses in many cases has been dissected at physiological scale, and many component traits contributing enhanced adaptation have been identified, which plant breeders are using to develop new germplasm with specific characteristics. Use of transgene(s) to support conventional breeding has led to the development of transgenic events with improved adaptation to abiotic stresses, in some cases with no adverse effects on agronomic traits in chickpea, maize, peanut, rice and wheat. These transgenic events are in various stages of evaluation and characterization and when released will be a valuable resource either as cultivars or use as source materials to transfer these transgene(s) into locally adapted cultivars enhancing their adaptation to abiotic stresses.

Climate change analogs today provide a view of tomorrow's agriculture. They offer a virtual natural laboratory to innovate and test various technological options to develop climate resilience production systems for large-scale adoption by the farming community. The available information generated through this approach may also provide researchers an

opportunity to establish "atlas of climate sensitivities" for multiple crops in various regions to assist growers adopt a"cafeteria approach"to mitigate the adverse effects of climate change and variability.

Plant phenomics offers a suit of new technologies for accelerated progress in breeding crop germplasm with specific attributes, and to diagnose nutrient and environmental stresses to adapt appropriate crop management options to ameliorate these stresses in commercial agriculture.Various automatic high-throughput phenotyping platforms are available to accurately measure plant growth and development.The response to abiotic stresses can be dissected at physiological levels that will facilitate to accurately determine its genetic control, thus, providing new opportunities to enhance our knowledge of plant response to abiotic stresses, which may help develop improved germplasm with specific attributes. Such a rapid development in high-throughput assays allows researchers phenotype and genotype large sets of individuals. Linking this large data is a major issue for basic and applied genomic-centric biological research. Developing an appropriate platform that efficiently stores, adds, retrieves, and or modifies, queries it, and analyzes these datasets is needed; and use of "ChadoNatural Diversity" module (http://gmod.org/wiki/Chado_Natural_Diversity_Module) is recommended as it has inbuilt mechanisms to store large-scale phenotyping, genotyping and breeding data.

It is evident from the forgoing discussion that no one solution will suffice to fight climate change and variability effects. Suits of technological innovations including nutritionally enhanced climate-resilient crop cultivars will be needed to fight adverse impact of climate change variability on rural peasant lives, which will help produce food to feed the 9 billion mouths.

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