Chittaranjan Kole *Editor*

Genomics and Breeding for Climate-Resilient Crops

Vol. 1 Concepts and Strategies

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Dedicated to
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Foreword by M. S. Swaminathan

I am very happy that Prof. Chittaranjan Kole and other eminent authors have prepared two books on Genomics and Breeding for Climate-Resilient Crops. These are timely publications since climate-smart agriculture is the need of the hour. Many of the crops formerly known as coarse cereals are both very nutrient rich and climate smart. It would therefore be more appropriate to refer to them as climate-smart nutricereals. In this connection I give below the views I expressed in an editorial which I wrote for *Science* under the title "Gene Banks for a Warming Planet" (Swaminathan 2009).

"At the International Congress of Genetics in New Delhi in 1983, I stressed the need for a conservation continuum, beginning with the revitalization of conservation of domesticated plants by farm families in all countries, and extending to the establishment of an international genetic resource repository maintained under permafrost conditions. Since then, thanks to the spread of participatory breeding and knowledge-management systems involving scientists and local communities, on-farm conservation and gene banks have become integral parts of national biodiversity conservation strategies. For example, there are now over 125,000 genetic strains of rice, of which over 100,000 are in a cryogenic gene bank maintained by the International Rice Research Institute (IRRI) in the Philippines. This gene pool is invaluable for adapting one of the world's most important cereal grains to the consequences of global climate change.

We now largely depend on a few crops such as rice, wheat, corn, soybeans, and potatoes to sustain global food systems. However, their genetic homogeneity increases their vulnerability to abiotic and biotic stresses. If their production is affected by a natural calamity, their prices will increase and food-deficient countries are likely to face riots and worse. Important publications such as *Lost Crops of the Incas* and *Lost Crops of Africa* document the historic role of agrobiodiversity in ensuring food and health security. It has therefore become an urgent task to save vanishing 'orphan crops.' We also know that millets, tubers, and grain legumes are rich in micronutrients but require less irrigation than the major crops. These plants and others are also sources of genes that confer tolerance to drought, floods, and the increased salinity of soils.

Although plant conservation on farms and in the wild is the ideal approach to preserving genetic diversity in crop plants, these methods are constantly jeopardized by invasive species, human destruction of habitat, and market factors. Therefore, other preservation strategies become essential. There are many cryogenic gene banks around the world resembling that at IRRI, but each is very expensive to maintain. Now, thanks to an initiative of the Government of Norway and the Global Biodiversity Trust that began in 2007, the Svalbard Gene Vault located near the North Pole will conserve over four million accessions without the need for expensive cryogenics. The remote isolation and capacity of this facility should be sufficient to preserve a sample of the existing genetic variability of all economically important plants, a vast resource generated over the past 10,000 years of agricultural evolution."

Mahatma Gandhi used to say that "nature provides for everybody's need, but not for everyone's greed." Thus, we find in nature halophytes which are salinity tolerant, xerophytes, which are drought resistant, and many other crops adapted to different agroecological conditions. We should conserve this genetic wealth of inestimable value. We should also promote anticipatory research in order to learn how to scientifically checkmate the adverse impact of unfavorable weather. This book provides guidelines for such work.

I thank Prof. Chittaranjan Kole for this labor of love in the cause of sustainable food security. I also congratulate all his authors. I hope these books will help to make life better for people everywhere.

Prof. M. S. Swaminathan
Member of Parliament (Rajya Sabha),
India and Emeritus Chairman,
M S Swaminathan Research Foundation

Reference

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Foreword by Loren H. Rieseberg

In response to the remarkable rise of food prices in 2008, *The Economist* published an article titled, "Malthus, the false prophet: The pessimistic parson and early political economist remains as wrong as ever." The authors argue that neo-Malthusian worries about our ability to feed 9.2 billion people in 2050 are mistaken, and that advances in agricultural productivity will be sufficient to feed the world.

I am less optimistic. Growth in crop yields has been decelerating for some time, a trend that is likely to be exacerbated by climate change and regional water scarcity. Nonetheless, the Food and Agriculture Organization of the United Nations contends that 90 % of the necessary increase in crop production globally must come from higher yields, since there is little opportunity for expanding the agricultural land base.

Should we be worried about this? I think so. While I may not make it to 2050, I have two small children who will (I hope). My wife tells me that we should not worry about things we cannot change. However, as genomicists, agronomists, and plant breeders, we have the knowledge and tools to develop more productive, sustainable, and resilient crops (and thus perversely prove *The Economist* to be correct in their dismissal of Malthus and predictions of a food crisis later this century).

The *Genomics and Breeding for Climate-Resilient Crops* provides a blueprint for meeting this most important challenge. The first volume discusses how new genomic tools and resources can be used to accelerate breeding, with the overall goal of maximizing crop productivity while minimizing resource use and environmental damage. An especially promising approach, in my view, is the use of genomic tools to identify and introduce valuable alleles from the wild relatives of crops into elite cultivars. It is this untapped variation in wild species—housed in seed banks around the world—that has the greatest chance of providing quantum jumps in yield.

The second volume is a natural extension of the first, focusing on the key traits (drought tolerance, heat tolerance, water use efficiency, disease resistance, nitrogen use efficiency, nitrogen fixation, and carbon sequestration) necessary for climate-resilient agriculture. Any hope we have of ameliorating the impact of climate change on crop productivity rests on our ability to manipulate these traits.

This twin book project is timely, as the world is slowly waking up to the fact that a global food crisis of enormous proportions is brewing (indeed, I suspect that it will arrive long before 2050). As a consequence, these volumes are likely to form the basis of new courses on climate change and agriculture at academic institutions, to influence policy-makers worldwide, and to provide motivation and guidance to funding agencies. With sufficient investment in agricultural research and public breeding programs, I hope that my worries about filling 9.2 billion bellies are unfounded and that human ingenuity will once again trump Malthusian pessimism.

Loren H. Rieseberg
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Foreword by Calvin O. Qualset

We are in the midst of a new era in crop improvement by genetic means with unprecedented ability to manipulate genes beyond Harlan's genepool compatibility circles. Still, wide hybridizations are difficult to produce, but the ability to introduce genes by parasexual means widens the genepool to include "any gene from any species" in the breeders' repertoire. Genomics provides the bases for such events. On the other side of the technological advances is the realization that our globe is under change due to anthropological causes, and perhaps by other forces. Climate change is a reality, but also is the reality that climate change is not predictable on a day-to-day or even annual basis. Agriculturalists will tell you that climate change occurs every year—sometimes for a succession of years. Our natural resources include atmospheric properties that effect climate change and our other natural resources—soil, biological, and water—are subjected to perturbations that can be detrimental and ultimately affect the sustainability of humankind.

It is a pleasure to see this comprehensive two-volume treatment dedicated to the subject of modifying the resiliency of crops to mitigate the impacts of long-term climate changes. But how about short-term climatic effects? These are the reality in agriculture, and we cannot say that crop breeders have been unaware of the short-term effects. Research programs over many decades have been dedicated to fitting crops to their environments, much as natural selection has adapted organisms to their environments. For example, latitudinal adaptation of crops or forest trees is genetically determined. The genetic bases are generally understood and those genetic effects may be exploited to produce climate-resilient crops. As early as 1921 Mooers' classic paper "Agronomic Placement of Varieties" (Mooers 1921) showed how crop management decisions could stabilize and maximize the performance of crop varieties that differed in their genetic potentials, such as maize. He pioneered what is now known as the regression approach to visualizing genotype × environment interaction that has become a mainstay for characterizing genotype performance in variable environments. He was able to show that certain varieties had greater resilience than others and that "placement" of varieties should match their potential and the environmental potential that he called soil quality.

Coping with the vagaries of environment has long been an issue in food, feed, and fiber production and these two volumes are relevant to the current concerns about climate change and to the stabilization of sustainable yields. Now, more than ever, integration of scientific approaches is necessary to mitigate the impacts of climate on crop production, including soil science, pest control, water management, human nutrition requirements, recognition of climatic variances, and, yes, socio-economic factors. Most of these topics are found in these two remarkable volumes, written by global experts.

I encourage the reading of the chapters on history, principles, and methods in Volume 1, as well as the innovative chapters on critical crop traits for resilience in Volume 2.

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Reference

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Foreword by Ronald L. Phillips

As crop scientists, we are inherently charged with preserving, protecting, and defending the world's food supply, much like the oath that a US President may take when assuming office. Our profession takes seriously the need to *preserve* the germplasm that exists around the world—evidenced by the major seed banks for a large variety of crops. We also do our best to *protect* the food supply by responding to various biotic and abiotic stresses via conventional breeding and genetic engineering approaches. *Defending* the world's food supply takes various forms such as policies that provide open access to valuable materials or via intellectual property rights that encourage the development of new types of products, including those for feed, fiber, biofuels, nutritionally improved types, and even medical applications.

The environment causes many difficult situations—and always has—such as the dust bowl in the US during the 1930s. Drought still plagues many areas of the globe and is one of the myriad of conditions that cannot be well-predicted in advance. Then we have the complication that the genotype being grown interacts with the environment in a very complex fashion making difficult the development of improved types.

Genomic approaches add to our understanding of many of the environmental issues. Data from genomic sequencing now provide the basic framework on which to gain information for enhancing the understanding of such phenomena as genotype $\times$ environment interactions, gene expression related to environmental stresses, and provide clues as to how to apply the information for the betterment of agriculture. Marker-assisted selection is being employed in most modern plant-breeding programs with considerable success. For example, the *Sub1a* gene that provides impressive flooding tolerance in rice can be transferred from one variety to another by marker-assisted conventional breeding in 2.5 years or less.

In addition to various challenges traditionally faced by crop scientists, we now have the specter of even more extreme and variable conditions under which crops are grown. Climate change is a reality; however one of the debatable questions deals with whether it is man-made. Do you believe it or not? My question is "Does it matter?" Underpinning any belief about climate change is the fact that climate determines the very future of the world's food supply. Research must be focused on mitigating the often devastating effects of climate, which appear to be increasingly serious.

In this unique book project, the need is emphasized for meaningful and efficient phenotyping, including innovative techniques, as well as the use of model organisms such as *Arabidopsis*, *Medicago truncatula*, and *Lotus japonicas*. The effectiveness of plant breeding is well established; improved methods of phenotyping and testing for various traits will only enhance the contributions of plant breeding. Attention to the thousands of accessions of various crops in germplasm banks in terms of phenotyping would speed the movement of such materials into prebreeding programs. As with the search for durable disease resistance, breeding for "durable" resilience to important climate traits will require detailed understanding of the underlying genetics reviewed in these volumes. The shift of flowering time is a trait of interest with considerable underlying genetic information. Selection for flowering in the cooler part of the day, for example, may avoid some of the dramatic effects of high temperature on grain production.

These volumes on Genomics and Breeding for Climate-Resilient Crops are carefully designed to provide up-to-date insights on research accomplished as well as what is needed to preserve, protect, and defend our food supply. There is a logical and systematic progression of thought throughout the two volumes and numerous ideas are presented on climate change topics. As the Earth's temperature is rising, huge ice caps are melting, highs and lows of rainfall are hitting extremes, and carbon dioxide is changing the pH of oceans, we must take action on developing a comprehensive program to reduce the effects of climate threats on our food supply. International cooperation is needed, and these volumes reflect the international interest in the goal of developing climate-resilient crops. The environmental events that bring dramatic headlines in news programs and magazines demand that crop scientists employ the most effective technologies to circumvent the reduction in food supply due to climate. This is especially important given that another billion people exist on this planet every 14 years, and they must have an adequate food supply. This two-book project is a welcome contribution to the future of genomics and breeding for climate-resilient crops.

Ronald L. Phillips
Regents Professor Emeritus
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Foreword by J. Perry Gustafson

The UN projects that by 2050 World food production will need to increase by a minimum of 70 % to feed a projected World population of more than nine billion. This 70 % increase will not be enough to improve the diets of the one billion hungry people in the world; it will only be enough to keep the same diet the world has today. It is clear that extraordinary improvements in agricultural productivity will be necessary. World food production has been steadily increasing from approx. 2.94 billon metric tons (BT) in 1961 to approx. 8.27 BT in 2007. Most importantly, this dramatic increase in food production was produced on approximately the same amount of land currently under production as was under production in 1950. Thus, the increase in production was the result of improved crop cultivars, crop technology advances, and better management practices. Projections indicate that world food production between now and 2050 can be increased to meet population demands for improved dietary standards provided that existing and newly developed technology is utilized to genetically improve cultivars, and that the world works very hard to improve crop management. This all needs to be accomplished without causing any additional adverse effects on the environment, and while clearly avoiding the cultivation of new land. Plant breeders will have to pay close attention to the effects of global warming on food production. However, the chapters in this book clearly show that current advances in technology are capable of doing so and of dramatically decreasing the time to deliver genetic improvements into the field. These include techniques, which bypass some traditional approaches to seed production. Introducing gene complexes by genetic manipulation from related species has a long and successful history and with the addition of new genetic engineering techniques will continue into the future. Arguably, the carefully coordinated application of all existing and new technologies discussed in the two volumes will be critically important to feed an ever-increasing population, sustaining the productivity of arable lands, and maintaining our fragile environment.

J. Perry Gustafson
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Preface

Climate change is expected to enormously affect life on the Earth. It will cause drastic changes in the environment and ecology and thus will severely impact agriculture. Therefore, it poses a serious challenge for global food security. It is expected to cause drastic changes in agroclimatic conditions including temperature, rainfall, soil nutrients and health, and incidence of pathogens and pests leading to striking reduction in crop yields due to global warming, water scarcity, changes of rainfall patterns resulting in increasingly frequent drought and flood, and other extreme weather events. Plant pathogens and pests may also evolve quickly with more virulent pathotypes and biotypes and so may extend their geographical spread leading to epidemics and severity due to climate change. Furthermore, elevated CO_2 levels will also reduce the nutritional quality of most crops and some crops may even become more toxic due to changes in the chemical composition of their tissues. Climate change will also cause elevation of greenhouse gas emission. The most grave and still unknown concern, however, involves the critical effects that interactions among various biotic and abiotic excesses or paucities will have on crops and cropping systems making the task of feeding a world population of nine billion by 2050 extremely challenging.

Several eminent scientists from different parts of the world are planning to put significant effort into combating or mitigating the threat to food security due to climate change. We organized an international workshop on Climate Change during the 20th International Conference on the Status of Plant and Animal Genome Research (PAG conference) held during January 2012 in San Diego, California and established the International Climate Resilient Crop Genomics Consortium (ICRCGC) with a membership of over 30 active scientists from over ten countries (http://www.climatechangegenomics.org). Recently, many more scientists have become interested in this critical topic and we organized a special international workshop on Genomics and Breeding of Climate-Resilient Crops for Future Food Security during the 6th International Crop Science Congress held during August 6–10, 2012 in Bento Goncalves, Brazil followed by a brain-storming discussion to formulate the future strategies and work plans for combating climate change.

We recently organized another two workshops on this subject in January 2013 in San Diego, California during the 21st PAG conference. ICRCGC is now preparing a white paper for more serious and broad dialogs to initiate international and multi-disciplinary efforts to combat climate change using genetic resources and advanced genomics and breeding tools.

The central strategy of combating climate change will obviously involve the development of climate-resilient crop cultivars with broader genome plasticity allowing wider adaptability, broader genome elasticity with potential for high response to phenotypic, chemotypic, and molecular selection and above all durable and robust resistance to biotic and abiotic stresses. However, genomics and breeding for climate-resilient crops are relatively new fields of study and research and given the seriousness of the threats of climate change will obviously be included in course curricula in academic institutes and in frontier programs of agricultural research organizations at national and international levels. This topic is expected to be of increasing interest to policy-makers, social activists, and both public and private sector agencies supporting agricultural research. There are a few critical and comprehensive reviews on this subject and a number of publications are also available on the assessment of the impact of climate change on agriculture and suggested strategies to circumvent the severe effects to ensure food security. These deliberations are, however, scattered over the pages of newspapers, newsletters, journals, and web sites. Hence, a compilation of narratives on the concepts, strategies, tools, and related issues was felt to be lacking and this was the guiding force behind the inception of this two-volume work on Genomics and Breeding for Climate-Resilient Crops.

Volume 1 "Genomics and Breeding for Climate-Resilient Crops: Concepts and Strategies"

Volume 1 of this book deliberates on the basic concepts and strategies of genomics and breeding for developing climate-resilient crop varieties. In recent years considerable gains have been made in our understanding of plant genome organization and gene expression. In large part this has been achieved through the study of "model species," i.e., species in which genetics and genomics are more tractable than in many crop plants. The best known and most developed of these models is *Arabidopsis thaliana*, the DNA sequence of which was published in 2000. Subsequently, a number of different model species have been developed and the number of crop species that feed the world with sequenced genomes has also increased.

Progress in developing the resources, tools, and approaches to allow more rapid development of improved crops has been significant in the last decade. These include genomics, transcriptomics and metabolomics as well as nondestructive, dynamic high-throughput phenotyping (phenomics), and novel

approaches to germplasm characterization and population improvement. It is timely therefore to provide a detailed analysis of where we are now and what progress can be expected in the near future. This description of the "state of the art" is presented in Volume 1.

The first chapter of Volume 1 by Abberton provides an introduction to the potential damaging effects of climate change on agriculture leading to future food insecurity and narrates the required integrated approaches to address this serious threat. It elucidates on the role of plant improvement and the requirement of judicious deployment of genomic tools for utilization of genetic diversity and precision high-throughput phenotyping as supplementary strategies for developing climate-resilient crop varieties.

At the heart of crop improvement are genetic resources, their collection, characterization, and utilization. The contribution of Pignone and Hammer (Chap. 2) shows ways in which the use of genetic resources can be targeted at the challenges of a changing climate. Genetic resources offer a vast reservoir of important novel traits and allelic variation for traits. Increasingly, genomics tools are being brought to bear on the variation collected in genebanks.

De Pace and coauthors in Chap. 3 detail modern methods of identifying traits for incorporation into selection criteria for new cultivars and to uncover the underlying genetic control of variation in these traits. They present reasons as to why paleoclimate and vegetation-type reconstruction from fossil records and species vicariance help in understanding the long-term dynamics of plant features and trait evolution associated with dispersal and climate changes. Comparative genomics demonstrated as alleles for those plant features (i.e., plant morphology and phenophase alteration), and for biotic (response to bacterial and fungal pathogens) and abiotic (i.e., drought, flooding) stress resistance are still part of the standing genetic endowment of the living gene pools of the crop and forest plant species and allied wild relatives. Therefore, recapitulation of evolutionary and domestication processes using various genomic tools will provide innovative molecular breeding methods to explore and select the genetic variants needed for forest and crop adaptation to climate change pressures.

Subsequent chapters deal more explicitly with how modern genetics and genomics can be used within crop improvement programs. Dwiyanti and Yamada (Chap. 4) describe genetic mapping and identification of quantitative trait loci (QTL) for traits involved in responses to changing climate. Generally, QTLs related to climate change response are complex and largely affected by environmental conditions. Nevertheless, researchers have succeeded in identifying several major QTLs and applying the knowledge in crop breeding. Various genomics tools are now available in crop species that have been the subject of most research, particularly maize but now increasingly rice and other cereals. Progress in wheat has been slow due to the limited extent of genetic variation in the crop and its hexaploid nature, but next-generation sequencing (NGS) approaches are changing this rapidly. Genome sequencing with ever-increasing speed and reducing costs brings with it the potential for "genotyping by sequencing," which when allied with sophisticated statistical approaches is likely to allow the potential of genome-wide or genomic selection

(GWS) to be realized more effectively. Knowledge of QTLs in model species and advances in genomic tools can be applied to crop plants with limited genomic information.

Adaptation to climate change would require convergence of appropriate technologies, policies, and institutional innovations and Chap. 5 by Prasanna and coauthors focuses on some of the promising genomic tools and strategies that can enhance time- and cost-effectiveness of breeding for climate-resilient major cereal crops, particularly maize. They deliberate on employment of modern breeding strategies such as high-density genotyping, whole-genome re-sequencing, high-throughput and precise phenotyping, and genomics-assisted breeding including genome-wide association studies, breeder-ready marker development, rapid-cycle genomic selection, marker-assisted recurrent selection, and crop modeling.

The strategy for climate-resilient agriculture should be to maximize crop production with minimal or no damage to the environment. This would demand changes in the approaches to crop improvement, and in the deployment of recent techniques involving genomics in crop research. Designer crops have to be developed with enhanced efficiency in the use of radiation energy, nutrients, and water; they also have to fit the system of conservation agriculture including zero tillage. Talukdar and Talukdar (Chap. 6) describe recent changes in strategies and approaches to crop breeding highlighting the progress of genomic tools in meeting the challenges of climate change in agriculture.

Of course a major modern tool for the improvement of specific traits is genetic engineering. To date, this technology has been widely employed but only for a few traits in a small number of crops. Developments in genetic engineering have been instrumental for commercial application in the production of transgenic plants for biotic and abiotic stresses. It has tremendous potential in agriculture and especially for important traits related to climate change. Yadav and coauthors (Chap. 7) address the challenge of deploying this technique for important complex traits that are central to climate adaptation.

New approaches to selection are required to take full advantage of the pace at which new genomic knowledge is being acquired. Ceccarelli and coauthors (Chap. 8) and Murphy and coauthors (Chap. 9) explain how participatory breeding, where farmers are involved in the selection process, can be focused on adaptation.

In Chap. 8, Ceccarelli and coauthors show how climate changes have affected humanity for a long time, and how crops and people have reacted and adjusted to changes. As biodiversity has sometimes been negatively affected by modern crop production, they discuss how participatory plant breeding, by exploiting specific adaptation and farmers' knowledge, can positively contribute not only to crops' adaptation to climate changes but to increase biodiversity and to increase production directly in the hands of the farmers, thus also improving the accessibility and availability of food.

Murphy and coauthors (Chap. 9) explain how participatory breeding can be focused on adaptation. Evolutionary breeding has been shown to efficiently increase fitness, disease resistance, and related yield components in self-pollinating cereals while maintaining a broad-based genetic diversity in the field. Through

buffering of biotic and abiotic stresses, increased on-farm genetic diversity can be important to maintaining yield stability across time and space. Inclusion of on-farm selection of diverse evolutionary breeding populations within and across fluctuating environments has the potential to greatly increase the scope of genotypically plastic cultivars capable of adaptation to unpredictable climate-induced environmental change.

Modern crop improvement in both its genomics and phenomics components is increasingly data rich. Edwards (Chap. 10) summarizes the role and importance of bioinformatics in integrating these data and converting them to usable knowledge has been emphasized greatly over the past two decades and is now being increasingly reflected in the public sector and commercial programs throughout the world.

Chapter 11 by Lybbert and coauthors deals with the critical issue of international collaboration and the importance of international funding to facilitate the exchange of knowledge and germplasm required to achieve success in global crop development. The benefits of climate-resilient crops are often complex and the adoption of these crops will require international collaboration and coordination of public and private sectors. Support for networking and funding of collaborative research and extension activities will determine global success in the development of climate-resilient crops and their impact on food security.

However, the importance of regulation and intellectual property is pervasive in the area of modern crop improvement and not restricted to genetic engineering. These issues are considered by Blakeney (Chap. 12). Similarly, sociopolitical consideration extends across the whole of the application of genomics, its translation into the development of cultivars and farmers' access to them and the ability to incorporate them into their operations as enumerated by Hughes and coauthors in Chap. 13.

Volume 2 "Genomics and Breeding for Climate-Resilient Crops: Target Traits"

In many cases breeders seek improvements in yield and yield per unit of input, however, factors limiting yield are many and various and often these factors must be addressed directly in targeted approaches. Volume 2 elucidates the genomic and breeding approaches for genetic improvement in the major target traits covered in its 13 chapters.

In the majority of crop species, the timing of flowering represents a key adaptive trait, with a major impact on yield. In Chap. 1, Bentley and coauthors review the genetic determinants and environmental cues that influence flowering time in a range of crop species. They deliberate on the consequences of climate change on crop adaptation mediated by flowering and discuss the breeding targets and methodologies to mitigate detrimental yield.

Plant growth and development are largely dependent on the root system due to its crucial role in water and mineral uptake affecting overall plant growth and

architecture. Most agricultural crops have a remarkable level of genetic variation in root morphology that can be harnessed for improving crop adaptation to several abiotic stresses. The importance of roots and roots traits (size, architecture, interactions with soil, exudation, etc.) has long been recognized, but progress has been slow due to difficulty in phenotyping and screening techniques. However, genomics approaches allied to the development of noninvasive dynamic imaging techniques capable of phenotyping root traits and largely effecting QTL identification to facilitate marker-assisted selection will bring significant new opportunities for crop improvement as enunciated in Chap. 2 by Silvas and coauthors.

Two of the major abiotic stresses involve responses to low or high temperatures. Because of the need to feed an ever-increasing global population, attempts at agricultural production are being extended into marginal locations, including those at higher altitudes where growth conditions are suboptimal due to the cold stresses commonly encountered. Amongst the traits associated with survival under such conditions, the acquisition of genes for freezing tolerance is considered of primary importance with gene improvement targeted specifically at the timeliness of engagement, the maintenance, and the subsequent release of cold acclimation mechanisms necessary to ensure a winter survival appropriate to the region of crop growth, followed by rapid recovery to ensure good crop yields once the threats of encountering further freezing temperatures are diminished. Humphreys and Gasior (Chap. 3) deliberate the detailed effects of cold on crop growth and development and ultimately on crop yield, and the strategies and tools for developing crop varieties with improved tolerance to winter and freezing.

Porch and Hall (Chap. 4) describe crop improvement with respect to heat tolerance. Many current environments experience high temperatures that reduce crop yield, and projected increases in temperature could reduce grain or fruit yield by about 10 % per °C increase in temperature. Yet, relatively little effort has been devoted to breeding for heat tolerance. However, for a few crop species, heat-resistant cultivars have been bred by conventional hybridization and selection for heat tolerance during reproductive development. The successes that have been achieved are described and provide blueprints, whereby heat-resistant cultivars could be bred for many annual crop species. Molecular approaches to enhance breeding for heat tolerance are also discussed.

A major focus of this volume is on water: drought stress and water use efficiency important for interactions with soil including effects on flooding propensity. Crop improvement has had limited success in developing new cultivars with enhanced adaptation to drought-prone environments, although it has been pursued for various decades. Research on the mechanisms underlying the efficient use of water by crops and water productivity remains essential for succeeding in this endeavor. They may be improved through genetic enhancement. Advances in genetics, "omics," precise phenotyping and physiology coupled with new developments in bioinformatics and phenomics can provide new insights into traits that enhance adaptation to water scarcity. Chapter 5 by Ortiz provides an update on research advances and breeding main grain crops for drought-prone environments.

For several decades now, plant breeders have been selecting for high water use efficiency as a way to increase agricultural productivity in water-limited environments. Water use efficiency is the ratio between carbon gain (photosynthesis) and water loss (transpiration), which inherently occurs during stomatal opening. High water use efficiency is generally associated with greater drought tolerance, but this does not always equate to greater productivity. There are few studies that have demonstrated improvements in water use efficiency that lead to improved yields. Chapter 6 by Bramley and coauthors describe the multifarious hydraulic and biochemical processes controlling plant water loss and photosynthesis. They show that water use efficiency is predominantly driven by plant hydraulic properties, and genes that are mostly involved in gas exchange. Genetic variation for these properties exists in agricultural crops, but research needs to be directed towards examining the influence of high water use efficiency on yield production in targeted environments.

The enormous diversity in rice and its adaptation to contrasting hydrological and edaphic conditions made it one of the crops most acquiescent to genetic manipulation to keep up with the increasing adversities of climate change, including the increasing flood incidences predicted by several climate models. A classical example is presented in Chap. 7 by Ismail, summarizing the progress in breeding for flood tolerance in rice and prospects for future improvements to cope with further deteriorations projected in rainfed lowlands of the tropics. A case was presented where deployment of the *SUB1A* gene that confers tolerance to submergence during the vegetative stage resulted in considerable impacts in farmers' fields in flood-prone areas, with yield advantages of 1 to over 3 t ha^{-1} following 4–18 days of complete submergence. This is a classic example of the use of genomic tools to resolve current issues and cope with further adversities of climate change, while keeping up with the rising demands for more food.

Clearly responses to biotic stresses, and postharvest losses, are crucial aspects in maintaining yield in many environments and often diseases and insects are a major cause of biomass loss.

Chapter 8 by Bariana and coauthors summarizes the role of genomics in the breeding of disease-resistant crop cultivars. Various selection technologies used in cereal and pulse improvement programs are discussed. Current information on disease response linked markers is reviewed in light of their implementation. The potential use of whole genome molecular scanning in breeding disease-resistant crops is also explored. The role of information management in programs aimed at the application of genomics to crop improvement is emphasized.

Agricultural research has for decades focused on gathering crucial information on the biochemical, genetic, and molecular realms that deal with plant–insect interactions in changing ecosystems. Environmental conditions, which include the overall conditions of climate change, are a reality that needs to be considered as one of the crucial phenomena of changing ecosystems when planning future crop improvement, security, and/or pest management strategies. In the context of climate change in Chap. 9 Emani and Hunter attempt to integrate past and present research in classical and molecular breeding, transgenic technology, and pest

management. The integrated approach will direct present research efforts that aim at creating plant–insect pest interaction climate change models that reliably advise future strategies to develop improved insect-resistant, climate-resilient plant varieties.

One of the foundations of the increases in crop productivity in the past has been improved nutrient availability, especially nitrogen and phosphorus. The increases in crop production that are needed to meet the demands of a growing world population will require greater supply and uptake of essential nutrients by plants. Even in the absence of climate change, there is a need to improve the nutrient use efficiency of our present cropping systems to make better use of nonrenewable resources and to minimize the adverse environmental effects of the over-use of fertilizers. Moreover, in many parts of the world, the nutrient concentration of staple food crops is low, and significant gains in the health of communities can be achieved by biofortification of grain. Breeding crop varieties that are better able to use poorly available sources of nutrients in the soil or which can respond better to inputs of nutrients is an important aspect of increasing nutrient use efficiency. The uptake and use of nitrogen and phosphorus by crop plants are complex processes and to date, there has been limited success in improving nutrient use efficiency using conventional approaches. Advanced genetic techniques allied to traditional plant breeding may play an important role in improving nutrient use efficiency. McDonald and coauthors present a review on the importance of improving nitrogen use efficiency and on research accomplished so far for that purpose in Chap. 10.

Nitrogen assimilation and fixation underlie the advances of the green revolution and limit the impact of both agriculture and environmental plant productivity on carbon sequestration. For a number of years the potential importance of legumes in crop rotations across many agroecosystems has been recognized. However, the limited extent to which this potential has been realized has been disappointing. Legumes do not just contribute in terms of food, feed, and fertility but are also important as fuel wood and could help more with respect to carbon sequestration. However, their key attribute and a major reason why they are so important for the future of world agriculture is the nitrogen-fixing symbioses they form with nodulating bacteria. Genetic and genomic tools have been applied powerfully in recent years to understand the control of the legume–rhizobia interaction utilizing model legumes, particularly *Medicago truncatula* and *Lotus japonicus* and the great challenge is to deploy this information in the improvement of the major grain and forage legumes (Lightfoot, Chap. 11). However, the world's major nitrogen sink crops are the grasses and cereals. Equally, the world's major carbon sink crops are trees and wetland plants. These crops and plants could be made more efficient in their use of nitrogen and consequently, water. Scientists have tried many transgenes in many crops to achieve these improvements with good rates of success. Stacks of genes, and new sets of transgenes hold promise to deliver significant improvements across the world's major crops. On the basis of these discoveries efforts to develop genetically, or even transgenically altered environmental plants might also help to slow global warming.

Carbon sequestration in plants has been proposed as a possible moderator or solution to the rising levels of atmospheric carbon dioxide (CO_2) threatening to alter global temperature and climate. Chapter 12 by Cseke and coauthors examines the different mechanisms of carbon sequestration within the Earth's natural carbon cycle with a special focus on events associated with plant development. This chapter outlines the specific chemical and biological processes that allow plants to capture, allocate, and provide for long-term storage of CO_2 in the form of both above-ground and below-ground biomass. They specifically examine the contribution of mycorrhizal and other soil community-level interactions as an important reminder that healthy soils are required for the uptake of nutrients needed for efficient carbon sequestration. This chapter provides a perspective on molecular approaches to enhancing carbon sequestration in biological systems.

Gases that trap heat in the atmosphere are referred to as greenhouse gases. Four major greenhouse gases that are abundant in the atmosphere today are carbon dioxide, methane, nitrous oxide, and fluorocarbons. These are naturally occurring greenhouse gases because they are potentially essential to keeping the Earth's temperature warm. However, man-made activities have increased the number of these gases, resulting in more heat getting trapped in the atmosphere. Among these gases, carbon dioxide has the highest concentration in the atmosphere. Chapter 13 by Abberton deliberates on greenhouse gas emission and carbon sequestration.

The chapters of Volume I "Genomics and Breeding for Climate-Resilient Crops: Concepts and Strategies" were contributed by 38 scientists from 14 countries including Australia, China, France, Germany, India, Italy, Japan, Kenya, Nigeria, Netherlands, Philippines, UK, USA, and Zimbabwe. The chapters of Volume 2 "Genomics and Breeding for Climate-Resilient Crops: Target Traits" were contributed by 48 scientists from nine countries including Australia, Brazil, Germany, India, Japan, Philippines, Sweden, USA, and UK. Altogether 84 scientists from 16 countries authored the 26 chapters of the two volumes. I wish to extend my thanks and gratitude to all these eminent scientists for their excellent contributions and constant cooperation.

I have been working with Springer since 2006 and edited several book series, and I have developed a cordial relationship with all the staff involved. I wish to thank Dr. Christina Eckey and Dr. Jutta Lindenborn for their constant guidance and cooperation right from the planning up to the completion of this book project. It was highly enriching and comfortable to work with them all along.

I must thank my wife and colleague Phullara, our son Sourav, and our daughter Devleena for their patience over several months as I put in extra time on this project and also for assisting me with the editing of these two books as always.

I feel myself fortunate that several pioneering scientists of the field of plant and agricultural sciences from around the world have been so kind to me over the last three decades of my professional career. Five such legendary scientists, Profs. M.S. Swaminathan, Loren H. Rieseberg, Calvin O. Qualset, Ronald L. Phillips, and J. Perry Gustafson, have kindly penned the forewords for this work and given readers a rare opportunity to learn from their precise assessment of the problem of

climate change and vision for the future road map of genomic and breeding research required to attain sustainable food security in the future. I express my deep regards and gratitude to them for sharing their wisdom and philosophy and also for all the generosity, affection, encouragement, and inspiration they showered upon me!

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Abbreviations

AAAS	American Association for the Advancement of Science
AATF	African Agricultural Technology Foundation
AB	Advanced backcross
ABA	Abscisic acid
ABF	ABRE binding factor
ABF3	ABRE binding factor 3
ABF4	ABRE binding factor 4
ABI5	ABA insensitive 5
ABL	Advanced backcross line
AB-QTL	Advanced backcross-QTL
ABRC1	ABA-responsive complex 1
ABRE	ABA-responsive element
ACIAR	Australian Center for International Agricultural Research
ACP	African, Caribbean, and Pacific
ACPFG	Australian Center for Plant Functional Genomics
ACTA	Anticounterfeiting Trade Agreement
ADH	Alcohol dehydrogenase
Adh1	Alcohol dehydrogenase 1 gene
AEBC	Agriculture and Environment Biotechnology Commission (of UK)
AFLP	Amplified fragment length polymorphism
AGAMOUS	*AGAMOUS* (plant homeotic) gene
AGRA	Alliance for a Green Revolution in Africa
AHK1	*Arabidopsis* histidine kinases 1
AI	α-Amylase inhibitors
AlaAT	Alanine aminotransferase
ALR	Aldose-aldehyde reductase
AMBIONET	Asian Maize Biotechnology Network
AMPA	Aminomethyl phosphonate
ANP	Anaerobic polypeptide
AP1	*APETALA1* gene

AP2	*APETALA2* gene
APX	Ascorbate peroxidase
ARE	Anaerobic response element
ARIPO	African Regional Intellectual Property Organization
Asc	*Alternaria* stem canker resistance gene
*At*HSP17.6A	*Arabidopsis thaliana* heat-shock protein 17.6 A
AtMRP5	*Arabidopsis thaliana* multidrug-associated gene
*At*MYB2	*Arabidopsis thaliana* MYB transcription factor 2
AtMYB2	*Arabidopsis thaliana* MYB oncogene for protein B2
AtMYC2	*Arabidopsis thaliana* MYC oncogene for protein C2
*At*MYC2	*Arabidopsis thaliana* MYC transcription factor 2
AtNHX1	*Arabidopsis thaliana* vacuolar gene encoding Na+/H+ antiporter
ATP	Adenosine-5′-triphosphate
ATPase	Adenosine triphosphatase
*At*SR1	*Arabidopsis thaliana* signal responsive 1
AVP1	*Arabidopsis* vacuolar pyrophosphatase 1
BAC	Bacterial artificial chromosome
BAR	Basta N-acetyltransferase
BAR	*BAR* gene
BC	Backcross
BFGR	Biofuel Feedstock Genomic Resource
BIK1	*Botrytis*-induced kinase 1
BIL	Backcross inbred line
BIM	Bayesian interval mapping
BLAST	Basic local alignment search tool
BLUP	Best linear unbiased predictor
BMZ	Bundesminister für Wirtschaftliche Zusammenarbeit
BSA	Bulk segregant analysis
Bt	*Bacillus thuringiensis*
bZIP	Basic leucine zipper
C4H	Cinnamate-4-hydroxylase gene
CAFC	Court of Appeals for the Federal Circuit of (US)
CAGE	Cap analysis of gene expression
CaM	Calmodulin
CAM	Crassulacean acid metabolism
CaMV	Cauliflower mosaic virus
CaP1F1	*Capsicum annum* pathogenesis induced factor 1
CAPS	Cleaved amplified polymorphic sequence
CAT	Catalase
CBD	Convention on Biological Diversity
CBDC	Community Biodiversity Development and Conservation Network
CBF	C-repeat (CCAAT) binding factor

CBF	C-repeat binding factor gene
CBF1	C-repeat binding factor 1
CBF3	C-repeat binding factor 3
CBF4	C-repeat binding factor 4
CBL	Calcineurin B-like molecules
CBOL	Consortium for the Barcode of Life
CBP60g	Calmodulin binding protein 60g
CC	Composite cross
CCA1	Circadian clock-associated 1 protein
CCAFS	Climate Change Agriculture and Food Security
CCD	Charge-coupled device
CCT	CONSTANS, CONSTANS-LIKE, TOC1 domain
cDNA	Complementary-DNA
CDPK	Calcium-dependent protein kinase
CEV1	Constitutive expression of the vegetative storage protein 1
CEV1	Constitutive expression of vegetative storage protein 1 (VSP1) gene
Cf	*Cladosporium fulvum* resistance gene
CFC	Chlorofluorocarbon
CGIAR	Consultative Group on International Agricultural Research
CGPBRI	Center for Genomics, Proteomics and Bioinformatics Research Initiative
CHO	Chinese hamster ovary
chyB	Beta-carotene hydroxylase gene
CIAT	International Center for Tropical Agriculture
CIM	Composite interval mapping
CIMMYT	Centro Internacional de Mejoramiento de Maiz y Trigo (International Maize and Wheat Improvement Center)
CIPK	CBL-interacting protein kinase
CO	*CONSTANS* gene
codA	Choline oxidase
COI1	Coronatine insensitive 1
COP	Conference of the Parties to the Convention on Biological Diversity
COR	Cold-responsive/regulated
COR	Cold-responsive/regulated gene
COR14b	Cold-responsive gene 14b
COR15a	Cold-responsive gene 15a
cor6.6	Cold-responsive gene 6.6 gene
CP	Coat protein
CPB	Centralized, nonparticipatory plant breeding
CPR5	Constitutive expressor of PR genes
CRoPS	Complexity reduction of polymorphic sequences
CRT	C-repeat

CSACC	Commission on Sustainable Agriculture and Climate Change
CSIRO	Commonwealth Scientific and Industrial Research Organization
CSP	Cold-shock protein
CTD	Canopy temperature depression
CTR	Constitutive triple response
CuZn-SOD	Copper-zinc superoxide dismutase
CWR	Crop wild relative
CYP71A15	Cytochrome P450 group 71, subfamily A gene 15
CYP71E1	Cytochrome P450 group 71, subfamily E gene 1
CYP79A1	Cytochrome P450 group 79, subfamily A gene 1
CYP79B2	Cytochrome P450 group 79, subfamily B gene 2
CYP79B3	Cytochrome P450 group 79, subfamily B gene 3
DAG	Diacylglycerol
DAMP	Damage-associated molecular pattern
DArT	Diversity array technology
dCAPS	Derived CAPS
ddNTP	Dideoxynucleotide triphosphate
DH	Doubled haploid
Dhfr	Dehydrofolatereductase
DHPLC	Denaturing high-pressure liquid chromatography
DMO	Dicambamonooxygenase
DMR	Downy mildew resistant
DnaK1	Chaperon protein DNA K1
DND	Defense, no death
DOE	Department of Energy
DPSIR	Driving forces-Pressure-State-Impact-Response conceptual framework
DRE	Dehydration-responsive element
DREB	Dehydration-responsive element binding gene
DREB	Dehydration-responsive element binding protein/factor
DREB1	Dehydration-responsive element binding 1 gene
DREB1A	Dehydration-responsive element-binding transcription factor 1A
DREB2A	Dehydration-responsive element binding protein 2A
DREB2B	Dehydration-responsive element binding protein 2B
DRIP	DREB-INTERACTING PROTEIN DRIP
DSB	Double-strand break
DTI	DAMP-triggered immunity
DTMA	Drought Tolerant Maize for Africa
DUS	Distinctiveness, uniformity, and stability
EBA	Extended Board of Appeals of the European Patent Organization
EcoTILLING	Eco-TILLING

EDR1	Enhanced disease resistance 1
EDS1	Enhanced disease susceptibility 1
EEA	European Environment Agency
EgAGL1	*Eucalyptus grandis AGAMOUS*-like MADS-box gene 1
EgAGL2	*Eucalyptus grandis AGAMOUS*-like MADS-box gene 2
Ehd1	Early heading date 1 locus
eIF4E	Eukaryotic translation initiation factor 4E
EPB	Evolutionary participatory breeding
EPSPS	5-Enolpyruvylshikimate-3-phosphate synthase
e-QTL	Expression-QTL
erd10	Early response to dehydration 10
EREBP1	Ethylene response element binding protein 1
ERF	Ethylene-responsive element binding factor
ERF2	Ethylene responsive factor 2
ESI	Electric spray ionization
EST	Expressed sequence tag
ETR1	Ethylene receptor 1
EU	European Union
EVG	Evergrowing
EVG-d	Evergrowing recessive allele
EW	Epicuticular wax
F5H	Ferulate 5-hydroxylase
F5H	Ferulate 5-hydroxylase gene
FACE	Free-Air Concentration Enrichment
Fad	w3-Fatty acid desaturase
FAO	Food and Agriculture Organization (of the United Nations)
FARA	Forum for Agricultural Research in Africa
Fe-SOD	Iron superoxide dismutase
FpCBF6	*Festuca pratensis* C-repeat binding factor 6 gene
FpVRN1	*Festuca pratensis* vernalization 1 locus
Fr-A1	Frost resistance 1 locus located at wheat chromosome 5A
Fr-A2	Frost resistance 2 locus located at wheat chromosome 5A
FR-H1	*Frost resistance 1* locus of barley (*Hordeum vulgare*)
FR-H2	*Frost resistance 2* locus of barley (*Hordium vulgare*)
FT	*FLOWERING LOCUS T* gene
G × E	Genotype × environment
GA	Gibberelic acid
GAI	Gibberellin insensitive
GAI	Gibberellin-insensitive gene
GATA1	GATA-1 transcription factor binding to DNA sequence GATA
GBS	Genotyping-by-sequencing
GCED	Gross carbon emissions from deforestation
GCP	Global Cassava Partnership
GDP	Gross domestic product

GE	Genotype × environment
GEBV	Genomic estimated breeding value
GEI	Genotype × environment interaction
GGE	Greenhouse gas emission
Ghd7	Grain number, plant height, and heading date 7 locus
GHG	Greenhouse gases
GIPB	Global Partnership for Plant Breeding and Capacity Building
GL	Genotype × locations
GLM	Generalized linear model
GLS	Grey leaf spot
GM	Genetic modification/Genetically modified
GMO	Genetically modified organism
GMP	Global Maize Program
GNA	Snow-drop lectin
GO	Gene ontology
GOLD	Graphical overview of linkage disequilibrium
GOX	Glyphosate oxidoreductase
GPAT	Glycerol-3-phosphateacyltransferase
GPX	Glutathione peroxidase
GR	Genetic Resources
GRC	Germplasm regression combined
GRULAC	Group of Countries of Latin America and the Caribbean
GS	Genomic selection
GS	Glutamine synthetase
GST	Glutathione S-transferase
GUA	Genotype unit area
GWAS	Genome-wide association studies
GWS	Genome-wide selection
GY	Genotype × years
H3K4Me3	Histone H3 lysine 4 trimethylation
HCN	Cyanhydric acid
HCT1	Hydroxycinnamoyl transferase gene
Hd1	Heading date 1 locus
Hd2	Heading date 2 locus
Hd3a	Heading date 3a locus
Hd3b	Heading date 3b locus
Hd6	Heading date 6 locus
HDD	Host direct duplication
HDR	Homology-directed repair
Hero	*Heterodera rostochiensis* resistance gene
HFC	Hydrofluorocarbon
HIC	High carbon dioxide
HIR	Haploid induction rate
HIS	Heat susceptibility index

HOS 1	High expression of osmotically responsive gene *hos 1*
hpRNA	Hairpin-RNA
HR	Hypersensitive response
HRM	High-resolution melting
HSE	Heat-shock element
Hsf	Heat-shock factor
HSF1	Heat-shock factor 1
HSF3	Heat-shock factor 3
HsfA1	Heat-shock factor A1
HsfB2a	Heat-shock factor B2a
HSI	Heat-susceptibility index
HSIGFD	HSI for grain fill duration
HSITGW	HSI for thousand grain weight
HSP	Heat-shock protein
Hsp17.7	Heat-shock protein 17.7
Hsp21	Heat-shock protein encoding gene 21
Hsp90	Heat-shock protein 90
HTPP	High-throughput phenotyping platform
HVA1	*Hordeum vulgare* LEA gene
HVA1	*Hordeum vulgare* LEA protein
HvCBF2	*Hordeum vulgare* C-repeat binding factor 2 gene
HvCBF4	*Hordeum vulgare* C-repeat binding factor 4 gene
HVR	Hypervariable region
I-2	Immunity to *Fusarium* wilt race 2
IAASTD	International Assessment of Agricultural Knowledge, Science and Technology for Development
IAEA	International Atomic Energy Agency (of the United Nations)
IBM	Intermated B73 × Mo17 population
ICARDA	International Center for Agricultural Research in the Dry Areas
ICGS	International Cocoa Genome Sequencing Consortium
ICRCGC	International Climate-Resilient Crop Genomics Consortium
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
IDRC	International Development Research Center
IFAD	International Fund for Agricultural Development
IFPRI	International Food Policy Research Institute
IGC	Intergovernmental Committee
IITA	International Institute of Tropical Agriculture
IM	Interval mapping
IMT1	Myo-inositol O-methyltransferase
Indel/INDEL	Insertion/deletion
INRA	National Institute of the Agrarian Reform
InsP	Inositol phosphate
IP	Intellectual Property
IP3	Inositol trisphosphate

IP6	Inositol hexaphosphate
IPCC	Intergovernmental Panel on Climate Change
IPGRI	International Plant Genetic Resources Institute
IPK	Institut für Pflanzengenetik und Kulturpflanzenforschung
IPPC	International Plant Protection Convention
IPR	Intellectual Property Rights
IRRI	International Rice Research Institute
ISAAA	International Service for the Acquisition of Agri-biotech Applications
ISR	Induced systemic resistance
ISSR	Intersimple sequence repeat
ITPGRFA	International Treaty on Plant Genetic Resources for Food and Agriculture
ITWGPGRFA	International Technical Working Group for Plant Genetic Resources for Food and Agriculture
IUCN	International Union for the Conservation of Nature and Natural Resources
JA	Jasmonic acid
JA-Ile	Jasmonic acid-isoleucine
JAZ	Jasmonate ZIM domain protein
JERF	Jasmonic ethylene-responsive factor
JERF	Jasmonic ethylene-responsive factor gene
JGI	Joint Genome Institute
KIN	Kinetin-induced protein
kin1	Kinetin-induced 1 gene
kin2	Kinetin-induced 2 gene
LAG	Longevity-assurance gene 1
LAI	Leaf area index
LBD	Lateral organ boundaries
LBD	*Lateral Organ Boundaries Domain* gene
LD	Linkage disequilibrium
LDH	Lactose dehydrogenase
LEA	Late embryogenesis abundant protein
Lea	*Late Embryogenesis Abundant* gene
LFY	*LEAFY* gene
LG	Linkage group
LpCBF	*Lolium perenne* C-repeat binding factor gene
LRR	Leucine-rich repeat
LSD1	Lesion-simulating disease resistance 1 gene
MAB	Marker-assisted breeding
MABC	Marker-assisted backcrossing
MADS	MCM1/AGAMOUS/DEFICIENS/SRF
MAGIC	Multiparent advanced generation cross
MALDI	Matrix-assisted laser desorption ionization

MAP	Microtubule-associated protein
MAP	Mitogen-activated protein
MAP65-3	Microtubule-associated protein 65-3
MAPK	Mitogen-activated protein kinase
MAPK4	Mitogen-activated protein kinase 4
MAPKK	A protein kinase that phosphorylates MAPK
MAPKKK	A protein kinase that phosphorylates MAPKK
MARS	Marker-assisted recurrent selection
MAS	Marker-assisted selection
MDG	Millennium Development Goals
MET	Multienvironment trial
Mi	*Meloidogyne incognita* resistance gene
MIcroSAtellite	MISA tool
MIM	Multiple interval mapping
MKK1	Mitogen-activated protein kinase kinase 1
MKK2	Mitogen-activated protein kinase kinase 2
MKK3	Mitogen-activated protein kinase kinase 3
MKK4	Mitogen-activated protein kinase kinase 4
MKK5	Mitogen-activated protein kinase kinase 5
MKK8	Mitogen-activated protein kinase kinase 8
MLO	Mildew resistance locus *o*
MMR	Meiotic mismatch repair genes
MnSOD	Manganese superoxide dismutase
mPing	Miniature Ping transposable element
MPK3	Mitogen-activated protein kinase 3
MPK4	Mitogen-activated protein kinase 4
MPK6	Mitogen-activated protein kinase 6
MPK8	Mitogen-activated protein kinase 8
MPSS	Massively parallel signature sequencing
mQTL	Metabolite level QTL
mRNA	Messenger-RNA
MSG	Melanesian Spearhead Group
MSV	Maize streak virus
MV	Methyl viologen
MYB	Myeloblastosis transcription factor
MYBRS	MYB recognition sequence
MYC2	MYC recognition sequence 2
MYCRS	MYC recognition sequence
NADP	Nicotinamide adenine dinucleotide phosphate
NAM	Nested association mapping
NARS	National Agricultural Research System
NAS	National Academy of Sciences
NASA	National Aerospatial Agency

NB	Nucleotide binding
NBRP	National BioResource Project
NCBI	National Center for Biotechnology Information
NCED	9-*Cis*-epoxycarotenoid dioxygenase
ncRNA	Noncoding-RNA
NDVI	Normalized differential vegetation index
NERICA	New Rices for Africa
NGO	Nongovernmental organization
NGP	Next-generation population
NGS	Next-generation sequencing
NHEJ	Nonhomologous end joining
NHX	Intracellular Na^+/H^+antiporter protein
NICRA	National Initiative for Climate-Resilient Agriculture
NIL	Near-isogenic lines
NMR	Nuclear magnetic resonance
NOA1	Nitric oxide-associated protein 1
NPR1	Nonexpressor of pathogenesis-related protein 1
NUE	Nitrogen use efficiency
NWP	Nairobi Work Program
OECD	Organization for Economic Cooperation and Development
ol-2	*Oidiumneo lycopersici* resistance 2
OPEC	Organization of the Petroleum Exporting Countries
ORA59	Octadecanoid-responsive *Arabidopsis* AP2/ERF 59
ORF	Open-reading frame
OsCDPK7	*Oryza sativa* calcium-dependent protein kinase 7
OsHKT1; 5	*Oryza sativa* high-affinity potassium transporter gene
OsMADS26:GR	*Oryza sativa* MADS-glucorticoid receptor
OsTOP6A3	*Oryza sativa* DNA topoisomerase 6A3 gene
OsTOP6B	*Oryza sativa* DNA topoisomerase 6B gene
P5CS	Delta 1-pyrroline-5-carboxylate synthetase
pad3	Phytoalexin deficient 3
PAGE	Polyacrylamide gel electrophoresis
PAMP	Pathogen-associated molecular pattern
PAP23	Purple acid phosphatase gene of *Arabidopsis*
PAT	Phosphinothricinacetyl transferase
PCR	Polymerase chain reaction
PDC	Pyruvate decarboxylase
PDR	Pathogen-derived resistance
PEN1	Penetration protein 1
PEN2	Penetration protein 2
PEN2	Penetration protein 2
PEN3	Penetration protein 3
PEN3	Penetration protein 3
PFC	Perfluorocarbon

PGR	Plant genetic resources
PGRFA	Plant Genetic Resources for Food and Agriculture
PI	Proteinase inhibitor
PIP	Phosphatidylinositol 4,5-biphosphate
PIPRA	Public Intellectual Property Resource for Agriculture
PLC	Phospholipase C
PLT	Patent Law Treaty
PMR4	Powdery mildew resistant 4
PopCEN1	Poplar ortholog of *CENTRORADIALIS*
pot-1	Potyvirus resistance 1
PPB	Participatory plant breeding
Ppd1	Photoperiod 1 locus
Ppd-B1	Photoperiod 1 locus on wheat chromosome B
Ppd-D1	Photoperiod 1 locus on wheat chromosome D
Ppd-H1	Photoperiod 1 locus of barley
Ppd-H2	Photoperiod 2 locus of barley
PR	Pathogenesis-related protein
PR1	Pathogenesis-related protein 1
PRGA	Participatory Research and Gender Analysis
ProDH	Proline dehydrogenase
PRR	Pseudo-response regulator
PRR	Pattern recognition receptor
PRSV	Papaya ring spot virus
PsMLO1	*Pisum sativum* mildew resistance locus 1
PTGS	Post-transcriptional gene silencing
PTI	PAMP-triggered immunity
PUE	Phosphorus uptake efficiency
Pup1	Phosphorus uptake 1 locus
PVPA	Plant Variety Protection Act
PVS	Participatory variety selection
Q-PCR	Quantitative-PCR
QTL	Quantitative trait locus/loci
R & D	Research and development
RAD	Restriction site-associated DNA
RAPD	Random(ly) amplified polymorphic DNA
RAS1	Response to ABA and salt 1 locus
RD	Responsive to dehydration
rd17	Responsive to dehydration 17 gene
RD22	Responsive to dehydration 22 gene
RD22	Responsive to dehydration 22
rd29A	Responsive to dehydration 29A gene
RFLP	Restriction fragment length polymorphism
RGA	Repressor of GA
R-gene	Resistance gene

RGL	RGA-like
RGL1	RGA-like 1 gene
RIL	Recombinant inbred line
RLK	Receptor-like kinase
RNAi	RNA-interference
RNA-seq	RNA-sequencing
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RuBisCO	Ribulose-1,5-bisphosphate carboxylase-oxygenase
RUE	Radiation use efficiency
RUR	Roundup Ready
SA	Salicylic acid
sad	Saponin deficient mutation
SAGE	Serial analysis of gene expression
SAMPL	Selective amplification of microsatellite polymorphic loci
SAR	Systemic-acquired resistance
SBSTA	Subsidiary Body for Scientific and Technological Advice (of the UNFCCC)
SCAR	Sequence-characterized amplified region
SCF	Skp, Cullin, F-box containing complex
SCOF-1	Soybean zinc-finger protein
SCP	Standing Committee on the Law of Patents
Sd1	Semidwarf 1 gene
SDS	Sodium dodecyl sulfate
SeeD	Seeds of discovery
SEMS	Standard evaluation systems
SG	Selective genotyping
S-gene	Susceptibility gene
SHI	*SHORT INTERNODES* gene
sHsp	Small heat-shock protein
siRNA	Small interfering-RNA
SK1	Snorkel 1 locus
SK2	Snorkel 2 locus
SKC-1	Shoot K^+ concentration 1 locus
SlMLO1	*Solanum lycopersicum* mildew resistance locus *o1*
SLR1	Slender rice 1 locus
SLRL1	SLR-like 1 locus
smRNA	Small-RNA
SMRT	Single molecule real time
SNAC	Stress-responsive NaC1
SNP	Single nucleotide polymorphism
SnRK1	Sucrose nonfermentation 1 (SNF1)-related kinases
SOC1	Suppressor of overexpression of *CO1*
SOD	Superoxide dismutase

SON1	Suppressor of nim1-1
SOS1	*Salt Overly Sensitive 1* gene
SOS-2	Salt overly sensitive-2
SOS-3	Salt overlay sensitive 3
SPA	Sanitary and Phytosanitary Agreement (of the WTO)
SPICE	Stratospheric Particle Injection for Climate Engineering
spl7	Rice spotted leaf (lesion-mimic) gene
SR1	*Signal Responsive 1* gene
SRAP	Sequence-related amplified polymorphism
SSD	Single-seed descent
SSI2	Suppressor of salicylic acid-insensitivity 1
SSR	Simple sequence repeat
SSRIT	Simple sequence repeat identification tool
StEREBP1	*Solanum tuberosum* EREBP1 gene
Stg1	Stay-green 1 locus
Stg2	Stay-green 2 locus
Stg3	Stay-green 3 locus
Stg4	Stay-green 4 locus
STPD	Sorbitol-6-phosphate dehydrogenase
STS	Sequence-tagged site
STY1	*STYLISH1* gene
Sub1	Submergence 1 locus
Sub1A	Submergence 1A gene
Sub1B	Submergence 1B gene
Sub1C	Submergence 1C gene
sw-5	Spotted wilt resistance 5 gene
SWP PRGA	System Wide Program on Participatory Research and Gender Analysis
TaCBF14	*Triticum aestivum* C-repeat binding factor 14 gene
TaCBF15	*Triticum aestivum* C-repeat binding factor 15 gene
TaCBF16	*Triticum aestivum* C-repeat binding factor 16 gene
TAIL	Tropically adapted inducer line
TAIR	The *Arabidopsis* Information Resource
TASSEL	Trait analysis by association, evolution, and linkage
TCE	Traditional cultural expression
T-DNA	Transfer-DNA
TF	Transcription factor
TILLING	Targeted induced local lesions in genomes
TK	Traditional knowledge
Tm	Tobacco mosaic virus resistance
TMV	Tobacco mosaic virus
TOF	Time-of-flight
TOM1	Tobamovirus multiplication protein 1
TOM3	Tobamovirus multiplication protein 3

TP	Training population
TPE	Target population of environments
TREB	Tax-responsive element-binding protein
TRF	Tandem repeat finder
TRIPS	Trade-Related Intellectual Property System
TRIPS	Trade-Related Aspects of Intellectual Property Rights (of WTO)
UNDP	United Nations Development Program
UNFCCC	United Nations Framework Convention on Climate Change
UPOV	International Convention for the Protection of New Varieties of Plants
USAID	US Agency for International Development
USDA	United States Department of Agriculture
USPTO	United States Patents and Trademarks Office
UV	Ultraviolet
Ve	*Verticillium* resistance gene
VGT	Vegetative to generative transition
Vgt1	Vegetative to generative transition 1 locus
VIGS	Virus-induced gene silencing
VNTR	Variable number of tandem repeats
VRN	Vernalization
VRN-1	Vernalization 1 locus
VRN2	Vernalization 2 locus
VRN3	Vernalization 3 locus
VRN-A1	Vernalization 1 locus on wheat chromosome 5A
VRN-B1	Vernalization 1 locus on wheat chromosome 5B
VRN-D1	Vernalization 1 locus on wheat chromosome 5D
VRN-H1	Vernalization 1 locus of barley
VRN-H2	Vernalization 2 locus of barley
VRN-H3	Vernalization 3 locus of barley
WARDA	West African Rice Development Association (now The Africa Rice Center)
WCS120	Wheat cold-shock 120 gene
WCS19	Wheat cold-shock 19 gene
WEMA	Water-Efficient Maize for Africa
WGR	Whole-genome resequencing
WGS	Whole-genome shotgun/Whole-genome scanning
WIPO	World Intellectual Property Organization
WRKY22	*WRKY* transcription factor 22
WRKY29	*WRKY* transcription factor 29
WRKY70	*WRKY* transcription factor 70
WTO	World Trade Organization
WUE	Water use efficiency
Xa	*Xanthomonas* resistance gene

XETC	Xyloglucanendotrans glycosylases
ZAT 12	A zinc-finger protein
ZCCT1	Zinc-finger-CCT domain 1 gene
ZCCT2	Zinc-finger-CCT domain 2 gene
ZFN	Zinc-finger nuclease
Zmt-12	Zinc deficiency-induced mortality-12 locus
Zmt-2	Zinc deficiency-induced mortality-2 locus

Contents

Chapter 1
Introduction

Michael T. Abberton

Abstract Climate change, coupled with the effects of population growth, excessive consumption, and environmental degradation, represents a challenge to future food security around the globe. To meet this challenge will require integrated approaches across the food system and in many other areas. Plant breeding is a central component of this response and has the potential to drive progress towards sustainable intensification. To do so effectively will require the judicious deployment of genomic tools, including DNA sequencing, allied with the focused utilization of germplasm diversity and precision high-throughput phenotyping.

1.1 Impact of Climate Change

The consequences of anthropogenic climate change are now being seen and have been predicted with greater or lesser degrees of certainty into the future. Global mean temperatures are increasing and precipitation patterns changing. The challenges facing the world have been enunciated many times over the past decade and include the concept of facing a "perfect storm" (Beddington 2009) in which concerns over food and water security and climate change, driven in part by population growth and exacerbated by environmental pressures and a decline in ecosystem services become more severe by 2030 and beyond. Such predictions have become statements of the consensus of opinion.

Increased environmental variability, for example in terms of increased frequency of episodes of environmental stress outside the range associated with adaptation, will have ecological significance. For example, interannual temperature variability is predicted to increase by 100 % for Central Europe by 2071–2100; with

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winter rainfall increasing and summer rainfall decreasing (Schär et al. 2004). Whilst water deficit is referred to frequently as an outcome of climate change, episodes of intense rainfall leading to flooding now occur more regularly in many countries and are likely to increase in frequency bringing other major challenges to national economies. In addition, prolonged summer droughts due to climate change will increase soil compaction and as a consequence will later increase opportunities for surface flooding.

1.2 Challenges to Food Production

The concept of "sustainable intensification" or "getting more with less" has become equally prevalent over this same period: the need to increase the production and productivity of agricultural systems whilst simultaneously ameliorating their impact on biodiversity, soil quality, air, and water pollution. At the present time more than one billion people are undernourished or malnourished.

Although food production per se is only part of the total "food system" and therefore an increase in food production can only be part of the answer to the need to increase food security, it is nonetheless likely to be an important part. Again, breeding of new crop varieties is only part of the chain through which improved varieties are utilized by farmers; other factors include agronomy and the farm system, the seed system, extension agencies, private sector dealers and the wider involvement of input and market supply chains. However, genetic improvement is a cornerstone of agricultural development as a central means by which research in genetics and genomics is translated to impact on farmers' fields.

The major challenges include:

1. Developing crops with improved drought tolerance and enhanced water use efficiency (WUE)
2. Improving tolerance of saline soils
3. Tolerance of floods and related consequences of changes in rainfall patterns
4. Increasing nutrient use efficiency and reducing pollution of water and air
5. Enhancing yield and guarding against losses from biotic and abiotic stresses

Drought is an important environmental factor limiting the productivity of crops worldwide. Climate change models predict greater variability in rainfall patterns and increased periods of summer drought will affect many regions. The growing demand for crops exhibiting greater drought tolerance and water use efficiency is reflected in the increasing emphasis on selection of varieties better able to tolerate prolonged periods of water deficit.

Problems of soil salinity are a major constraint to crop development in many drier areas of the world. In 1980, in excess of 3×10^6 ha of arable land were considered saline. This area has more than doubled in the past two decades (Malhotra and Blake 2005) and it is estimated that salinity in soil affects about 7 % of the land's surface. For many regions, the design of salt-tolerant crops is

considered a priority. Unfortunately, there are only a few naturally occurring salt-tolerant higher plant species. Rising sea-levels, and increased wind speeds both, a likely consequence of climate change, will also induce more instances of coastal flooding and increased salinity and desiccation stresses affecting particularly low-lying locations and coastal areas found typically in the UK and elsewhere.

Detailed studies have illustrated the importance of rooting depth and the vertical variability of root function on soil water uptake. They have also highlighted that the porosity of soil should not be considered a fixed parameter, but is actually under the influence of the vegetation (Macleod et al. 2007). Recently there have been a number of laboratory studies published, which describe how roots do change soil hydraulic properties (Macleod et al. 2007). The studies have demonstrated a change to the water release characteristics, which tend to be associated with an increased number of larger pores in the rhizosphere and an increase in water repellence. This follows from the observation that root activity tends to increase the number of large pores at the root–soil interface. The generation of soil structure by roots has been widely reported in the literature and there is evidence that this phenomenon is influenced by plant species.

Although water stress is the most intensively researched physical stress to root growth, field data show that it may not always be the most critical. Various physical stresses may act in combination to limit root elongation. Hypoxia, water stress, and mechanical impedance to root growth will change with the water content of the soil and their relative importance will depend upon the degree of soil compaction.

Many of the world's ecosystems are characterized either by suboptimal nutrient availability, ion toxicities, or both (Lynch and St Clair 2004) and the majority of world agriculture is conducted with low fertility inputs on soils with poor availability of P and other nutrients. Whilst inherent nutrient use efficiencies may increase under elevated CO_2 (Drake et al. 1997), potential gains in net primary productivity may be limited by nutrient availability and/or uptake, both of which may be affected in contradictory ways, depending on the climate variable considered. The reduction in transpiration by C_3 plants under elevated CO_2 may reduce transpiration-driven mass flow of (mobile) nutrients such as Ca to roots. Conversely, the increased C allocation to below-ground processes, including root biomass, may have a positive impact on productivity depending on nutrient availability. The poor understanding of how many aspects of root morphology and function, including root architecture and exudation, are likely to respond to climate change means that the identification and selection of adaptive traits remains difficult. Furthermore, traits conferring adaptation to one stress may incur negative "trade-offs" with respect to other stresses (Lynch and St Clair 2004). This is particularly so for root adaptations. For example, low P induces alterations in root architecture that enhance top soil foraging (Lynch and Brown 2001), but may consequently result in greater susceptibility to drought stress (Ho et al. 2004). Likewise, increased specific root length may result in decreased root life-span (Eissenstat 1997).

Prioritization of breeding objectives is complicated by the gaps in our understanding of the range and magnitude of adaptations likely to be required as the impacts of climate change become more acute.

1.3 Promise of Genomics and Breeding

In recent years considerable gains have been made in our understanding of plant genome organization and gene expression. In large part this has been achieved through the study of "model species," i.e., species in which genetics and genomics are more tractable than in many crop plants. The best known and most developed of these models is *Arabidopsis thaliana*, the DNA sequence of which was published in 2000. Subsequently, a number of different model species have been developed and the number of crop species (e.g., rice, soybean) with sequenced genomes has also increased.

Progress in developing the resources, tools, and approaches to allow the more rapid development of improved crops has been significant in the last decade. These include not only genomics, transcriptomics, and metabolomics but also noninvasive dynamic high-throughput phenotyping (phenomics) and novel approaches to germplasm characterization and population development. At the heart of germplasm improvement are genetic resources, their collection, characterization, and utilization. Genetic resources offer a vast reservoir of important novel traits and allelic variation for traits. Increasingly genomics tools are being brought to bear on the variation collected in genebanks (McCouch et al. 2012). New approaches to selection are required to take full advantage of the pace at which new genomic knowledge is being acquired.

A range of genomics tools are now available in those species, which have been the subject of most study, particularly in maize but now increasingly in rice and other cereals. Progress in wheat has been slow due to the limited extent of genetic variation in the crop and its hexaploid nature but next-generation sequencing (NGS) approaches are changing this rapidly (Berkman et al. 2012). Genome sequencing with ever-increasing speed and reducing costs brings with it the potential for "genotyping by sequencing" which when allied with sophisticated statistical approaches is likely to allow the potential of genomewide selection (GWS) to be realized more effectively (Heffner et al. 2009).

Of course a major modern tool for the improvement of specific traits is genetic engineering. To date the importance of this technology has been widespread for a few traits in a small number of crops. Clearly genetic engineering has proven to be a controversial topic in some parts of the world and has engendered much debate ranging well beyond technical issues. However, the importance of regulation and intellectual property is pervasive in the area of modern plant breeding and not restricted to genetic engineering).

Modern breeding in both its genomics and phenomics components is increasingly data-rich. The role and importance of bioinformatics in integrating these data

and converting them to usable knowledge has been emphasized greatly over the past 10–20 years and is now being reflected increasingly in public sector and commercial programs throughout the world.

There is an urgent need for increased public sector resources to be dedicated to the development of new varieties of crops for the tropics and subtropics. Genetic improvement approaches could be complemented by research to explore the potential of introduced species and ecotypes and allied with modeling of climate change scenarios to facilitate breeding targeted to the needs of regions most affected.

In many cases breeders seek improvements in yield and yield per unit of input. However, factors limiting yield are many and various and often these factors must be addressed directly in targeted approaches.

A major focus should be placed on water to develop climate-resilient crop varieties. Drought stress, water use efficiency, and root characteristics are important for interactions with soil including effects on flooding propensity.

Phenotypic selection for improved drought tolerance, or for yield under drought stress conditions, is widely accepted as difficult. Episodes of drought stress in natural environments are highly variable in their timing, duration, and severity, making it difficult to identify traits that confer a predictable advantage across stress environments (Passioura 1996). Direct selection for drought tolerance has been carried out in the field and indirect methods have also been used, but success has been limited. Where insufficient genetic variation is available to achieve any significant improvements in drought resistance from within a species, increasingly new allelic variants are being sought from wild relatives adapted to drier environments.

Many genes involved in plant adaptations to drought stress and desiccation-tolerance also confer improved salinity tolerance. New sources of salt tolerance are required as are more efficient techniques for identifying salt-tolerant germplasm so that new genes for salt tolerance can be introduced into crop cultivars. The mechanisms of salt tolerance remain poorly understood, despite salinity being studied in a range of glycophytic and halophytic plants. It is associated with a range of physiological adaptations, including ion compartmentation and the production of compatible solutes. Salt-tolerant plants may use vacuolar sodium storage and synthesize organic osmotic protectants including carbohydrates such as trehalose and fructans, and protein protectors such as glycine betaine and various compounds capable of scavenging reactive oxygen species like superoxide dismutase and glutathione peroxidase (Malhotra and Blake 2005). For legumes, an important consideration is the impact of salt on symbiotic nitrogen fixation. Free-living rhizobia are frequently more salt tolerant than their host, although the symbiotic process itself is sensitive to salinity. The transfer of gene(s) for salt tolerance into wheat following hybridization with the related (salt tolerant) species *Thinopyrum bessarabicum* (King et al. 1997) demonstrates the potential of introgression as a breeders' tool for transferring salt tolerance from wild into a crop species.

Water use efficiency, defined as the ratio between plant (usually shoot) production and transpiration is one measure of the ability of a plant to perform well under

incipient drought. This can also be defined as the yield of product/water consumed. Condon et al. (2004) identified three key processes that can be exploited in breeding for higher water-use efficiency (1) moving more of the available water through the crop rather than it being wasted as evaporation from the soil surface or drainage beyond the root zone or being left behind in the root zone at harvest; (2) acquiring more carbon (biomass) in exchange for the water transpired by the crop; (3) partitioning more of the achieved biomass into the harvested product. These processes are not independent and targeting specific traits to improve one of the processes may have detrimental effects on the other two. Direct evaluation of WUE, which requires precise measurement of individual plant growth and water consumption, is not feasible in the field, making selection for WUE difficult within a breeding program. However, carbon isotope discrimination provides a robust, if indirect, method of identifying variation in WUE and is increasingly used in breeding programs (Rytter 2005).

In considering climate change adaptation and mitigation we need to remember that food security depends on more than arable and horticultural agriculture. Grasslands cover about 70 % of the world's agricultural area. They have a crucial role in terms of food production and in the delivery of ecosystem services such as water supplies, biodiversity, and carbon sequestration. For a number of years the potential importance of legumes in many agroecosystems, but also the limited extent to which this potential has been realized, has been recognized. Legumes do not just contribute in terms of food, feed, and fertility but are also important as fuel wood and with respect to carbon sequestration. However, their key attribute and a major reason why they are so important for the future of world agriculture is the nitrogen-fixing symbioses they form with nodulating bacteria. Genetic and genomic tools have been applied powerfully in recent years to understand the control of the legume–rhizobia interaction utilizing model legumes particularly *Medicago truncatula* and *Lotus japonicus* and the great challenge is to deploy this information in the improvement of the major grain and forage legumes. The importance of roots and root traits (size, architecture, interactions with soil, exudation etc.) has long been recognized but progress has been slow. However, genomics approaches allied to the development of noninvasive dynamic imaging techniques capable of phenotyping root traits brings significant new opportunities.

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Chapter 2
Conservation, Evaluation, and Utilization of Biodiversity

Domenico Pignone and Karl Hammer

Abstract In 1984, Plant Genetic Resources (PGR) were defined by the International Undertaking of the FAO as the entire generative and vegetative reproductive material of species with economical and/or social value, especially for the agriculture of the present and the future, with special emphasis on nutritional plants. Within the last 100 years, with powerful support from the "plant genetic resources movement" since about 1970, large collections of PGR have been built up in genebanks as a reservoir for present and future tasks for breeding research and plant breeding. The disappearance of landraces and other materials, including in their centers of diversity, became evident (genetic erosion). The speeding up of the loss of species (extinction) initiated the global biodiversity discussions in the early 1980s. Extinction and genetic erosion are both driven by climatic change. Impending more significant damage through climatic change and global warming has led to conservation activities, which are largely based on in situ methods. But they are also useful for the conservation of wild relatives of crop plants and complement the ex situ approach of the genebanks.

The uses of PGR for improving the ability of crop plants to cope with the results of changing climate are described. They include newly introduced crop plants from wild progenitors, neglected and underutilized crops, and new plant combinations. The evaluation of PGR has to be intensified in order to obtain material with the desired characteristics (heat resistance, drought resistance, salt resistance, stress resistance). Germplasm enhancement is necessary to obtain material that can be used by breeders.

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PGR and their uses in breeding research and breeding have to be considered as an important basis for the fight against the various effects of climate change.

2.1 Climate Change and the Birth of Agriculture

It is interesting to note that men were forced into agriculture at the end of the last glacial age. Richerson et al. (2001) hypothesized that agriculture had become possible in the Holocene due to the climatic change that accompanied the end of the glacial era. In fact, in earlier times, even in the late Pleistocene, agriculture could have not been possible due to the extremely variable climate of that period, and the low atmospheric CO_2 levels. The so-called Younger Dryas event led to the success of the experiments with farming as it created a superior and more stable availability of food and better utilization of land resources. It is evident that on the same parcel of land, a larger population of farmers can be fed than of hunter-gatherers. Further support for this hypothesis comes from the fact that agriculture was "invented" almost simultaneously in disparate parts of the world separated by great distances (Merrill 1930; Diamond 1997).

The subsequent spread of human populations away from the areas in which the primitive domesticates had been produced and, therefore, into different environments, forced the adaptation and selection of genotypes that better suited the new ecoclimatic conditions (Pignone 2009). The resultant interventions through human-driven selection based mostly on cultural factors such as preference, religion, and superstition, although performed in a nonscientific way (at least in the sense of how we now define the word science), increased the differentiation of the various crops (Hammer 1984, 1988). These two processes, i.e., adaptation and human selection, gave rise to the great wealth of agricultural biodiversity that characterized the agricultural systems until the "green revolution" in the mid-twentieth century.

The first explorer and utilizer of this gigantic store of useful agricultural traits was Nikolai Vavilov (1926a). During a series of travels to the different parts of the newly constituted Soviet Union and beyond, he happened to notice that different varieties of each crop were cultivated in different areas of the country. Moreover, he recorded that the closer he got to the Caucasic regions, the greater the observed level of crop diversity. He hypothesized that in the areas where a crop had originally been domesticated the level of genetic diversity should be the highest, since reduction in diversity was a result of adaptation to climates that became different over time (Vavilov 1935). Travels to the five continents (Vavilov 1997) led him to formulate his hypothesis of the "Centers of origin" of crops, a hypothesis that modern molecular biology approaches have demonstrated to be largely veritable (Salamini et al. 2002).

He was among the first scientists to recognize the utility of such diversity for breeding new varieties, possibly influenced by the experience of the Italian agronomist Nazareno Strampelli, who had initiated a breeding campaign aimed at assuring

sufficiency of wheat in Italy (Giorgi and Porfiri 1998; Brandolini and Vaccino 2012). He devised the concept of storing all the diversity he had collected in a single site thus giving birth to the idea of a gene bank as early as 1925. The modern concept of gene bank was elaborated many years later during the 1968 FAO Technical Conference in Rome. The latest development of this concept is the Global Seed Vault built in the Svalbard archipelago, aimed at conserving a duplicate of all the plant germplasm of the world (some six million samples) in a vault built deep in the permafrost at a high enough altitude to survive even if all the ice of the world should melt. The Global Seed Vault can be seen as a sort of modern Noah's Ark for seed-propagated crop varieties, representing a part of our responsibility for the future (Jonas 2002).

2.2 Climate Change and Paradigm Shift in Agriculture

2.2.1 Shift of Agricultural Areas

As many indicators suggest, global warming will drastically affect agriculture. This change will be particularly evident in particular parts of the Planet. The Mediterranean region is already experiencing the effects of climatic change due to peculiar meteorological conditions. The main effect, besides warming, is the change in the precipitation pattern (Ruosteenoja et al. 2003). In fact, even if the total amount of precipitation does not change drastically, the number and intensity of rainfall will. In this scenario, the Mediterranean region will be a hot-spot of climate change (IPCC 2007). In the last decades, a reduction in winter precipitation has been observed over large areas of the Mediterranean region. This trend is particularly evident in the East Mediterranean region (Oikonnomou et al. 2008).

Many Mediterranean crops are quite well adapted to long dry seasons, but the composition of agricultural systems in the Mediterranean area has changed much over the last decades. For instance, horticultural crops have grown in importance over traditional rain-fed ones, like wheat, olive, or pulses. The need for irrigation of horticultural crops implies a better use of water resources. Achieving this goal implies many different actions: to rationalize water accumulation in natural and artificial reservoirs, to downsize the amount of water used in irrigation through water-saving methods, to reduce the use of underground water so as to limit their salinization, especially in coastal areas, to reutilize waste waters, and, last but not least, to breed plants with improved water use efficiency. This latter goal is also based on a more efficient use of species' biodiversity and therefore is based on the availability of vast reservoirs of germplasm, not only plant diversity, but also microbial diversity. In fact, specific microbial strains can interact with the root system in order to improve its capability of catching soil water (Bianco and Defez 2011).

Nonetheless, it is possible to hypothesize that in the next 30 years there will also be a shift in the plants cultivated in the Mediterranean. An example could be *Abelmoschus esculentus*, a plant already cultivated in southern and eastern Mediterranean areas and well adapted to dry conditions. The constant immigration flux towards Europe and consequent cultural shifts will possibly lead to a more extended cultivation of this crop also in countries, like Italy or Spain, where it was traditionally absent, as some experience in local markets demonstrates.

2.2.2 Temperature Change

Similar reasoning applies to temperature increase. It is estimated that average temperature will increase between 0.2 °C and 0.3 °C per decade (IPCC 2007). This appears to be a very low value, but it is not. Recent data on temperature show that the Mediterranean is going to face milder winters and warmer summers, with a higher frequency of extreme phenomena such as heat waves. This change will affect agricultural crops in two ways: warmer winters may affect the production of those crops needing vernalization to achieve their full productive potential, and heat waves can affect pollination and fruit development as well as quicken the dehydration of cereals, leading to shriveled seed. Under different scenarios a reduction of the crops' production between 30 and 70 % has been predicted, in the absence of interventions (Burney et al. 2010).

Once more, breeding efforts should be directed at plants possessing escape mechanisms, such as lesser sensitivity to cold, faster and earlier pollination and ripening.

2.2.3 Change in Atmospheric CO_2 Levels

Higher levels of atmospheric CO_2 can have a beneficial effect on plants, since it can contribute to a more efficient production of carbohydrates useful for plant energetic needs. As a matter of fact, increased levels of CO_2 at the beginning of the Holocene are supposed to have been one of the triggers of agriculture (Beerling 1999).

This aside, there are not only direct consequences of the increased level of CO_2 and numerous implications for plant growth (Morison and Morecroft 2006) but also indirect changes have been reported, such as for example, sea level rises (Braasch 2007).

2.2.4 Spread of Pests/Diseases

Temperature is recognized as the principal abiotic factor directly affecting herbivorous insects. Temperature, in fact, affects the development, survival, distribution, and abundance of plant-eating insects (Bale et al. 2002). Plant species with a large geographic range will tend to be less affected. Fleming and Volney (1995) pointed out that the quality changes induced in plants by drought stress, in association with the effect of temperature increase on insects' biology might damage the natural host–parasite equilibrium. Larval development and feeding habit could be drastically changed by the suggested average temperature increase. Even though this change could be partially compensated by a more rapid plant growth, also as a consequence of increased CO_2 levels, the combination of these two effects cannot easily be predicted (Cannon 1998).

Another important effect of temperature increase is observed in the distribution range of the insects, which are more frequently observed in areas in which they were not previously present (Bale et al. 2002). This phenomenon is a general one relating to all living organisms and remarkably consistent with the shifting of isotherms (Walther et al. 2002). The expansion/change of insect-pests' range and distribution is not a problem limited to crops alone, but has a bearing on vegetation biodiversity as a whole. In fact, many invasive species possess common traits that allow them to take advantage of the different elements of global change. The presence of new invader species or changes to the prevalence of autochthonous ones would alter the equilibrium of the basic ecosystem with consequences for biodiversity (Dukes and Mooney 1999).

Similar to herbivorous insects and pests, other insects with importance to agriculture and biodiversity are also affected by climate change, such as, for instance, pollinators. In fact, changes in insect range should not only be appreciated from the view point of new species entering a given environment, but also from the view point of species not finding the new environment suitable to their biological needs. A study on butterflies of northern Spain (Stefanescu et al. 2003) indicates the consequences of climate change on butterfly population abundance.

The role of pollinators cannot be underestimated. Climate changes may affect the reproduction biology of pollinators, as also cultivation conditions can. In cases where domesticated honeybees are not bred close to a field, it has been demonstrated that the number of visits by wild bees drops in proportion to the distance to the natural habitats (Levy 2011). Recent evidence demonstrates that in terms of wild bees, problems attributed to monoculture can be overcome by pollinators feeding on weeds (Winfree et al. 2011). An alternative could be to plant multicrop fields within a distance capable of ensuring the correct feeding by the wild insects. This becomes particularly important when new crops are introduced on which pollinators are not accustomed to feed.

The present literature data are too limited to evaluate in detail the overall impact of climate change on terrestrial productivity and crop production (Roy et al. 2001). Anyhow, most likely the impact of this change will be observed through the effect

of plant diseases on crop production, and mainly in yield losses, in the efficacy of disease management strategies, and in the geographical distribution of those diseases (Chakraborty et al. 2000).

2.2.5 *Disappearance of Landraces and Crop Wild Relatives*

Genetic erosion has also reached plant genetic resources in remote areas and centers of crop diversity (Brush 1999). Ex situ conservation methods have been complemented by in situ efforts to counteract the ongoing trend (Hammer 2003). On-farm conservation has come into the focus of researchers (Brush 2000). There was already a sharp decline in collecting missions organized by IPGRI/Bioversity in the last decade of the past century (Thormann et al. 2012). Only relatively late did new interest in landraces emerge (Zeven 1998) and lead to concerted actions and a new awareness (Veteläinen et al. 2009).

Crop wild relatives (CWRs) are less endangered than landraces. A special approach has to be developed toward their maintenance and use. Large programs have been established (Maxted et al. 2008). CWRs can easily be included into programs for the conservation of wild species, a topic which is very important in the general biodiversity discussion (Heywood and Dulloo 2006). In general, the loss of landraces due to climatic changes is greater than that of the CWRs.

2.3 How Can Plant Genetic Resources Help?

With the advent of intensive agriculture, and monoculture in particular, the ancient varieties have progressively been replaced by modern high-performing varieties. Moreover, the number of such modern varieties is much lower than the number of local varieties cultivated previously. The result of this change in the structure of crop fields has been the loss of genetic diversity.

In the 1960s the scientific community realized the urgent necessity of preserving the declining crop genetic variation by putting in action measures of preservation. The “Plant Genetic Resources Movement” was developed (Pistorius 1997) and it helped to guide large collection, conservation, and utilization programs. The central theme was how to combat the threat of mass starvation and to provide solutions to world food problems (Jackson et al. 1990). A first success was the Green Revolution showing on a highly selected genetic basis of two crops (mainly dwarfing genes in wheat and rice) that plant breeding can produce dramatic yield increases. Strong support from internationally financed institutions within the CGIAR system was necessary to create the conditions and manage the operations. But there have been unexpected social costs and other obstacles connected with the Green Revolution technology and many peasant farmers in the Third World could not participate in its success. Another surprise was the loss of genetic resources, especially in

developing countries. New efforts were necessary to secure global food security (Rosegrant and Cline 2003). A kind of new Green Revolution was proposed based on different ways of increasing diversity (Cleveland and Soleri 1989). Yield was always the decisive criterion for progress and methods were developed for this important improvement (Austin et al. 1980). With the promise of increasing wheat production for the world (Braun et al. 1998), a strategy for better use of crop genetic diversity has to be developed (Hoisington et al. 1999). In a recent review (Ortiz et al. 2008), CIMMYT (The International Maize and Wheat Improvement Center) as one of the most important CGIAR (Consultative Group on International Agricultural Research) centers and a main promoter of the Green Revolution, summarized its efforts in terms of wheat genetic resource enhancement discussing the main breeding objectives for wheat which included yield potential, stability and wide adaptation, disease resistance, water use efficiency and drought tolerance, heat tolerance and end-use quality. In a concluding remark they state that "... the wealth of genetic resources available in the wheat gene pools (including wild species) will be among the important sources available to plant breeders in their quest for high and stable yielding wheat cultivars that meet the end use quality demands at a time of limited resources and global warming."

2.3.1 Search for Adaptation Characters

Besides the availability of such vast wealth of genetic resources, the use of plant germplasm in crop improvement continues to be a significant challenge (Glaszmann et al. 2010). In fact, it is difficult to dig into germplasm collections consisting of thousands of samples in order to find the few genetic traits useful to breeding. In the last decades many strategies have been developed to solve this challenge.

Some 25 years ago the concept of core collection was developed. A core collection is a subset (under 3,000 samples) of a vast germplasm collection intended to be quite representative of the whole genetic variation present in the original collection (Frankel 1984). The subset size is intended as a sort of genetic platform of a more manageable size on which to perform gene hunting. The indicators used to develop such a platform are mostly the geographic origin and morphological traits considered to be representative of the genetic diversity (Brown 1989). Citing the author, "One aim of the core is to build up a body of information on a restricted 'reference' set of lines" (Brown 1989).

The concept of a "reference set" is a further refinement of the "core collection" idea. It refers to a smaller set (under 500 samples) well characterized at both phenotypic and genetic level that can be shared among different groups in order to achieve converging results. Within the Generation Challenge Program much support has been devoted to developing such reference sets from the findings of different coordinated groups (Glaszmann et al. 2010).

All forms of germplasm collection are not merely intended to conserve genetic resources threatened by extinction. In the recent genetic concept they are rather intended as a biological platform on which to perform genetic studies. Such bio-platforms have the same importance to the scientific community as large hardware facilities, like the synchrotron, have for physics. From this viewpoint the collections are the vehicle geneticists can use to define the position and function of Mendelian genes and quantitative trait loci (QTLs). An example of such utilization is provided by Mathews et al. (2008).

2.3.2 Search for New Genes/Alleles/Epialleles (Genetics)

The analysis of plant physiology has allowed the identification of traits able to confer resistance to abiotic stresses. Some of these traits are substantially variable: root traits, water use efficiency, transpiration efficiency, stem water soluble carbohydrates, etc. (Latha et al. 2004; Dwivedi et al. 2005). Once the useful traits have been identified, different approaches can be used to identify the genetic determinants of those traits. To access this vast domain of traits it is essential to have a precise description of the crop genomes (Glaszmann et al. 2010). The availability of saturated genetic maps of principal crops is an essential prerequisite to identify, for instance, QTLs and treat them as Mendelian genes. Some of these QTLs are directly associated with resistance to environmental factors (Bidinger et al. 2005; Mathews et al. 2008).

The access to germplasm collections has also allowed the identification of whole genotypes better adapted to specific environmental conditions. As an example, the use of some 150 different local varieties of wheat has allowed the identification of salt-tolerant genotypes useful as donors of this trait in breeding programs (Badridze et al. 2009).

At the same time, with different approaches, it has been possible to investigate the role and function of single genes in the plant response to general or specific abiotic stress. Recent times have seen the focus switch from genes to their control. In fact, plant growth is strongly influenced by interaction with the growing environment and its stresses, which often results in the reduction of productivity. This process involves the alteration of expression of a large number of genes. For this reason the study of genetic interaction in a genotype is becoming more and more important.

Kobayashi et al. (2008) indicated that in common wheat a gene known as *WABI5* acts as a transcriptional regulator of the *Cor*/*Lea* genes in multiple abiotic stress responses. The genes *Cor* (cold-responsive) and *Lea* (late embryogenesis abundant) are, in fact, a gene family coding for proteins, which are reported to promote the development of freezing tolerance acting as protectors of cellular components.

The role of epigenetics in contributing to stress tolerance appears to be more important than previously estimated. Rahaie et al. (2010) reported on the role of MYB elements in the response to salinity and drought stresses. The MYB elements

are a family of transcription factors, a rather large one with tens of members different in length and nucleotide sequence, which is known to modulate a vast range of genes during different phases of plant development, and in response to external stimuli (Yanhui et al. 2006). The general picture that comes from these studies is that crop adaptation to abiotic stresses implies the functional alterations of the expression of a large number of genes, all synergically involved in plant protection, and of their regulatory elements. In this view the role of transcription factors is gaining importance.

In fact, there is enough evidence that epigenetic knowledge can be of great interest to plant breeders, not only for identifying candidate genes for the selection of promising genotypes, but also as a source of new useful characters to develop new varieties. It is now evident that endogenous epigenetic elements regulating plant genes can form heritable genetic variants named epigenes, which not only regulate true genes but also other elements of the intragenic space (like transposons), which are involved in the modulation of the genetic information (for a review and insights, please refer to Mirouze and Paszkowski 2011).

2.3.3 New Breeding Strategies (Traditional vs. Biotech)

The challenges facing food production in the forthcoming scenario of population growth, climate change, a paradigm shift of agriculture, etc., have brought into focus the debate on the use of genetically modified organisms (GMOs) as an answer to these challenges (Gepts 2002; Hammer and Teklu 2008). The political position of different countries in this respect is very diversified, ranging from liberal growth to being complete forbidden. Nevertheless, new strategies are needed to improve crop plants in a faster and more efficient way. In the last decades genetic studies have produced several new tools for plant breeders (Callow et al. 1997) but a full exploitation of the genetic potential of crops present in the plant collections is still far from being achieved. Direct genetic manipulation of the genome of a given variety has the great advantage of rapidly adding a new single trait to a well-stabilized and performing genotype, while crossing may introduce unfavorable characters that need backcrossing and selection cycles to be removed.

Scientists have been exploring new possibilities of producing GMOs that do not fall within the categories that are under strict control of the authorities (Waltz 2012). A recent issue of Nature Biotechnology (volume 30, no. 3, 2012) reports on many of these new techniques and implications. Some of them had not been intended for the production of GM plants, but as tools for better exploring gene functions. One of them, the zinc finger nuclease (ZFN) technique was originally intended to better dissect gene functions by inducing double-strand breaks in DNA at specific sites through the use of engineered nucleases. The use of these modified enzymes actually produces a site-directed mutagenesis that can extend its effects over a certain span of DNA length (Townsend et al. 2009). The ZFN technique can, therefore, be used to directly modify the function of a specific gene through

mutagenizing it. The difference to classical mutagenesis is that the latter produces mutations at random in the genome and in a number of sites at the same time which is difficult to control. This is one of the reasons why classical mutagenesis has found limited application.

The potential of these new techniques for manipulating plant functions through direct DNA manipulation often does not fall within the classical GMO definition, and could therefore obtain better acceptance from both Governments and consumers.

2.3.4 The Role of Crop Wild Relatives

Crop wild relatives have long been considered an excellent source of useful genes for plant breeding (Harlan 1976; Hawkes 1977; Hajjar and Hodgkin 2007; Sonnante and Pignone 2008). In fact, during the domestication and subsequent selection processes many traits have been unintentionally lost, so that the crops of today have a reduced genetic base. This phenomenon has been particularly intense for cereals (Feuillet et al. 2007). For some CWR, large collections were established at an early time together with the methods to secure their maintenance under agricultural/horticultural conditions, for example for *Aegilops* (Hammer 1980). The fast development in this genus is outlined in a recent publication (Kilian et al. 2011). The work with CWR has made good progress over the last few years (Maxted et al. 2008). CWR collections are still not as abundant as those of crop landraces but some improvements are also being made in this respect (e.g., Veteläinen et al. 2009).

Besides the reproductive barriers between crops and their relatives the main problem in gene transfer from wild to cultivated plants is at the level of hybrid meiosis. The differentiation of genomes, in fact, does not allow much recombination between genomes that are even slightly different. Over the years, though, a series of genes have been discovered that reduce the capability of the meiotic control systems to recognize differences, thus favoring chromosome pairing also in interspecific hybrids. Wheat scientists are familiar with the *Ph* gene, which has recently also been proposed as a preventive measure against the introduction of transgenes into wild wheat relatives (Weissmann et al. 2008).

Unfortunately genes favoring homeologous pairing are rarer in other genera. Nevertheless, the improved knowledge of plant genetic mechanisms allows one to also manipulate the meiotic pairing control system. Tam et al. (2011) produced increased meiotic pairing between chromosomes of *Solanum lycopersicum* and the wild relative *S. lycopersicoides* by silencing the *S. lycopersicum* endogenous MMR (meiotic mismatch repair) genes using RNA interference-induced silencing. RNA interference (RNAi) is a mechanism normally present in cells and aimed at the regulation of gene transcription. It is based on small RNAs that bind to messenger RNA and through a variety of mechanisms reduce the expression of that character.

It appears evident that the exploitation of CWR can also depend on biotechnology even though not through a classical GM approach.

It is interesting to note that the exploitation of wild crop relatives' diversity is not a prerogative of scientists and breeders. Pujol et al. (2005) reported that in fields of cassava landraces (*Manihot esculenta*), a vegetatively propagated crop, a higher than expected level of heterozygosity for clonal propagation can be found. They observed that farmers regularly introduce plants in the fields arising from sexually produced seeds. Among these plants showing a low level of heterosis, there is a few that are highly heterotic. Farmers select these plants. A similar mechanism has also been proposed for the observed heterosis in artichoke (Sonnante et al. 2007).

2.3.5 Domesticating New Crops

The statement made by Anderson (1967) seems to be true that not a single new (important) domesticate is known to have been produced for millennia. Despite the advantages of polyculture (Altieri 1991, 1994), agricultural monoculture prevails, leading to extinction and genetic erosion (Khoshbakht and Hammer 2010).

But we can rely on serendipity in the exploration of biodiversity (Iltis 1988) and plant genetic resources to initiate new developments and eventually even establish new crops. It took more than 100 years to develop the new successful triticale from the cross of wheat and rye, creating the first man-made cereal (Guedes-Pinto et al. 1996). New developments are underway including a combination between wheat and barley (×*Tritordeum* Aschers. et Graebn.) and other relatives of wheat (Mujeeb-Kazi and Rajaram 2002). Wheat, in evolutionary connection with many related species, is clearly becoming a super-domesticate in the sense of Vaughan et al. (2007). Other world crops such as maize, barley, tomato, banana, sugarcane, *Vigna*, *Phaseolus*, and rice are following the same lines (see Vaughan et al. 2007).

Considering that 30 crops nourish mankind—some scientists even refer to only seven columns of world nutrition (Brücher 1982)—we come to the conclusion that this limited number seems insufficient in the light of biodiversity considerations. The approximately 7,000 cultivated plants known to date (excluding ornamentals) (Hanelt and IPK 2001), most of them largely forgotten or neglected and underutilized, form a good basis for the development of future new crops resilient to the effects of climatic change.

Taking the large amount of domestication efforts and their reported outcome (Hanelt and IPK 2001), it is difficult to imagine completely new development. Kiwifruit, *Actinidia deliciosa*, a delicious fruit, experienced its primary domestication in China about 1,200 years ago. It developed into a world crop as late as 1937, when commercial cultivation started in New Zealand (Ferguson 2007). There are several other examples of minor crops gaining world importance.

But there are some new developments in connection with the side effects of global warming. Saline agriculture is making progress and uses a number of traditionally used halophytes but also some newly used species (Lieth et al.

1999). Palmer's salt grass (*Distichlis palmeri*) from North America has wheat-like grains, which may be cooked or roasted. At one time it was even thought to be extinct. But it was redetected and proposed as a crop for saline soils in Arizona (Yensen 1987). CAM (Crassulacean Acid Metabolism) plants with good drought resistance, Cactaceae especially from the genus *Opuntia*, have been selected as new vegetable plants and for their fruits (Scheinvar 2007).

2.4 Current Status of PGR Utilization

The basic preconditions to make PGR available for the present and future needs of mankind have been achieved in a powerful international action (FAO 2010). Large amounts of plant material (cultivated and wild) have been collected, conserved ex situ (mainly in gene banks) or in situ/on farm. Characterizations and evaluations have been done and selected material has been transferred to farmers and breeders. Finally, the selected material has been used for germplasm enhancement by a growing scientific community for better use in plant breeding. There was certainly progress in the use of PGR for breeding purposes directly or indirectly aimed at improving characters useful for enduring climate change.

2.5 Conclusions

More than 20 years ago Jackson et al. (1990) reported on a workshop entitled "Climatic change and plant genetic resources." Their conclusions were alarming and prognostic at the same time and they have been taken, in a form only slightly changed and supplemented with some additional points, as the basis for the brief discussion below. Their prognoses were given just in the highly developed "plant genetic resources movement" (Pistorius 1997) during the forcefully emerging biodiversity approach (Solbrig et al. 1994). Their prognoses should be a a guideline for discussions in decision-makers cyrcle (FAO 2008).

1. In view of the significant climate changes that have already occurred or are still to be expected (IPCC 2007), the role of PGR will increase. Research and management of PGR has to be further developed and improved, particularly with respect to their collection, conservation, and utilization strategies. Nevertheless, the number of samples gathered during collecting trips supported by IPGRI shows a sharp decline after 1985, the proposed end of the plant genetic resources movement (Thormann et al. 2012).
2. Climate models and global vegetation patterns are an integral part of biodiversity research. Global biodiversity conservation in the face of climatic changes becomes the most important task. Mostly endangered vegetation areas such as mangroves are included here (see point 3 in Jackson et al. 1990). A wealth of

biodiversity can be found in the so-called biodiversity hotspots (Zachos and Habel 2011). The centers of diversity for cultivated plants, a concept of Vavilov (1926b), obtained much less attention, with a few exceptions, for example in connection with on-farm conservation (Brush 2000). A comparison between hotspots and diversity centers for cultivated plants is still missing. But despite notable progress, biodiversity still declines (Stokstad 2010).

3. The conservation of ecosystems plays an important role. This is also true for ecosystems of cultivated plants in centers of diversity (Brush 2000), and provides a new aspect to the concentration on the gene-ecological model of genebanks (Frankel and Bennett 1970; Hawkes et al. 2000).
4. Priority should be given to the tropical areas because these areas are mostly affected by global warming. Several of the International Agricultural Research Centers are situated in those areas. Their mandate crops include those from the tropics.
5. More than 7,000 plant species are cultivated. Another 115,000 species can be considered as PGR (Hammer 2003). This material is urgently needed for establishing new genetic combinations towards super-domesticates (Vaughan et al. 2007) and also newly domesticated plants in the light of global warming and interconnected changes.
6. Intensive work is necessary for a better characterization and evaluation of the infraspecific diversity of crop plants and CWRs. A better understanding of ecogeographic variation is needed (Guarino et al. 2005). These are important results based on which the plant breeder can select new cultivars (Becker 2000; Hammer et al. 2012).
7. Screenings should be intensified for drought tolerance, raised temperatures, and salinity. Adequate progress has still not been made with respect to stress. Both agronomy and plant breeding still have to find the right approach.
8. Physiological research has to be intensified also with respect to photosynthetic rates and water use efficiency. Plant breeders need support from physiologists to radically change crops from C_3 photosynthesis to C_4 photosynthesis. It is known, that C_3 plants have enzymes and pathways for C_4 photosynthesis. Such genes have already been successfully expressed in rice (Mitchell and Sheehy 2006).
9. Plant growth simulation models can also be useful for plant breeders. They will help to understand the nexus of environmental changes and the tasks of the plant breeder.
10. Apart from agronomical research, plant breeding will have the important task of using PGR to improve crops enabling them to cope with global warming and its interconnected effects. A major task is and will be the successful integration of genetic information from PGR into established crops. For the combination of primitive germplasm and advanced breeding material, germplasm enhancement was developed as a new approach (Ortiz 2002; Hajjar and Hodgkin 2007). Usually this approach cannot be performed by the plant breeder responsible for the new cultivars. Breeding research of the International Agricultural Centers,

many National Centers, Universities, and other research bodies is the indispensable precondition for successfully using PGR.

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Chapter 3
Identification of Traits, Genes, and Crops of the Future

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Abstract Studies on vulnerability and resilience of forest plant communities and crop species have multiplied with the growing realization that societal and scientific response is necessary to adapt to climate change impacts. The DPSIR (Driving forces, Pressure, State, Impact, Response) conceptual framework provides one of the simplest structure of indicators required to connect and model the dynamic systems of the causative and correlative components of climate envelopes and the genetic and genomic complexities regulating adaptive plant response to fluctuating environments and climate. Paleoclimate and vegetation type reconstruction from fossil records and species vicariance help in understanding the long-term dynamics of plant features and trait evolution associated with dispersal and climate changes. Comparative genomics demonstrated as alleles for those plant features (i.e., plant morphology and phenophase alteration), and for biotic (response to bacterial and fungal pathogens) and abiotic (i.e., drought, flooding) stress resistance are still part of the standing genetic endowment of the living gene pools of the crop and forest

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plant species and allied wild relatives. The data gathered so far are beginning to offer important insights into the candidate genes and gene networks for resistance to pests and disease outbreaks favored by the changing climate, and tolerance to the perturbed climatic components such as air temperature. Genomic scans have resulted in some remarkable discoveries, including genes sensing the amount of fall and winter chilling hours affecting the annual plant rhythms such as bud dormancy break in trees, and the master genes for plant adaptation to the changing patterns of the annual distribution and intensity of rainfalls and consequent drought or flood hitting crops. Application of the current genetic discoveries and technological advances in genomics will allow the many long-standing questions about the nature of adaptation to be answered and assist with the implementation of innovative breeding methodologies for plant trait enhancement in forest plant communities and farmer's fields to increase adaptive response and resilience to global warming.

Dedication

This chapter is dedicated to the great plant geneticist Prof. G. T. Scarascia Mugnozza and to his students and collaborators Girolamo Fanizza and Ivana Greco who enhanced with their friendship and support our earlier steps in plant breeding and genetics.

3.1 Introduction

3.1.1 Definitions of Climate Change

According to the Intergovernmental Panel on Climate Change (IPCC) usage, climate change refers to an alteration in the state of the climate that can be identified (e.g., using statistical tests) by the persistence for an extended period (decades or longer) of modified mean and/or variability of the climate properties compared to a previous reference period. It refers to any significant change in climate over time, whether due to natural variability or as a result of human activity (IPCC 2007a). This definition differs from that proposed by the United Nations Framework Convention on Climate Change (UNFCCC), where climate change refers to a change of climate that is attributed directly or indirectly to human activity that alters the composition of the global atmosphere and that is in addition to natural climate variability observed over comparable time periods.

The dominant human activity or driving force for climate change is fossil-fuel combustion (because of its carbon dioxide emission) (EEA 2000). Other activities that contribute to greenhouse gas (GHG) emissions are agriculture, land use changes (including deforestation), waste disposal to landfills, and industrial processes such as cement production, refrigeration, foam blowing, and solvent use. Gases and particles emitted from aircraft directly to the upper troposphere and lower stratosphere also contribute to climate change.

The greenhouse effect is a natural phenomenon. However, over the past century atmospheric concentrations of anthropogenic GHG—carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O) and halogenated compounds such as

chlorofluorocarbons (CFCs), hydrofluorocarbons (HFCs), perfluorocarbons (PFCs), and sulfur hexafluoride—have risen, and a considerable increase, in historic terms, in global mean temperatures has been observed.

Since the beginning of the Industrial Revolution, carbon dioxide levels have jumped from 280 parts per million (ppm), a level characteristic of the interglacial periods, to 389 ppm at the end of 2009, resulting mostly from the human activities of agriculture, deforestation, and burning fossil fuels. Agriculture accounts for 10–12 % of total global anthropogenic emissions of GHG, half of which is due to emissions of CH_4 (5.5 %) and N_2O (6 %), respectively. N_2O emissions from soils and CH_4 from enteric fermentation constitute the largest sources, 38 % and 32 % of total non-CO_2 emissions.

Since 2000, the growth rate in CO_2 concentration has increased more rapidly than in the previous decades (Canadell et al. 2007). Similar trends have been observed for CH_4, N_2O, and other GHG (Spahni et al. 2005; IPCC 2007a).

Cars, power plants, factories, and homes release about 5.5 giga metric tons of carbon dioxide every year (Gt C per year) as fossil fuels such as coal, oil, and natural gas are burnt to produce energy. Another 1.6 Gt C per year are released by deforestation, especially in the tropics. Some of this carbon dioxide is absorbed by oceans, plants, and other natural "sinks." But about 3.2 Gt C per year remains in our atmosphere every year.

There is growing evidence that GHG emissions from human activities are causing an enhanced greenhouse effect. These gases, which may remain in the troposphere from a decade to centuries, act to heat the planet because of absorption and re-radiation of infrared radiation.

This causes pressure on tropospheric physical properties such as temperature increase igniting global warming, which lead to climate change (NAS 2008). Since 1880, average global temperatures have increased by 0.8 °C (NASA) and during the past three decades, average global temperatures have climbed about 0.2 °C per decade. Between 1990 and 2100, if emissions continue to increase, temperatures are expected to rise between 1.1 °C and 6.4 °C.

According to Gerlach (2011), five recent studies suggested that volcanoes worldwide (such as Alaska's Shishaldin) emit, on average, between 0.130 and 0.440 Gt C per year. "But in 2010, anthropogenic emissions of the planet-warming gas were estimated to be a whopping 35 Gt C per year. Individual events—such as Mount Pinatubo, whose major eruption in 1991 lasted about 9 h—can produce CO_2 at the same rate that humans do, but they do so only for short periods of time. It would take more than 700 Mount Pinatubo-sized eruptions over the course of a year to emit as much carbon dioxide as people do, the study notes" "(Sid Perkins, 2011, http://news.sciencemag.org/sciencenow/2011/06/scienceshot-volcano-co2-emission.html)".

However, "within hours of the Mount Pinatubo eruption which started in the afternoon of April 2, 1991, the plume of gas and ash released had penetrated the stratosphere, eventually reaching an altitude of 21 miles. Three weeks later, an aerosol cloud had encircled the earth, and it remained for nearly 2 years. Twenty million metric tons of sulfur dioxide mixed with droplets of water, creating a kind of gaseous mirror, which reflected solar rays back into the sky, counteracting the

warming caused by the volcanic GHG emission. Throughout 1992 and 1993, the amount of Sunlight that reached the surface of the earth was reduced by more than ten per cent (Specter 2012)." This fact has suggested the possibility of geoengeenering efforts (see below) to tinker with the planet to mitigate global warming and reduce the effects of climate change.

Since publication of the fourth assessment report of IPCC (2007a), the debate on the security implications of climate change has intensified, and climate change is emerging as a world security threat in the twenty-first century also in relation to the potential long-term effects on rising sea levels, severe droughts, melting of the polar caps, and more frequent and devastating natural disasters [http://content.usatoday.com/communities/sciencefair/post/2011/11/defense-science-panel-climate-a-national-security-threat/1 (last checked Oct 22, 2012)].

Without feasible intervention, environmental vulnerability to climate alteration will undermine human security and societal stability with unprecedented depletion of natural resources (water, land, terrestrial and marine biodiversity) driving violent conflicts. Evidence has been given on the relation between climate change and armed conflict, in line with some narratives about the evolution and collapse of civilizations (Scheffran et al. 2012). Only the development of a risk mitigation approach will promote a reduction of the climate change impact on human security and elicit measures for the adaptive response needed for resilience at all levels in the biosphere.

Civil society sees critical links between climate change and national security, energy independence, and the economy (The Pew project http://www.pewenvironment.org/campaigns/pew-project-on-national-security-energy-climate/id/8589935509). On the basis of projection studies, IPCC has high confidence that changes in the climate will be visible in the changing state of rainfall patterns, sea levels, permanence of glaciers, ocean temperatures, etc. (IPCC 2007b). An increase in the frequency and severity of extreme climate events, such as severe droughts, floods, heatwaves, erratic storm behavior, tropical cyclones, wildfires, excessive sea level rise, deteriorating glaciers, shift in agriculture ranges, and warming of the oceans are expected. Their modeling studies suggest that increasing frequency of crop loss due to extreme events, such as droughts and heavy precipitation, may overcome the positive effects of moderate temperature increase (see Sect. 5.4.1 in IPCC 2007b). For forests, elevated risks of fires, insect outbreaks, wind damage, and other forest disturbance events are projected, although little is known about their overall effect on timber production (see Sect. 5.4.1 in IPCC 2007b). These factors can have serious consequences for food and forestry production and may result in conflicts over water, crop failures, famine, disease, mass migration of people across borders, and country instability (Sen. J Walker) with impacts also on fossil energy availability and distribution. Climate change is a "threat multiplier" making the problems that already exist worse.

Whatever the cause of climate change, in this chapter we will address questions related to genetic and breeding technologies for the identification of traits, genes, and crops of the future designed as a response to (a) keep greenhouse gas levels and tropospheric temperature from rising any higher (decelerate the GHG release driving force of climate change), (b) increase crop and forest adaptation to the

current and future state of the Earth's environment components, which will contribute to mitigation of the impact of climate changes on sea levels, ocean temperatures and currents, glacier water reservoirs, biodiversity, agricultural productions, forest services, advancement of the front line of forests, etc.

In the above context, we will examine the deployment of adaptive genes in agricultural crops and agroforestry plantations including nonfood and carbon-neutral bioenergy crops that will contribute to the production of biofuels, which will substitute those derived from fossil sources.

Adaptation is the practice of identifying options to adapt to climate change after evaluating them in terms of criteria such as availability, effectiveness, efficiency, feasibility, benefits, and costs. Plant adaptation to agricultural and forestry ecosystems through deployment of adaptive genes to biotic and abiotic stresses under climate changes, is the most effective, efficient, feasible, and cost effective strategy for durable benefit. Proposals on future evolutionary adaptation of crop plants requires understanding of the ecology of their wild ancestors and the selective pressures that cultivators exerted when they began manipulating plants and shaping agricultural environments for plant domestication. Evolutionary processes under domestication are known for several field crops and some fruit tree crops and can give (1) important insights into general questions in evolutionary ecology and the population genetic dynamics that link the wild crop relatives to earlier ancestors, and (2) suggestions to devise options to adapt crops to the predicted climate change trends.

The chapter is organized into five sections connected in a DPSIR conceptual framework (see Appendix).

Firstly, we describe the framework and then we develop major themes of the chapter related to the adaptation strategies under climate change.

In the second section, we discuss the ecological context of past adaptations to climate change as resulting from fossil records, and the evolutionary and domestication processes that can experimentally be recapitulated for selecting, using genomic tools, adapted genotypes shaped by the changing climate.

We show in the third section that our knowledge on the biology and ecology of forest trees, fruit trees, and annual crops needs support from genetics and genomics mainly to gain information on genotype fitness and genetic variation for phenotypic plasticity and adaptation as detected by the reaction norm and "genotype × environment" interaction studies. That will help in the appreciation of trait evolution and understanding of the reproductive ecology of the domesticates themselves, enlightening how evolution might proceed in the future by breeding. Studying phenotypic response under domestication in different environments shaped by climate change, will provide hints as to the gene actions necessary to cope with the response to the climate-induced stresses and on future directions of forest tree population management to help dispersal and adaptation to more hospitable climates.

In the fourth section, which comprises the bulk of the chapter, we focus on the genetics and genomic quest to find genes endowing plant resilience to

environmental changes. We focus on the molecular genetic approaches to discover genes for adaptation to biotic and abiotic stresses in fodder crops and forest species.

Finally, the options available to breeders to develop cultivars better adapted to current and predicted climate change conditions are briefly discussed. Here we argue that understanding past genetic events that shaped the genome of modern crop species in the light of population genetics and ecological principles is the key to identifying traits, genes, and crops to direct future plant evolution to face stressors generated by climate changes. Phenology, yield potential, and tolerance to biotic and abiotic (drought and submergence) stresses are the traits to consider for producing new breeding materials. Selection of genotypes adapted to the current and most tangible effects of global warming and their adoption by farmers and foresters will mitigate the impact of heatwaves and flooding caused by unusual heavy storms in several parts of the world.

3.1.2 The DPSIR Conceptual Framework to Devise Climate-Resilient Crops Through Genomics and Breeding

The driving forces that exert pressure on physical atmospheric properties (the Earth's surface temperature increases through the enlarged carbon footprint and consequent global warming) and ignite *climate changes* affecting the state of the Earth's environmental components (rainfall pattern, frequency of storms and flooding events, sea level, ocean temperatures, glacier retention), are the activities that increase GHG emission affecting the tropospheric temperature. This has an impact on several of the Earth's biotic components (biodiversity) in natural (forests, reserves) and man-made (agricultural and agroforestry) ecosystems affecting host plant phenology, biomass productivity, vulnerability to fungal and bacterial pathogens and pests, equilibriums in species community composition in forests, and biodiversity assemblages in terms of extent and composition at the level of genes, populations, species, including the wild crop relatives. Researchers have documented that signs of spring such as animal breeding and the blooming of flowers are occurring, on average, 2.3 days earlier over the course of each decade for the past half-century.

Mahlman (1997) and IPCC (2007a) classified the relative certainty of driving forces on climate change into several categories. The "virtually certain facts" include:

- Atmospheric concentrations of GHG would continue to rise, largely caused by human activities such as burning of fossil fuel and changes in land use.
- Changes in other radiatively active substances (e.g., sulfur aerosols) and increased cloudiness caused by greater evaporation in a warmer climate may offset some of the greenhouse effect.

The pressure on global warming of the Earth's surface has increased and during the past three decades average global temperatures have climbed about 0.2 °C per decade. If emissions continue to increase, temperatures are expected to rise between 1.1 °C and 6.4 °C by 2100.

The stratosphere would continue to cool as CO_2 levels rise and that would mean that global mean concentrations of water vapor in the lower troposphere would increase (approximately 6 % per 1 °C warming) altering the state of rainfall patterns towards an increase in global precipitation of 2 ± 0.5 % per 1 °C warming, erratic seasonal rainfall distribution and intensity, impacting agricultural ranges and systems and crops' exposure to disease outbreaks. Higher latitudes in the Northern Hemisphere are expected to experience above-average increases in both temperature and precipitation. Further, changes in mean climate would probably be accompanied by changes in the frequency and magnitude of climate extremes; globally, this would include an increased probability of warm events and decreased probability of cold events.

Human societal response to mitigate the impact of changes in the state of the environment aims to understand ecosystem vulnerability to foster adaptation in the biotic component. Measures span from structural intervention in regard to the driving force (GHG emissions) by international agreements to limit GHG emissions and promotion of renewable energy sources for electricity and heating (i.e., biomethane from waste and biomass residuals from agriculture activities), soil fertilization (i.e., charcoal), and transportation (biofuels from biomass harvested from dedicated carbon-neutral bioenergy crops), geoengineering intervention to reduce the pressure on global warming by adding enough aerosol or light-colored, highly reflective particles to the atmosphere to reflect sunlight and cool the planet, and interventions for human, crop, and forest adaptation to the current and future state of the Earth's environment.

Each step of the conceptual framework depicted in Fig. 3.3 of the Appendix, is based on projected regional changes, which are then used as a basis for "driving force models" that predict future CO_2 release, "pressure models" that forecast future temperature increase in the atmosphere, "impact models" that estimate the effect on the quality of human life, and various "response models" to mitigate the predictions on climate changes from the other models of the framework. However, for the fifth major assessment of climate science by the IPCC, due to be released in 2013, the climate models scientists are now working with, are likely to produce wider rather than smaller ranges of uncertainty in their predictions (Maslin and Austin 2012). The climate models may have reached their limit because they cannot capture all the factors involved in a natural system, and those that they do capture, are often incompletely understood. For example, the expected accumulation of GHG used as an input variable in any climatic model is based on economic models that predict global fossil fuel use over the next 100 years, given broad assumptions about how green the global economy will become; but evidence from the 2008 economic crisis has shown how difficult it is to predict changes in the economy (Maslin and Austin 2012). Efforts to reduce GHG emissions are related to the reliable assessment of emissions, and uncertainty about the prediction interval of

carbon emission estimates need to be reduced. Carbon emissions from fossil fuel combustion and cement production are relatively well quantified (they grew by 0.51 Gt C (5.9 %) in 2010 and reached a record high of 9.1 ± 0.5 Gt C) (Peters et al. 2012). However, emissions from land use change are the most uncertain component of the global carbon cycle, and policies to reduce emissions from deforestation would benefit from clearly and independently derived estimates of carbon emissions (Harris et al. 2012). Gross carbon emissions from deforestation (GCED), as opposed to net emissions, which include forest regrowth, are defined as the area of gross forest loss multiplied by the carbon stock of the forest before clearing. Estimation of GCED using FAO ground-based data and the bookkeeping model, were 2.8 ± 0.5 Gt C per year for the period 2000–2007 and 2.2 Gt C per year for the period 2000–2010. The emergence and widespread use of multiresolution remote sensing imagery (satellite observations of gross forest cover loss) to track land-cover change provide an independent and more accurate benchmark for monitoring global progress on reducing GCED (Harris et al. 2012) without resorting to the FAO data and using only satellite-based analyses of the geographic distribution of tropical forest carbon stocks and of both the location and quantity of tropical deforestation, assessed GCED of 0.81 Gt C per year for the period between 2000 and 2005 due to the loss of 43 million hectares of forest. This result is about one-third of some previous estimates. In fact, the equivalent value estimated by other authors (Baccini et al. 2012) using maps of tropical forest carbon stocks based on multisensor satellite data calibrated with spatial information on the location of deforestation, and the FAO data, was of 2.22 Gt C per year. The magnitude of the difference remains surprising, particularly given the similarity between the underlying forest carbon stock data (Zarin 2012). Despite those complications, the present models can reproduce natural climate variability over the past 150 years, and have provided an essential test of the theoretical link between CO_2 and global temperatures. In addition, the model prediction of the 2–5 °C future temperature increase by doubling current CO_2 in the atmosphere has been stable for all models proposed in the last 20 years (Maslin and Austin 2012). The science is getting close to achieving unbiased assessment of tropical deforestation and forest degradation emission reductions and defining objective quantification of GCED.

On that basis, there is no excuse for inaction, and the UNFCCC and AAAS scientists suggested approaching the climate change problem from three directions—by combining efforts to (A) reduce greenhouse gas emissions and energy consumption, with strategies for (B) human adaptation to climate change as well as (C) technologies designed to mitigate impacts.

(A) The realization of a global response by re-enforcing international agreements to limit GHG emissions (i.e., negotiate a new climate treaty by 2015 as agreed in Durban, South Africa in 2011) and actions distinct from and complementary to CO_2 measures to reduce tropospheric ozone and black carbon pollutants, can slow the rate of climate change and help keep global warming below 2 °C relative to preindustrial levels in the near term. Those interventions are

expected to provide enhanced warming mitigation in the Arctic and the Himalayas, and reduce regional disruptions to traditional rainfall patterns—in addition to their local health and local-to-global agricultural benefits (Shindell et al. 2012).

A more precise evaluation of carbon losses from deforestation and forest degradation will affect the pace of intervention for reafforestation using improved genetic resources and agriculture land use with genetically improved crop varieties to reduce the need for new land from deforestation. In fact, other tools exist today for local response to keep greenhouse gas levels and tropospheric temperature from rising any higher. They are based on next-generation energy solutions and innovative genetic technologies for capturing or "sequestering" carbon. Trees are the main living natural CO_2-sequestering organisms. Global warming can prolong the growth condition in fall but leaf senescence may be impeded and one can take advantage of this extra time for carbon sequestering. Delaying leaf senescence and falling or anticipating leafing in deciduous trees may enhance the carbon sequestration ability of trees. Embracing cutting-edge research to find phenology-modifying genes will address the "genotype × environment interaction" issue of how to change plant phenology by breeding in long-lived plants (i.e., forest plant species) under the environmental variability forced by global warming, providing a greener way for capturing or "sequestering" carbon compared to geoengeenering solutions. Substitution of fossil fuels with carbon-neutral and renewable energy resources from biomass (i.e., dedicated bioenergy crops), as well as burning fuels efficiently, are ways of mitigating the driving force (by decreasing the GHG emissions) of climate change. Disposing of the sequestered carbon by transforming it into bioenergy (heat, biofuel, biochar, etc.) will be a response to the energy-related climate question. Soil fertilization with biochar will further displace fossil fuel-derived energy for the industrial production of fertilizers. Exploration of the controversial idea of geoengineering, i.e., deliberately tinkering with the planet to curb the effects of climate change by sucking carbon dioxide out of the atmosphere, seeding the Earth's stratosphere with sunlight-blocking particles, aerosols reflecting far-red light, etc., have been proposed and an experiment planned by 2012 has recently been stopped because some questions remain unanswered, such as: how practical are such approaches? What are the geopolitical implications? And what are the risks of these approaches compared to staying on our present course?

(B) Human adaptation to climate alteration can include changing settlement patterns and farming and flood-management practices. Improving the energy efficiency of cars, trucks, planes, buildings, appliances, and manufacturing processes may also hold the key to reducing vulnerability to the impact of our climate future. The Subsidiary Body for Scientific and Technological Advice (SBSTA) of the UNFCCC, through The Nairobi work program (NWP) assist developing countries to (1) improve their understanding and assessment of impacts, vulnerability and adaptation to climate change, and

(2) make informed decisions on practical adaptation actions and measures to respond to climate change on a sound scientific, technical and socioeconomic basis, taking into account current and future climate change and variability.

The proposed delivery of adaptation actions are: capacity building, climate-resilient development planning, communications and awareness-raising, disaster risk reduction, knowledge management, pilot adaptation programs or projects, risk and vulnerability mapping, and training.

(C) Proposed technologies designed to mitigate the impacts of alteration of the environmental state on agriculture and forestry plant species, are essentially based on hydrogeological structural intervention to avoid disasters (i.e., flooding, landslides, scarcity of irrigation water). However, deployment of adaptive genes in forest plantation and crop varieties, has also been considered as a sustainable and effective measure to (a) reduce the effect from severe drought or increased aqueous vapor in the troposphere on the crops, (b) take advantage of increased CO_2 availability in the troposphere and increased air temperature in crop and forest plant species, and (c) decrease the vulnerability or susceptibility of crop and forest plant species to invasive pests and pathogens or to the outbreak of strains of endemic pathogenic fungi and bacteria or parasitic insects, mites, and nematodes that may cause agricultural system and agroforestry range shift. Alleles for phenophase alteration, and biotic and abiotic stress resistance are part of the standing genetic endowment of the living gene pools of all the crop and forest plant species and allied wild relatives. Their homoalleles have been pivotal as standing genetic variation within the gene pools of the wild relatives for (a) the "domestication-related" gene revolution that has led to the extant crop species, and (b) the variation of the forest front-line during the past glaciation cycles. The big question is whether human development, crop resilience, and adaptive capacity can compensate for increasing exposure and sensitivity to climate change.

Temperature has a critical role in causing body size evolution within species of birds and mammals, as observed over geological and shorter time span. According to thermoregulatory hypothesis for the conservation of metabolic heat, which is at the basis of Bergmann's rule (body size tends to be inversely related to ambient temperature), changes in body size are expected. Temperature-related changes in relative growth of forest tree species and farm animals will be constrained by allometric laws and inheritance of traits related to body size.

Climate warming can also change the disturbance regime of forests by extending the range of some damaging insects, as observed during the last 20 years for bark beetles in the USA or the pine processionary moth in Europe. The latter has displayed a northward shift of 27 km per decade near Paris, a 70 m per decade upward shift in altitude for southern slopes, and 30 m per decade for northern slopes in Italian mountains.

Climate changes can lead to disruption of biotic interaction (e.g., predator/prey) and to changes of species composition as well as ecosystem functioning. Many studies show a high correlation between changes in species composition and recent

climate change, also via the frequency of weather-based disturbances, although many species community changes are also attributable to landscape fragmentation, habitat modification, and other nonclimate drivers. A real challenge will be distinguishing nonclimate impacts on biodiversity, such as natural disturbances (e.g., wild fires), pests, diseases, and pollution (e.g., soluble-nitrogen deposition), influencing the changes exhibited by species. Many animal and plant populations have been under pressure from agricultural intensification and land use change in the past 50 years, causing many species to be in decline. Habitat fragmentation or simply the absence of suitable areas for colonization, for example at higher elevations, also play an important role, especially in species extinction.

3.1.2.1 Response to Reduce Emissions Through Both Mitigation and Adaptation Measures

(A) Global response addressing the driving force of climate change

(A.1) Adoption of mitigation rules to reduce excess release of GHG.

The treaties that emerged from the 1992 Rio summit spawned a series of subsequent accords such as the UNFCCC, whose main assignment is to stabilize GHG emissions. Other assignments are related to the tracking of GHG emissions and sinks (held to create national inventories of GHG emissions, land use trends for afforestation, and carbon uptake by forests), promote and disperse climate-friendly technologies, promote sustainable land management (encourage efforts, such us genomics for breeding varieties endowed with genes conferring new adaptations to the environment, to advance sustainable agriculture and reduce deforestation), prepare for the impacts of climate change, etc. Within the UNFCCC framework, the Kyoto Protocol prepared in 1997 limits GHG emissions from industrialized countries. In 2005 the Kyoto protocol entered into force without the United States, which declined to ratify it. In 2009, China and India and other major developing countries agreed to reduce GHG emissions, and in 2011 it was agreed in Durban (South Africa) that a new climate treaty would be negotiated by 2015. Climate models help our understanding of complex climate processes, but they have reached their predictive limit. Therefore, it is time to stop waiting for further model improvement, and simply act and apply the response measures deduced from the current climate models (adopt more stringent international agreements to limit GHG emissions, comply with an emission-limiting treaty, assessment of ground-based atmospheric observations for verification of GHG emissions from urban regions, emission control measures distinct from and complementary to CO_2 measures, to reduce tropospheric ozone and black carbon pollutants (McKain et al. 2012), and measures that lessen car use thus increasing

walking and cycling, which in turn reduces obesity and heart attacks) (Maslin and Austin 2012).

(A.2) Provide livelihoods for local people through carbon credits.

(A.3) Assess the feasibility of Geoengeenering Technologies to keep Earth cool.

It could provide a radical fix for climate change (Cressey 2012; Long et al. 2012) but a controversial geoengineering field trial was cancelled in mid-May 2012, amid concerns about a patent application. The experiment planned by the SPICE (Stratospheric Particle Injection for Climate Engineering) research consortium, aimed at investigating if injection of reflective aerosols into the stratosphere could help to bounce some of the Sun's warming rays back into space (Murphy 2009; Kravitz et al. 2012). As part of this project, SPICE had planned to test a possible delivery system: pumping water up a 1-km-long hose to a balloon, where it would be sprayed into the sky (Cressey 2012).

(B) Local response addressing the driving force of climate change

(B.1) Cropland and forested area management

Use new crop varieties bred by deploying genes that provide resistance or tolerance to locally prevalent multiple biotic or abiotic stresses. Breeding for varieties with enhanced water use and nitrogen use efficiency for dedicated bioenergy crops in marginal lands is expected to mitigate deforestation for new cropland and promote reduction of CO_2 emissions by replacing fossil energy resources with renewable energy resources.

Develop and adopt new rice cultivars that emit less methane.

Determining what is the "best" genotype, or the "fittest" genotype for a particular environment, by proper evaluation of the reaction norm of different genotypes to a range of environments that the genotypes will experience under climate change in a given agricultural district or forested area.

Where crop and forest species exhibit threshold effects, where there is not a clear gradual transition between populations or species, but a stepwise change of phenotype in response to a gradual environmental change, the species vicariance in agriculture and forestry should be explored, through genomic and provenance studies, in order to realize managed relocation of germplasm. Examples of movement of species from one location to another for cropping purposes are the species introductions started by the Columbian Exchange, but for agroforestry purposes the introduction of Douglas-fir in substitution of *Castanea* stands in Italy have been realized, and assisted migration has been implemented for endangered species such as *Torreya taxifolia* in USA. Relocation of vicariant species is an adaptive option for maintaining biological diversity and ecosystem functionality when external causes such as climate change pose a threat to the local species. All the

plantations within the priority areas should be made by certified genotypes at the genome level using next-generation sequencing (NGS) and molecular markers. Ecotonal interaction should be favored between local and the related vicariant and introduced species (hybrid zones) for the development of adapted genotypes to the changing climate conditions.

Embracing cutting-edge research to find phenology-modifying genes to address the "genotype × environment interaction" issue and select new plant types with delayed leaf senescence and leaf falling or anticipated leafing in deciduous trees, which will enhance carbon sequestration ability under global warming and mild winters (temperatures > -3 °C) (Chmielewski et al. 2004; Catarcione et al. 2009).

Planting genetically selected tree progenies for long-term local adaptation in managed forest ecosystems, to increase genetic resilience and forest carbon stocks.

Monitoring genetic variation through ecogenomics tools and ground-based forest inventory data and remote-sensing measurements of forest attributes in order to have information on species assemblages, their genetic structure and departure from equilibrium, and on the actual and future contribution of individual species component to the estimates of national carbon stocks using allometric relationships.

(B.2) Agronomy

Improved agronomic practices that increase yields and generate higher inputs of carbon residue to increase soil carbon storage.

Extending crop rotations, notably those with perennial crops that allocate more carbon below ground.

Adoption of cropping systems with reduced reliance on fertilizers, pesticides, and other inputs because it reduces CO_2 and other GHG emissions during their production.

Use of rotations with legume crops, which reduce reliance on external N inputs although legume-derived N can also be a source of N_2O.

Provide temporary vegetative cover between successive agricultural crops, or between rows of tree or vine crops; these "catch" or "cover" crops add carbon to soils and may also extract plant-available N unused by the preceding crop, thereby reducing N_2O emissions.

Reduce off-site N_2O emissions by improved efficiency of N use through adjusting application rates based on precise estimation of crop needs (e.g., precision farming); using slow- or controlled-release fertilizer forms or nitrification inhibitors (which slow the microbial processes leading to N_2O formation); applying N when least susceptible to loss, often just prior to plant uptake (improved timing); placing the N more precisely into the soil to make it more accessible to crop roots; or avoiding N applications in excess of immediate plant requirements.

No-tillage systems to reduce CO_2 emissions from energy use in agriculture.

Increase availability of precursors for soil organic matter, the main carbon store in soil, by (a) retaining crop residues in the soil, (b) avoiding the burning of agricultural residues on site.

Crops and residues from agricultural lands can be used as a source of fuel, either directly or after conversion to fuels such as ethanol or diesel. These bioenergy feedstocks still release CO_2 upon combustion, but that is a recycled CO_2 which returned to the atmosphere after it was subtracted to be incorporated, through photosynthesis, into the crop biomass that is being used to produce bioenergy. The net benefit of these bioenergy sources to the atmosphere is equal to the fossil-derived emissions displaced, less any emissions from producing, transporting, and processing.

(B.3) Drainage of cropland

Drainage of croplands in humid regions can promote productivity (and hence soil carbon) and perhaps also suppress N_2O emissions by improving aeration; but any nitrogen lost through drainage, however, may be susceptible to loss as N_2O.

Draining wetland rice once or several times during the growing season reduces CH_4 emissions. Rice cultivars with low exudation rates could offer an important CH_4 mitigation option. In the off-rice season, CH_4 emissions can be reduced by improved water management, especially by keeping the soil as dry as possible and avoiding waterlogging. Adjusting the timing of organic residue additions (e.g., incorporating organic materials in the dry period rather than in flooded periods), CH_4 emissions can be reduced.

(B.4) Restoration of degraded lands

Erosion control, organic amendments, and nutrient amendments.

(B.5) Fire management

Mitigation actions involve (1) reducing the frequency or extent of fires through more effective fire suppression, (2) reducing the fuel load by vegetation management, (3) avoidance of on-site biomass burning (not to be confused with bioenergy, where biomass is combusted off-site for energy) or burning at a time of year when less CH_4 and N_2O are emitted. These actions contribute to lowering atmosphere forcing in several ways. Firstly, they reduce GHG, notably CH_4 and, to a lesser extent, N_2O (the CO_2 released is of recent origin, is absorbed by vegetative regrowth, and is usually not included in GHG inventories). Secondly, these reduce the generation of hydrocarbon and reactive nitrogen emissions, which react to form tropospheric ozone, a powerful GHG. Thirdly, less fires leads to a reduction in the production of a range of smoke aerosols, which can have either warming or cooling effects on the atmosphere; the net effect is thought to be in lowering radiative forcing. Reducing the frequency or intensity of fires typically leads to increased tree and shrub cover, resulting in a CO_2 sink in soil and biomass.

(B.6) Harmonization of land and food use

Allowing or encouraging the reversion of cropland to another land cover, typically one similar to the native vegetation. Conversion can occur over the entire land area (*set-asides*), or in localized spots, such as grassed waterways, field margins, or shelterbelts. Such land cover change often increases carbon storage.

Converting arable cropland to grassland typically results in the accrual of soil carbon because of lower soil disturbance and reduced carbon removal in harvested products. Compared to cultivated lands, grasslands may also have reduced N_2O emissions from lower N inputs, and higher rates of CH_4 oxidation. Switching from grain-fed beef, which "requires about 5,300 liters of water for each dollar's worth of grain fed to a cow, to grass-fed beef, which typically requires only the rainwater falling on a pasture," will contribute to agricultural sustainability (Perkins 2012). The FAO 2006 report concluded that livestock accounted for 9 % of the carbon dioxide, 37 % of the methane, and 65 % of the nitrous oxide that comes from human-associated activities. So, whether the steak comes from Argentina or the rancher down the road, it is, relatively speaking, a high-carbon food (Martin 2009). Therefore, a further reduction of GHG release and resources (i.e., water, energy) needed to produce the commodity will be achieved by shifting to a vegetable-rich diet compared to the use of processed and meat-based food.

A better organization of food production and use will be achieved by solving the locavore's dilemma. "Locavorism," "localism," or "eating locally" is: buying food from local producers. Locavores think that "sustainable farming" and "eating local" are the way to solve a host of perceived problems with our modern food supply system. By combining healthy eating and a high standard of environmental stewardship, it is expected that important economic benefits and increased food security within local economies can be achieved. However, several scientists argue that urban farms do more harm than good to the environment and suggest consuming not only locally grown food (Martin 2009; Glaeser 2011).

One recent UK report found that the GHG emissions involved in eating English tomatoes were about three times as high as eating Spanish tomatoes. The extra energy and fertilizer involved in producing tomatoes in chilly England overwhelmed the benefits of less shipping (Glaeser 2011).

But the most important environmental cost of metropolitan agriculture is that lower density levels mean more driving (Glaeser 2011). Some economists (Desrochers and Shimizu 2012) argue that encouraging localized supply, and thus diversified farming, strikes at the essence of agricultural development and socioeconomic progress. Modern animal and plant breeding, long distance trade in food, and modern production and processing technologies developed from the monocultures has made

possible the modern food system that distributes risk across regions, and the associated division of labor has delivered the financial means for risk management. But that specialization has also contributed to the incidence of diet-related diseases and there is evidence that above a certain point, growth in gross domestic product is not correlated with improved well-being (MacMillan 2012). A redesign of the whole food system to bring sustainability to the fore at all levels is advocated.

Considering the functional properties in addition to the nutritional value of the plant products used as food will reconcile local supply, socioeconomic progress, and mitigation of climate change. The incidence of obesity and related diseases including type 2 diabetes, coronary artery disease, fatty liver, some cancers and degenerative diseases, has increased dramatically in the last 25 years becoming a major costly health problem worldwide (Paladini et al. 2012). Genetic susceptibility and dietary misbehavior represent the pathogenic basis of this phenomenon leading to a chronic, highly detrimental, low-grade inflammatory status. While the genetic elements are difficult to dissect due to the polygenic inheritance of these complex diseases, dietary habits can be modified towards an enrichment in protective, bioactive micronutrients. For examples, nuts in general have been shown to be rich in total fat and unsaturated fatty acids, and their consumption has been shown to reduce the risk of diabetes as well as coronary disease in large epidemiological studies. The effect in reducing the blood concentration of some inflammatory parameters is also well documented. Nuts have also the great advantage of being an easy food to conserve and distribute.

Crop species such as hazelnut, walnut, almond, pistachio, etc., have peculiar adaptations to local environments and are linked to geographic districts scattered within and between European countries. Genetic improvement of local germplasm to enhance the yield traits of nut species grown locally for healthy food will benefit the local population; but when such improvements occur in several districts that have each adapted local germplasm for different nut species, other local human populations will also benefit. The final result will be a global availability of nuts harvested from trees raised through locally adapted germplasm requiring low energy input for crop management and commodity mobilization, providing increased carbon sequestration in the edible healthy fruits, and contributing to the recovery of near carbon-neutral energy from the burning of crop residues (nut shells, pruned branches) to heat nearby homes.

Local acceptance of fruits has been proven by stable ratings for nuts in the measurements “overall liking” and “desire to consume” by residents over a long exposure period, indicating that not only did the people like the nuts, but also that they wished to continue eating them. Therefore, the guidance to consume nuts on a regular basis appears to represent a sustainable behavior to reduce both cardiovascular diseases (Tey et al. 2011) and carbon emissions.

Planting trees can reduce emissions. Agroforestry (the production of livestock or food crops on land that also grows trees for timber, firewood, or other tree products), afforestation, and reafforestation using seedlings from selected ecotypes will further increase fixed carbon stocks and avoid soil erosion and flood damage when shelter belts and riparian zones/buffer strips with woody species are prepared. The standing stock of carbon above ground is usually higher than the equivalent land use without trees, and planting trees may also increase soil carbon sequestration.

Bioenergy from crops will contribute to a reduction of CO_2 release (Chum et al. 2011; Moomaw et al. 2011). Major feedstock sources for future biofuel production are likely to be high biomass-producing plant species such as poplar, pine, switchgrass, sorghum, and maize. One active area of research in these species is genome-enabled improvement of lignocellulosic biofuel feedstock quality and yield. To facilitate genomic-based investigations in these species, Childs et al. (2012) developed the Biofuel Feedstock Genomic Resource (BFGR), a database and Web portal that provides high-quality, uniform and integrated functional annotation of gene and transcript assembly sequences from species of interest to lignocellulosic biofuel feedstock researchers.

3.2 Adaptations to Climate Changes in Forest Species: Evidence from Fossil Records

3.2.1 Evidence from Fossil Records and the Importance of Leaf Longevity and Foliar Phenology for Adaptation to Climate Changes

Climate change is expected to subject forest plants to decreased moisture availability, increased temperature, increased climate variability, a changed atmospheric composition and if tree mortality rises, a more disturbed, open canopy. Physiological processes affected could include photosynthesis and respiration. Phenological processes affected could include bud burst, leaf longevity, defoliation (dehiscence), the onset of flowering, seed setting, and ripening.

The adaptive significance of leaf longevity and foliar phenology has long interested ecologists, biogeographers, and global modelers. In the context of global climate change, the phenology of deciduous tree species is crucial as it may affect their productivity in comparison to the evergreen trees.

Givnish (2002) evidenced that there are three types of patterns in leaf longevity and phenology: deciduous, leaf-exchangers, and evergreen.

Deciduous trees shed their leaves usually as an adaptation to a cold or dry season.

Evergreen trees do lose leaves, but not all at the same time in the way deciduous trees do; different trees shed their leaves at different times, so the forest as a whole looks green.

Leaf exchangers are evergreen perennials in which a new cohort of leaves emerges either simultaneously with, slightly later than, or slightly in advance of the shedding of the previous year's foliage (Milla et al. 2007). Leaf exchangers are particularly abundant in seasonally dry tropical and subtropical forests, although some species from the Mediterranean marquis and other environments also display this leaf phenology. Most Mediterranean leaf exchangers shed old foliage after the elongation of new shoots, but this is not a resource-demand driven senescence, because nutrient remobilization from old leaves frequently occurs after the demands of the new growth flush have been fulfilled. In general, mineral nutrients (e.g., N, P, K) are incompletely withdrawn from leaves (whether evergreen or deciduous) before they are shed, and the fraction not retranslocated must be replaced when new leaves are produced (Chabot and Hicks 1982).

The optimal leaf phenology can have important ecological consequences, from the level of individual leaves and plants to the entire biosphere.

The potential advantages of deciduous leaves are (1) a higher photosynthetic rate per unit leaf mass, (2) lower root costs during the unfavorable season, and (3) no leaf respiration during the unfavorable season. Deciduous leaves should have higher rates of photosynthesis per unit leaf mass during favorable conditions than evergreens, given their higher leaf nitrogen content and specific leaf area, higher intrinsic photosynthetic capacity, and the reduced internal competition for light and carbon dioxide (Givnish 2002).

The major advantages of evergreen leaves are (1) a longer photosynthetic season, (2) lower amortized costs of leaf construction, (3) lower amortized costs of replacing leaf nutrients, and (4) tougher laminae that can better endure frost, drought, and/or herbivore attack (Givnish 2002). Evergreen leaves often have to be tougher. Greater thickness facilitates withstanding frost or drought during the unfavorable season without suffering irreparable damage, and the low levels of leaf nutrients and high levels of chemical defenses help deter leaf consumption by herbivores. Given that nutrient remobilization from old leaves is incomplete before they are shed, the more frequently the leaves are shed, the higher should be the energetic costs associated with capturing nutrients needed to flush new leaves (Givnish 2002). This gives plants with evergreen foliage an ecological advantage, especially on nutrient-poor sites. For those reasons, it is generally recognized that seasonal drought can favor deciduous leaves, and that infertile soils can favor long-lived evergreen leaves. Evergreen (i.e., *Picea*) should outgrow deciduous trees (i.e., *Larix*) on highly infertile sites (based partly on lower nutrient-acquisition costs). Deciduous *Larix* should outgrow evergreen *Picea* on more fertile sites (Givnish 2002). While mean leaf weight per unit leaf area of evergreen spruce needles (approximately 8 mg cm^{-2}) was about twice as high as in the deciduous larch (Matyssek 1986), the needle nitrogen content and photosynthetic capacity per unit dry weight were markedly lower in the spruce than in larch. As high photosynthetic capacities are usually correlated with high stomata conductances, water demand in

larch needles was greater than in spruce needles, and spruce needles displayed a higher water use efficiency than those of larch (Matyssek 1986). The gymnosperm larch and the angiosperm beech are both deciduous, both achieve the same annual aboveground biomass increment per unit of foliage dry weight (2.4 kg kg^{-1}), and display low leaf weights per unit leaf area and high photosynthetic capacities (beech: 12 mg g^{-1} h^{-1}) (Matyssek 1986). Thus, it appears that foliage type similarity may increase phenotypic similarity for many physiological traits in phylogenetically different taxa (Matyssek 1986). Therefore, occurrence of convergent structural morphology (evergreen leaf-type) in different taxa may imply functional similarity (adaptation to poor soils in wet environments). Although there might be exceptions to such a tenet, it may be assumed that the structural features evolved independently, with the convergence in structure and function deriving from selective pressures that channel morphological change in response to the same environmental factors.

For example, the high precipitation/evaporation ratio at boreal and low-arctic latitudes leads to leaching and the formation of highly infertile, acid podsols with low rates of nitrogen fixation, which may partly account for the dominance of boreal forests with evergreen trees. Evergreen leaf and needle litter has a higher carbon–nitrogen ratio than deciduous leaf litter, contributing to a higher soil acidity and lower soil nitrogen content. In a temperate climate, these conditions favor the growth of more evergreens and make it more difficult for deciduous plants (that require more nitrogen in the leaves) to persist (Givnish 2002).

Efforts to lessen the impacts of climate change on forest biodiversity will depend on accurate forecasts of what will happen to the forest species community composed of deciduous and evergreen trees, if winters get warmer. Some hints come from simulation studies carried out to explain forest community assemblage during past warming events.

During much of the Mesozoic and Paleogene, polar deciduous forests rather than forests made of evergreen tree species, were an important biome occupying upwards of 40 % of the total land surface (Royer et al. 2005). Deciduous forests blanketed the polar regions up to 85° latitude for much of the Mesozoic and Paleogene (Jefferson 1982), an interval of Earth's history generally linked with high atmospheric CO_2 concentrations (Beerling and Royer 2002) and globally warm temperatures (Huber et al. 2002) (coldest month mean temperature >0 °C). Paleobotanists have speculated that the warm, dark winters associated with ancient polar forests selected for the deciduous habit because evergreens would lose too much carbon via respiration to remain competitive (Wang et al. 2003). Royer et al. (2003) tested this hypothesis and found that, although the deciduous taxa lost more carbon during the winter season than their evergreen counterparts, species from both leaf habits achieved comparable annual carbon budgets (evergreen = 23.7 ± 4.4 g C; deciduous = 21.9 ± 2.6 g C; $p > 0.67$), implying that deciduous species compensate for greater carbon losses incurred during the polar winters with greater net canopy carbon productivity relative to evergreen taxa. Royer et al. (2005) determined that the deciduous extant species [*Taxodium distichum* (L.) Rich (taxodioid), *Metasequoia glyptostroboides* Hu & Cheng (taxodioid), *Ginkgo*

biloba L. (gymnosperm)], exhibited a significant pulse in carbon uptake during the late summer and early autumn (August to mid-October) that enabled them to achieve annual carbon budgets similar to those of evergreen trees [*Sequoia sempervirens* (D. Don) Endl. (taxodioid) and *Nothofagus cunninghamii* (Hook.) Oerst. (angiosperm)], despite incurring higher carbon losses through annual leaf shedding. These taxa have long fossil records at the generic level (>65 Myr) and their ancestors were dominant elements in Cretaceous and Paleogene polar forests (Royer et al. 2005). Those results indicate that, from a carbon balance perspective, deciduous taxa have no clear advantage over evergreens and that leaf habit in ancient polar forests was not an adaptation to photoperiod (Royer et al. 2003). Moreover, it has been recognized that timely connected Antarctic polar forests appear to have contained a higher proportion of evergreens than their Arctic counterparts. Then considering that the ancestors and the extant deciduous and evergreen species have maintained an overall similar carbon budgeting and adaptation to soil fertility (low N for evergreen and high N for deciduous), other factors not directly related to intrinsic leaf traits determining carbon absorption (i.e., duration of leaf canopy and anticipation of leaf flushing in deciduous trees, amplifying feedbacks and far field influences) and N-use efficiency are required to fully understand the significance of the widely dispersed deciduous leaf habit in ancient polar forests. Unfortunately, function cannot always be inferred from the structure of hard parts that usually constitute the only direct evidence of extinct organismal morphology (Gould 1970). Studies across biomes with different evolutionary and climatic histories demonstrate that categorization in deciduous and evergreen leaf typology is unlikely to be correct because leaf lifespan is strongly correlated with physiological, whole-tree, and ecosystem processes (Givnish 2002). A paleontological view of leaf lifespan is now afforded through anatomical analyses of growth rings in fossil woods (Falcon-Lang 2000), providing a critical means of linking past and present forest physiology and accommodating other categories of phenologies affecting plant growth and leaf lifespan, such as the "evergrowing."

For instance, the length of the "shoulder" of the evergreen growing season relative to that for deciduous plants during the Mesozoic has not been determined from fossilized plant parts but can only be measured through comparisons with the phenology of potentially analogous extant taxa. The shoulder is the period during which evergreen competitors of deciduous trees can absorb water and photosynthesize after leaf shedding and before budbreak of deciduous plants (Fig. 10 in Givnish 2002). Deciduous species with a reduction of the fall-to-spring shoulder will be favored compared to the evergreen species striving in the same habitat when warm winters occur (coldest month mean temperature >0 °C). But how can deciduous trees reduce the fall–spring shoulder?

The best explanation will be that under the warmer climate of the Mesozoic and Cenozoic, the deciduous trees that once colonized the temperate forests below the polar circle possessed additional genes providing flexibility to winter budburst, going from December [as induced by the *EVG-d* allele in the "evergrowing" phenotype of *Corylys avellana* (Thompson et al. 1985; Catarcione et al. 2009)] to

the end of March due to other alleles differentially responsive to cumulative "chilling units" for new leaf flushing (Catarcione 2011). The time flexibility to budburst in a deciduous tree species, might have the advantages of a significant pulse in carbon uptake during the late summer and early autumn in addition to the advantage of the immediate compensation of the sudden carbon losses, occurring while shedding senescent leaves, through the rapid flushing of new leaves. In sites where the winter temperatures do not drop below −3 °C, the deciduous "evergrowing" phenotype is expected to nullify the fall–spring shoulder existing between the deciduous "wild-type" and evergreen species, and an almost continued uptake of carbon throughout the year will occur. The "evergrowing" phenotype is different from the three typologies of leaf phenology described above and consists of the elimination of the genetically controlled dormancy period that follows leaf shedding in deciduous trees to endure the rigid winter temperature. Under warm winters the dormancy period is not necessary and if the modified rainfall patterns favor mineral cycles that accumulate N and P in the soil, then evergrowing mutants in deciduous trees will greatly favor these plants and at the same time favor carbon accumulation in biomass. Then the development of mutants expressing the "evergrowing" leaf phenology could add explanatory issues to remedy the insufficient knowledge of adaptive factors to natural climate variability in the past (i.e., the excess of deciduous trees during the warming Mesozoic period in the Arctic), and provide reliable hints on the genetic traits modifiable for adaptation to the predicted or ongoing global warming.

Fossil pollen records revealed that the climatically demanding (thermophilous) broadleaf deciduous trees, such as *Corylus*, *Ulmus*, *Quercus*, and *Carpinus* could have been present in relatively warm and humid limestone areas of Slovakia (Willis et al. 2000; Jankovská and Pokorný 2008) and in central-eastern Europe (Western Carpathians) where many elements of forest biota survived the Last Glacial Maximum and formed a large-scale forest refugium.

Palynological studies at a site in northern Germany (Duvensee), emphasize the importance of hazelnut exploitation throughout the early Mesolithic, beginning with the first emergence of hazel pollen recorded in the late Preboreal (10300–9000 year BP calibrated) stage (Holst 2010). During that transition, a sudden rise in temperature that abruptly changed the ecosystems occurred (an increase of 7 °C in 50 years). Dispersal of thermophilous broadleaf species from ecological refugia allowed forests to replace the open lands in Europe. The northern European sites such as Duvensee hosted a continued seasonal settlement dating from the late Preboreal (8900 year BC calibrated) until the beginning of the Atlantic (6500 year BC calibrated). Duvensee had many logistical advantages for occupation comprising the availability of raw material food sources such as rich hazel stands. The large genetic basis for phenology might have contributed to the establishment of the hazelnut trees in that area. Extrapolations from nut remains at the Duvensee archeological site indicate the high potential of hazelnuts adapted to warmer temperatures in early Mesolithic subsistence farming. Hazelnuts met a substantial portion of the nutritional requirements, providing a concentrated, easy to shelve and transport fat storage throughout the year.

Nowadays, the cultivated and wild primary gene pools of *Corylus avellana* are concentrated from 37°N to 50°N (latitude) where winter temperatures fall well below −3 °C and are unsuitable for the "evergrowing" phenotype except in some territories such as Giresun (Turkey) were very early bud sprouting occur.

3.2.2 Phenology and Genetic Control of Phenophases

Amount and timing of the pollen release window during flowering is a delicate phenological phase in orchards and forest trees, and climatic changes could alter the blooming period. Disruptions in synchronization of floral phenology (increase of the time interval between maturity of male and female reproductive organs) disturb cross-pollination, which is crucial for seed set. To overcome this problem, farmers and agroforesters are forced to increase the number of new pollinizer plants in order to widen the time window and intensity of pollen release with the risk of allergenic effects. For this reason it is essential to study the genetic control of (a) allergenic protein synthesis in different trees, (b) allelic differences for allergenicity of the pollen, (c) number of microspore-bearing structures produced, and (d) temporal window of pollen release in the air, in order to select those with lower allergenic protein content and low mass pollen production in the seed-parent and moderate pollen mass release from the pollinizer plants.

In hazelnut, the EVG-d phenotype was described for the first time as a "nondormant" mutation by Thompson et al. (1985). The EVG-d phenotype has phenological similarity to the "evergrowing" trait coded by the recessive gene *evg* in peach (Rodriguez et al. 1994; Bielenberg et al. 2008). Empirical observations in hazelnut orchards indicate that the timing of later phenology events (i.e., bud flushing) can be contingent on the environmental cues regulating earlier life stages (i.e., cumulative chilling units from October to December). A clear description of the underlying genetic and physiological processes governing that type of phenological response to current and predicted climates has been given by Wilczek et al. (2010). QTL (quantitative trait loci) analysis of bud phenology permitted the identification of two major and population-specific genomic regions on linkage groups 8 and 9 containing genes controlling budburst in apple progenies (Celton et al. 2011), and up to 16 QTLs for bud phenological phases were mapped on the linkage map of *P. nigra* (Fabbrini et al. 2012). Several (five) QTLs for flowering time in maize were mapped, all corresponding to major QTLs for number of nodes. The inheritance of flowering time was dominated by the well-known QTL *Vgt1*, but the other QTLs for early flowering time correlated with fewer vegetative phytomeres, indicating the latter as a key developmental strategy to adapt the maize crop from the original tropical environment to the northern border of the temperate zone (Salvi et al. 2011). Transgenesis has also been used for inducing early flowering in tree species (*Citrus* sp.) (Endo et al. 2005).

3.3 Modeling Forest Biogeographic Patterns, Genetic Variation and Genomics for Domestication and Enhancement of Phenotypic Plasticity for Adaptation

The forests of the world continue to be threatened by climate change, population growth, and loss to agriculture. The ability to conserve natural forests and meet the increasing demand for fuel, biomass, wood, and paper under climate changes, depends on fundamental understanding of tree growth and adaptation through the application of traditional methods and genomics (Sederoff et al. 2009).

Current molecular methods to study adaptive genetic variation in forest trees need to be complemented by traditional field experiments in order to provide the information needed for predictive description of the forest dynamics induced by global warming (González-Martínez et al. 2006).

Established biogeography allows the description of patterns produced by past and contemporary natural selection in ecoregions. Common garden experiments (provenance, progeny, and clonal tests), are commonly used to gauge the effects of adaptive evolution of quantitative traits in forest tree populations in several ecoregions. Such studies are based solely on phenotypes and do not provide information on individual genes involved in adaptation, nor on how much phenotypic variation can be explained by variation in these genes (González-Martínez et al. 2006). Nevertheless, such studies serve to describe ecogeographic trends in relation to the role of ecological pressures (i.e., global warming), in shaping the spatial distributions of forest species and traits (Avise 2000).

When the ecogeographic information was placed into a broader temporal and climate context it was possible to reconstruct physiological and biological processes explaining taxa composition and ranges of ancestral forests as they appeared from fossil records (Royer et al. 2005). Similar approaches coupled with knowledge on the extant spatial distributions of tree genomes (species), allele and haplotype pools (population within species), may allow predictive modeling of scenarios of forest dynamics in terms of population genetics events consequent to dispersal (active range expansion of a forest species from an ancestral center) and vicariance (passive and human-directed introduction of tree taxa in areas beyond their natural dispersal range) induced by pressure from climate stressors.

3.3.1 Forest Biogeographic Patterns

Genetic variability is the basis for evolutionary change, but determining patterns of genetic variability in relation to climate change is possible only in extant populations which, in turn, may be used to infer the population dynamics underlying the range shift detected from the geographical distribution of fossil records of pollen and plant remnants.

Evidence from the fossil records (Davis and Shaw 2001) and from recently observed trends (Walther et al. 2002) shows that changing climate has had a profound influence on tree species' range expansion in some parts of the world, and contraction in others.

Plant migration to new regions occurs through passive seed dispersal and the establishment of seedlings in sites where conditions permit. As the climate warmed at the end of the last glacial interval, tree populations migrated and became established at higher latitudes. The patterns of migration during the past 25,000 years displaced tree taxa to new northern range boundaries, for example spruce shifted northward (*Picea* spp.) and *Quercus* spp. expanded from glacial refuges (Davis and Shaw 2001).

Fossil pollen indicates that Scots pine migrated across central Europe from the south as temperatures began to rise about 15,000 calendar years before present, expanding rapidly across the northern European plain at rates as fast as 150 km per century (Davis and Shaw 2001). By about 8,000 years ago, Scots pine was abundant as far north as southern Sweden; it then declined in the south while continuing to expand into northern Sweden, Norway, and Finland.

The distributions of many terrestrial organisms are currently shifting in latitude or elevation in response to changing climate (Chen et al. 2011), similarly to the reconstructed events that occurred at the end of the last glaciation.

It has been estimated that species distributions have recently shifted to higher elevations at a median rate of 11.0 m per decade, and to higher latitudes at a median rate of 16.9 km per decade. These rates are approximately two and three times faster than previously reported. The distances moved by species are greatest where the highest levels of warming occurred, with average latitudinal shifts being generally sufficient to track temperature changes (Chen et al. 2011).

The patterns and the rates at which forest tree species have already responded to recent warming and the temporal trend of the response (if linear or not), are important assessments because they are (a) indicative of the relative strength of gene flow and selection linked to both temperature change and dispersal range shifts, (b) measure ecological resilience, and (c) provide warning signals before a catastrophic ecological threshold is reached. In fact, when the selection-mediated responses is linear, a pronounced clinal pattern in organismal adaptations is generated whose regularity enter the domain of ecogeographic rules (Allen's rule, Bergmann's rule, etc.). In some instances, the critical factor underlying the observed gradient is related to the mid-domain effect rather than to climate, energy, and topography (Luo et al. 2011). Therefore, other rules regarding the latitudinal gradient of the range size pattern of organisms, such as Rapoport's rule on the decline in the geographic extent of species from high to low latitudes, have had little support from the range size pattern of forest trees.

When the forest tree response to a small change in forcing (air temperature) is strongly nonlinear, then, according to the climate "tipping point" theory, a qualitative change in the state of the plant community (i.e., dieback of the Amazon rainforest) or in the state of the environmental components such as melting of the Greenland ice sheet or the shift of the West African monsoon, is going to occur

(Lenton 2011). Simulations show that rising standard deviation (SD) of one ecosystem feature (plant range shift, microbial composition of the soil, lake eutrophication, shifts among vegetation types) when regional temperature change, could signal, a decade in advance, a regime shift in the water or terrestrial ecosystem due to climate change (Carpenter and Brock 2006; Lenton 2011). Therefore, the increase in variance or autocorrelation of fluctuations of the system, including the genetic variance of seedlings in the expanding range, may be used as general indicators of both reduced ecological resilience and as warning signals before a catastrophic threshold is reached behind which the result is the collapse of many natural populations under the deteriorating environment (Dai et al. 2012).

Rising SD of the forest ecosystem feature (range size shift), may intertwine with the fluctuation in population size and density, leading to reduced individual fitness and gene flow, exacerbating the nonadaptive conditions and population disappearance. It is common that variation in plant population size or density affect the average plant growth rate, a phenomenon known as the "Allee effect." When population size or density fluctuate due to changes in temperature, precipitation or both climatic factors, it is expected that (a) at high population densities there would be a reduced average growth rate because of resource competition, (b) at intermediate population densities the maximal growth rate will occur, and (c) at low population densities a negative growth rate will arise because of difficulties in adequate pollen flow (*strong Allee effect*) (Dai et al. 2012). The Allee effect has major impacts on the local dynamics and viability of populations especially during range shift under nongradual climate change; the intensity and stability of fitness to local environment and gene flow will impact population growth and will play a major role in the fate of the front-line populations (Davis et al. 2004).

A threefold genetic Allee effect can explain the reduced fitness observed in small populations of self-incompatible species during long-term dispersion (Willi et al. 2005). In fact, the simultaneous fitness consequences of inbreeding, genetic drift, and cross-compatibility occurring in such small-size natural populations, erode individual fitness causing negative population growth rates (Willi et al. 2005).

Disadvantages in small population caused by the Allee effect, may lead to a considerable decline in the duration of species coexistence. However, according to the Neutral Theory of biodiversity, dispersal limitation within a community indeed enhances coexistence, implying that in such situations, either the Allee effect is not important, or that other stabilizing mechanisms (i.e., rare species advantages, perhaps resulting from interspecific niche differentiation) oppose the Allee effect (Zhou and Zhang 2006). This information suggests that small changes in climate will greatly affect growth and survival of forest tree populations only when other factors (i.e., topography and land use) impede dispersal and linear range shift. Other driving forces of forest population fragmentation and reduction of forest population sizes are related to economic activities (production, trade and consumption of goods of plant origin), which may exert pressure on (1) the genetic resource of native plants endemic to restricted geographical areas and threatened forests (as consequence, for example, of planting industrial crops), and (2) habitat and environmental processes in forest areas threatened with clearance (Hertwich 2012).

Climatic unsuitability may induce contractive effects similar to those due to habitat alteration on loss of distributional area available to each species. A well-established empirical power-law relationship describing how the number of species relates to distributional area ($S = cA^z$, where S is the number of species, A is area, and c and z are constants) predicts adequately the numbers of species that become extinct or threatened when the area available to them is reduced by habitat destruction (Thomas et al. 2004). The ecosystem consequences of local species loss are as quantitatively significant as the direct effects of several global change stressors that have mobilized major international concern and remediation efforts (Hooper et al. 2012). In this case, maintaining contemporary forest productivities during global warming will require an extensive redistribution of genotypes across the landscape.

3.3.2 Patterns of Genetic Variation

Genetic differentiation in forests will occur between as well as within species. Discriminating among seedlings of different species to establish forest regeneration dynamics, or undertaking large-scale biodiversity surveys with limited access to taxonomic expertise can now be achieved by DNA barcoding, which is a method that involves sequencing a standard region of DNA in an organism to identify it as belonging to a particular taxonomic group. It differs from molecular phylogeny in that the main goal is not to determine classification but to identify an unknown sample in terms of a known classification. Although barcodes are sometimes used in an effort to identify unknown species or assess whether species should be combined or separated (CBOL 2009), it should be emphasized that the discriminatory power of the standard barcode is higher in situations that involve geographically restricted sample sets, such as studies focusing on the plant biodiversity of a given region or local area. A particular strength of the barcoding approach is that these identifications can be made with small amounts of tissue from sterile, juvenile, or fragmentary materials from which morphological identifications are difficult or impossible (Valentini et al. 2008). The barcoding approach has been used for forest species determination to assess the effectiveness of the proposed molecular markers for tree classification in Oaks, Conifers, and Oleaceae (Ferreira et al. 2011; Piredda et al. 2011).

To measure genetic variation within species, allozyme loci and cloned DNA markers served as valuable indicators of gene transfer within and among forest populations. They have been particularly valuable for comparative population genetic studies as they are fairly numerous and are codominant. Using isozyme data it was evidenced that botanical families with predominantly woody species [i.e., Fagaceae, Pinaceae and Myrtaceae (= *Eucalyptus*)] all had high genetic diversity values and exhibited little differentiation among populations (Hamrick and Godt 1996). In fact, forest trees are prevalently outcrossing long-lived perennials for which 65.5 % of the loci within the species were polymorphic (P_s), and express the highest genetic diversity within species (H_{es} = Hardy–Weinberg

expected heterozygosity) compared to short-lived perennials or annuals. However, the average genetic diversity residing among populations (G_{ST}) for each polymorphic locus, account for only 9.4 % of the total genetic diversity. Molecular genetic markers, although based on protein or nucleic acid polymorphism traceable to single genes, have however contributed little to our understanding of natural selection and adaptation in forest tree populations (González-Martínez et al. 2006). QTL mapping (see Sect. 3.4) is primarily a method to find genetic regions that are responsible for variation in complex traits, although it can also be used to study adaptive traits in forest trees. QTL very rarely explains a significant part of the total phenotypic variation associated with a trait of forest trees and the associated effects depend on the environment. Association mapping (see Sect. 3.4) in natural forest populations of conifers has been proposed as a powerful molecular method for the identification of genes that underlie complex traits and for characterizing their effect on complex phenotypes (Neale and Savolainen 2004), and poplars (*Populus* spp.) and conifers (e.g., *Pinus* spp., *Pseudotsuga menziesii* and *Cryptomeria japonica*) are the best candidates for nonclassical model eukaryotes for population, evolutionary and ecological genomic studies (Feder and Mitchell-Olds 2003; Neale and Savolainen 2004).

Current populations of temperate and boreal trees follow the pattern of much within population variability, but little differentiation among populations, suggesting a high degree of genetic exchange among populations. For instance, in loblolly pine (*Pinus taeda*) 4 out of 55 single nucleotide polymorphisms (SNPs) selected from adaptive trait and wood quality-related candidate genes, have shown a level of genetic differentiation among populations about sevenfold the genetic differentiation based on neutral nuclear microsatellite markers, and were probably affected by natural selection (González-Martínez et al. 2006).

Greater population differentiation for mitochondrial DNA markers has been found among present-day populations confined to isolated mountains as residuals of species abundant during the last glaciation.

Adaptation to local environments has been demonstrated in several common garden studies involving disparate population provenances of the same species such as Scots pine (*Pinus sylvestris*) from northern European countries (Sweden, Norway, and Finland), and white spruce (*Picea glauca*), lodgepole pine (*Pinus contorta)*, and red alder (*Alnus rubra*) from North America (Davis and Shaw 2001).

The existence of populations adapted to local environments may constitute the "foci" of "preadapted" genotypes to the niche opened in a new geographic range by climate change. Changing climate alters the fitness optimum for each population throughout the species range, but dispersal is likely to be at random with respect to the provenance and genetic endowment for adaptation possessed by the landing seed in the new niche. Differential survival during the course of seedling establishment selectively "sieves" out genotypes that do not tolerate local conditions. The arrival of seeds that are somewhat "preadapted" to the novel climate (e.g., seeds from more southerly populations during periods of climate warming) may contribute to adaptation of seedlings with proper genetic combinations for photoperiod and temperature responses suited to the novel growing season (Davis and Shaw 2001).

Examples of genes discovered in forestry germplasm using molecular genetics approaches or deployed in forest trees to promote and accelerate breeding for fast response to the need of adapted populations facing environmental modification induced by climate changes, are given in Neale and Kremer (2011), Harfouche et al. (2011) and in the next sections (see Sects. 3.3.2.1–3.3.2.4).

3.3.2.1 Conifers

Conifers represent a widespread group with an important ecological role in terrestrial ecosystems, including some species that also have a high commercial value (e.g., *P. taeda*, *P. radiata*, *P. sylvestris*, *P. pinaster*, *Cryptomeria japonica*, *Picea abies*, and *Pseudotsuga menziesii*). Conifers have a unique reproductive system with a haploid megagametophyte (the nutritious mother tissue of a seed) originating from a maternal gamete that can be used for direct sequencing and haplotype determination. In conifers, anonymous molecular markers typically show far less variation than adaptive traits when sampled in the same populations or across the same range (González-Martínez et al. 2004).

Conifers (i.e., *Pinus taeda* L.) and *Arabidopsis thaliana* (*At*) despite the great difference in form, ecological niche, evolutionary history, and genome size, share a high level of apparent homology for expressed sequence tags (ESTs), opening the *At* genomic resources as a reference for studying the genome in conifers (Kirst et al. 2003). QTLs for adaptive traits have been identified in Douglas-fir, loblolly pine, radiata pine, Scots pine, and maritime pine (González-Martínez et al. 2006).

With the combination of association genetics to dissect complex traits to individual genes and population genetic theory to test for selection, it has been possible to detect evidences for genes under various form of selection (purifying, balancing, or directional) in *P. balfouriana* (7 genes), *P. pinaster* (8 genes), *P. radiata* (8 genes), *P. sylvestris* (10 genes), *P. taeda* (79 genes), *P. abies* (24 genes), *P. mariana* (2 genes), and *Pseudotsuga menziesii* (118 genes) (Neale 2007). As already mentioned, 55 SNPs (approx. 7 %) in loblolly pine (*P. taeda*) have shown a level of genetic differentiation among populations sevenfold the species average, and were probably affected by natural selection (González-Martínez et al. 2006). Selection of candidate genes for adaptation have been undertaken, using gene-expression studies, for loblolly pine (Watkinson et al. 2003) and maritime pine (Dubos et al. 2003). Evidence exists that rare functional variants of large, favorable effect exist in breeding populations—a rare cinnamyl alcohol dehydrogenase mutant in a *P. taeda* elite genotype (Ralph et al. 1997) shows that such uncommon variants can have a sufficiently large impact on phenotypes to be targeted for improvement.

Alternative approaches to genomic selection (GS) prediction models (see Sect. 3.4.5) may perform differently for traits with distinct genetic properties (Resende et al. 2012b). The utility of genomic selection was demonstrated in a *Pinus taeda* population of c. 900 clonally replicated individuals screened at four sites, and genotyped for 4,825 SNP markers (Resende et al. 2012a). Generalized Linear Models

(GLM) was applied to estimate diameter and height at multiple ages using genomic random regression best linear unbiased predictor (BLUP). Accuracies of prediction models ranged from 0.65 to 0.75 for diameter, and 0.63 to 0.74 for height. Using genomic selection helps in eliminating field testing and reduce a cycle of genetic improvement by at least 50 %, leading to a gain in selection efficiency per unit of time that is 53–112 % higher than traditional breeding (Resende et al. 2012b). However, models generated at early ages did not perform well to predict phenotypes at age 6 years. These results demonstrate the feasibility and remarkable gain that can be achieved by incorporating genomic selection in breeding programs, as long as models are used at the relevant selection age and within the breeding zone in which they were estimated.

Using genomic selection, the magnitude of the breeding effort can be drastically reduced, by allowing the breeders not only to select superior genotypes early, but also precisely define the mating pairs most likely to produce an optimal allelic combination in the progeny (Toro and Varona 2010).

Achievements in forest tree genetic engineering in conifers (mainly *Pinus* spp.) have already been summarized by Merkle and Dean (2000). Hofig et al. (2006) described a system for inducing male sterility in *P. radiate,* which was based on overexpression of the stilbene synthase gene, STS, under the control of a *P. radiata* malecone-specific promoter. The pine promoter-STS construct might be useful for various genetic engineering experiments in gymnosperms.

3.3.2.2 Eucalyptus

Eucalyptus is the most widely planted hardwood crop in the tropical and subtropical world because of its superior growth, broad adaptability, and multipurpose wood properties. Plantation forestry of eucalyptus supplies high-quality woody biomass for several industrial applications while reducing the pressure on tropical forests and associated biodiversity (Grattapaglia and Kirst 2008). In-depth perspective is provided on the power of association genetics to dissect quantitative variation in eucalyptus (Grattapaglia and Kirst 2008), and QTL for adaptive traits have been identified in this species (Kirst et al. 2004; Thamarus et al. 2004).

The release of the draft *Eucalyptus grandis* genomic sequence (http://www.eucagen.org), has provided new tools for improving abiotic stress tolerance in trees, and RNA seq has recently been used to reveal genotype-specific molecular responses to water deficit (Villar et al. 2011).

Early achievements in *Eucalyptus* spp. genetic engineering have already been summarized by Peña and Séguin (2001). Recent transgenic experiments led to the overexpression of the choline oxidase (*codA*) gene from *Arthrobacter globiformis* which resulted in increased tolerance to NaCl in several lines of *E. globulus* (Yu et al. 2009).

Two AGAMOUS-like MADS-box genes, *EgAGL1* and *EgAGL2*, isolated from flower buds of eucalyptus are considered candidates for engineering of sterile eucalyptus (Kato and Hibino 2009).

Biotechnology-aided improvement of a highly productive tropical eucalyptus hybrid, *Eucalyptus grandis* × *Eucalyptus urophylla* has led to the development of cold-tolerant clones. The cloned trees have acquired freeze tolerance by the introduction of a plant transcription factor that upregulates the cold-response pathways and makes possible commercial plantings in the southeastern United States (Hinchee et al. 2009). Genomic selection to capture the missing heritability and accelerate breeding for growth and wood quality in eucalyptus has been applied by Resende et al. (2012c).

3.3.2.3 Poplar (*Populus* spp.)

Poplar is one of the few trees that are easily amenable to genetic transformation and in vitro regeneration. In many cases it can be propagated vegetatively and is capable of rapid growth, usually reaching 4–6 m within 2 years. Many (but not all) poplars are riparian species that exhibit fast growth, deep and extensive root systems, resistance to submergence, and a high demand for water, all of which are desirable characteristics for phytoremediation.

The poplar (*P. trichocarpa*) genome which is four times larger as compared to the *Arabidopsis* genome, has been sequenced and made publicly available (http://genome.jgi-psf.org/Poptr1_1/Poptr1_1.info.html).

QTLs for adaptive traits have been identified in poplar and willows (Casasoli et al. 2004). QTLs were detected also for the onset of growth cessation, the transition from shoot to bud, the duration of bud formation, and bud maturation, and eight and 16 QTLs, respectively, were mapped on the maternal and paternal map of *P. nigra* (Fabbrini et al. 2012).

Functional genomics research in a hybrid poplar genotype (*P. tremula* × *P. alba*) has been performed for metabolite profiling (Leplé et al. 2007).

Using next-generation Ecotilling and a set of 768 *P. nigra* trees, a variant allele carrying a premature stop codon in *HCT1* (a gene in the lignin biosynthetic pathway) has been identified (Marroni et al. 2011).

Genome-wide characterization of drought stress-induced miRNAs in *P. euphratica* has been recently reported. These miRNAs could be useful for tree breeding aimed at yield protection against drought stress (Bosheng et al. 2011).

A broad overview of transgenic *Populus* trees for forest products, bioenergy, and functional genomics has been given by Ye et al. (2011). Overexpression of a manganese superoxide dismutase (SOD) gene from *Tamarix androssowi*i in a hybrid poplar (*P. davidiana* × *P. bolleana*) resulted in enhanced SOD activity on exposure to NaCl, along with a remarkable increase in growth (Wang et al. 2010).

When the tomato jasmonic ethylene responsive factor (JERF) gene that encodes an ERF-like transcription factor was successfully expressed in a hybrid poplar (*P. alba* × *P. berolinensis*), the transgenic plants were significantly taller and more tolerant of higher salinity levels (up to 300 mM NaCl) than the controls (Li et al. 2009).

Another gene affecting plant tolerance to salt is *SP1*, which encodes a chaperone-like boiling-stable protein. *SP1* was cloned from *P. tremula* and its expression was induced by salt, cold, heat and desiccation stress (Dgany et al. 2004). The *SP1* orthologous gene from *P. euphratica* might have a role in the response to salt stress.

Chinese white poplar (*P. tomentosa*) expressing a chitinase gene from *Beauveria bassiana* (*Bbchit1*) exhibited increased resistance to a pathogenic fungus (*Cytospora chrysosperma*) (Jia et al. 2010).

Activation tagging, a forward-genetic approach, has been used to generate mutant lines and identify endogenes controlling key traits in poplar such as the gibberellin catabolism gene (GA 2-oxidase), which regulates tree stature (Busov et al. 2003, 2011) and the LATERAL ORGAN BOUNDARIES DOMAIN (LBD) gene, which appears to be a positive regulator of phloem formation during secondary growth in poplar (Yordanov et al. 2010).

Expression in poplar of the *A. thaliana GAI* and *RGL1* genes, which mediate gibberellin responses, resulted in dwarfing, altered root growth and gibberellin production, and unexpected metabolite profiles (Busov et al. 2006).

Root development was considerably improved in cuttings of a poplar hybrid (*P. tremula* × *P. alba*) that was transformed with an RNAi construct for an *A. thaliana* ABC transporter (AtMRP5).

The availability of woody biomass with high-syringyl (S) units in lignin increases the yield of biofuel per unit land area because of an increase in conversion efficiency (Weng et al. 2008). Overexpression of the *F5H* gene under control of the *A. thaliana* C4H promoter led to poplar with lignin comprising approximately 97.5 % (Stewart et al. 2009). Lignin has been modified by transgenesis also in hybrid poplar trees (*P. tremula* × *P. alba*) (Mansfield 2009).

Hoenicka et al. (2012) tested different strategies for promoting early flowering in poplar, aiming at the development of a system for biosafety studies on gene containment. Early flowering poplar containing the 35S::*LFY* or HSP::*FT* gene constructs allowed first approaches for the faster evaluation of gene containment. Early flowering has been attempted also by reducing the juvenile phase by gene silencing. RNAi has been used to downregulate a poplar ortholog of CENTRORADIALIS (*PopCEN1*), a gene that plays a key role in maintaining trees in a juvenile state (Mohamed et al. 2010). When poplars harboring an RNAi construct were grown under field conditions, four of the most strongly silenced lines produced inflorescences or floral buds within 2 years of planting, which was several years earlier than for wild-type trees.

RNAi suppression of two *Populus* genes, the SHORT INTERNODES (*SHI*) gene and the closely related STYLISH1 (*STY1*) gene, enhanced shoot and root growth and increased fiber length and the proportion of xylem tissue (Zawaski et al. 2011). Li et al. (2008) observed RNAi stability over 3 years for field-grown hybrid poplar clones (*P. tremula* × *P. tremuloides* and *P. tremula* × *P. alba*) transformed with four types of intron-containing hairpin RNA (hpRNA) constructs. The constructs expressed hpRNAs targeting the promoter or coding sequences of the *Arabidopsis rbc*S gene, suppressing, via RNAi, the expression of the *BAR* gene

already present in the transformed poplar genome. Only RNAi that was directed at the coding sequence was highly efficient at gene suppression.

3.3.2.4 Chestnut

Functional and neutral markers have been used to define the genetic mechanisms of variation at landscape level and to assess the existence of hybrid zones (Villani et al. 1999, 2010).

Simple sequence repeat (SSR) markers prepared by SSR sequence enrichment in the genomic library of Chinese chestnut (*Castanea mollissima*) were used to clearly separate into three different groups the accessions of the cultivated species, *C. mollissima*, *C. crenata*, and *C. sativa* (Inoue et al. 2009). Genetic regions harboring QTLs for adaptive traits have been identified in chestnut (Casasoli et al. 2004).

Chestnut have been transformed with antifungal genes to impart resistance to the chestnut blight fungus (*Cryphonectria parasitica*) (Merkle and Nairn 2005).

A more recent attempt to enhance resistance to *C. parasitica* in American chestnut involved overexpression of a potential antifungal gene, the oxalate oxidase (OxO) derived from wheat (Polin et al. 2006). It was assumed that overexpression of this gene in chestnut will confer resistance to the blight because the enzyme encoded by this gene metabolizes oxalic acid, which is essential for the necrotic ability of *C. parasitica*.

3.3.2.5 Future Applications

Future discovery of useful polymorphisms for MAS and breeding, will be achieved by genome-by-sequencing methodologies and genome-wide association studies (GWAS) carried out in populations sufficiently large to detect the effect of common and rare alleles (see Sect. 3.4). Rare functional variants, usually missed by GWAS, can be discovered by NGS.

Next-generation Ecotilling (Harfouche et al. 2012) holds great promise for accelerating the identification and utilization of beneficial mutations of large effect without the need for transgenesis.

Because of lower sequencing costs and the adoption of strategies for reducing genome complexity (i.e., sequencing the transcriptome by RNAseq), genotyping-by-sequencing (GBS) methods coupled with bulked segregant analysis, will become the method of choice to achieve, simultaneously, the preparation of high-density linkage maps, identification of informative multiple molecular markers, and assessment of haplotypes associated with the trait polymorphism.

Genomic selection will play a crucial role in supporting rapid decisions regarding which germplasm to deploy and the crosses to make to obtain families that are productive and/or adapted to a changing environment. However, for properties that are not observed in breeding populations or that are difficult to breed for, genetic engineering will be useful to support rapid trait enhancement (Harfouche et al. 2012).

3.3.3 Modeling

Parameters describing the structure of genetic diversity (between to within population genetic diversity partition) on extant populations, need to be included in bioclimatic models for predicting species distribution and adaptations under climate change.

"Climate envelope" models (or the climatic niche concept) have been proposed by Working Group II of the IPCC on the impacts of climate change (IPCC 2007b) as methods of choice for prediction of species distributions under climate change.

Climate envelope models use techniques that correlate current species distributions with climate variables to construct the climatic conditions that suit them through an understanding of species' physiological responses to climate change. This "envelope" can then be used to see where species could live under predictions of future climate (Cressey 2008). The bioclimate envelope modeling approach has its foundations in the "ecological niche" theory of Hutchinson (1957). The fundamental ecological niche comprises those environmental conditions within which a species can survive and grow. The climate envelope approach of matching distributions to climate is intrinsically appealing and has been validated by successfully predicting distributions of invading species when they arrive in new continents (Beerling 1993; Baker et al. 2000; Peterson and Vieglais 2001) or due to past climate. Huntley et al. (1989) fitted climate response surfaces to beech (*Fagus* spp.) distributions in Europe and eastern North America. They were able to simulate distribution patterns in Europe during the Holocene using the response surface derived for North America, and vice versa. This suggests that the North American and European beech populations have retained similar climatic tolerances since their separation between 25 and 10 My ago.

Bioclimatic models in their strictest form consider only climatic variables and do not include in their processing other environmental factors that influence the distribution of species, such as soil type, land-cover type, biotic interactions, adaptive evolution, dispersal limitation, competition for resources, historical chance, interpopulation genetic diversity for dispersal and adaptation (Beale et al. 2008).

Current predictions of potential distributions may differ greatly from actual future distributions due to migration limitations. The ability of a species to migrate at a sufficient rate to keep up with the changing climate will be dependent on the dispersal characteristics of individual species (Collingham and Huntley 2000). However, bioclimate envelope models do not account for species dispersal, but instead aim to predict the potential range of organisms under changed climate. The ability to migrate is a function not only of individual species' characteristics, but also the structure of the landscape over which dispersal is occurring, including the presence of natural barriers (such as mountain ranges) or the artificial fragmentation of habitats (through, for example, the growth of agricultural and urban areas or deforestation).

It has been proposed that physiologically based approaches are superior to correlative based bioclimatic envelope modeling methodologies, because species distributions as we observe them today might not be in equilibrium with the current climate as implied by the envelope modeling and might be determined by biotic interactions, physical barriers to dispersal, and human management (Woodward 1987). Therefore, the current realized niches may not represent absolute limits to species ranges and future distributions may show very different realized niches. For example, the present-day distribution of *Tilia cordata* reaches the northern limit in the British Isles. However, the current northern limit of this species is a relic from past climates in the period between 7000 and 5000 BP, made possible largely by the longevity of the species, and the actual estimations made by Woodward (1990) suggest that the present-day reproductive limit of the species is about 200 km south of the northern limit. In the case of yew (*Taxus baccata*), human exploitation has been sufficient to disrupt the large-scale equilibrium between distribution and climate at the European scale (Plaisance 1979).

Another ecological aspect that is overlooked when the climate envelope approach is used, is that species interact with each other in ways that deeply affect their viability (Zarnetske et al. 2012). Certain species impart particularly strong effects on others especially those involved in vertical interactions (e.g., consumers and their resources or predator–prey). Climate change should affect top consumers or predators more strongly leading to an increase in herbivores, and a decrease in plants. As a result, the community experiences an overall decrease in both species diversity and stability (Zarnetske et al. 2012).

On the basis of the above considerations, the use of bioclimate envelope models should form an important first step in a broader modeling framework. But the next step should be the application of a hierarchy framework for addressing the environment–biota relationship considering factors operating at different scales (Pearson and Dawson 2003). Thus, climate can be considered the dominant factor at the continental scale in determining range shift in a coarse grid (i.e., 50 km^2), while at more local scales factors including population genetic diversity, dispersal ability over long distance, topography, land-cover type, biotic interactions, and microclimate may become significant factors over species presence.

The complex dynamics of changing climate space and the potential for species to disperse through fragmented landscapes, need model systems integrating multiple interacting species, genetic diversity and gene flow among populations within species. Rapid climate changes impose stronger selection for adaptation to the new environment (i.e., air temperature increase) and long distance migration from those environments towards northern latitudes or higher altitude, where the average temperature may still match the migrant genotype ability to survive in a mild warming environment, is anticipated to occur in the future. Landscape-related factors such as habitat fragmentation and land use changes in territory encompassing the source population to the northern landing site, may impede

gene flow and can disrupt the linear interplay of adaptation and migration, affecting productivity and threatening the persistence of many species (Davis and Shaw 2001). The climate models IPCC are now working with for preparing the fifth climate assessment report, make use of significant improvements in understanding the complex processes ignited by climate change, but are likely to produce wider rather than smaller ranges of uncertainty in their predictions (Maslin and Austin 2012). Despite this, efforts should be made to integrate genetic data in frameworks such as those used for hierarchical modeling, which simultaneously account for climate-induced range shifts, migration into newly suitable areas, selection against phenotypes that are poor dispersers, or preference for genotypes adapted to local conditions (Pearson and Dawson 2003). The integrated models, may have a much more robust predictability power compared to models based solely on climate data.

3.3.4 Priority Traits for Genetic and Genomic Studies to Predict Dispersal Fate and to Monitor Genetic Diversity for Mitigating Impact

The magnitude of genetic variation in natural populations for traits likely to be critical to survival and reproduction in future climates, needs to be urgently explored for establishing plant community (i.e., forests) management favoring genotypes with stress tolerance genes, genes for new phenologies, or genes for new specialized compounds (i.e., phytohormones) biosynthesized in response to environmental cues affecting growth and development.

Many metabolites do not appear to be immediately required for survival; nonetheless, many may contribute to maintaining population fitness in fluctuating and geographically dispersed environments (Weng et al. 2012). A variety of compounds, from plant polymers (such as lignin, sporopollenin, and rubber which provide mechanical support, gamete protection, and wound healing) to pigments, flavors, volatile scents, and antimicrobials, mediate an array of interspecies interactions that attract pollinators and seed dispersers or deter pathogens and increase robustness against herbivores (Weng et al. 2012). Several secondary metabolites have a commercial importance because there are relevant pharmaceuticals derived from trees including terpenes and steroids with antineoplastic (from the bark *Taxus baccata*), antiprostatic hyperplasia (from the bark of *Prunus africana*) (Leakey 2012), and adaptogenic (*Gingko biloba* and *Panax ginseng*) drug activities (De Luca et al. 2012).

Discovering genes for biosynthesis of phytochemicals with pharmaceutical properties will be important for engineering pathways in plant cells, to be used for in vitro tissue culture factories, microbial systems, and alternative crop species (i.e., *Corylus avellana*) for improving the production of existing medicines that currently require overexploitation of endangered species such as *T. baccata* (Bestoso et al. 2006; Safari et al. 2012).

Changes in biological diversity within and between forest species induced by global warming, affect forest ecosystem functioning in terms of biomass production (primary productivity) and nutrient cycling (Cardinale et al. 2012). However, within species adaptive processes to local environment are extremely important to avoid contraction of the effective population size with detrimental effect on the chance of the species persistence in the forest ecosystem challenged by global warming. The effects of increased temperature on plant metabolism and interaction with the biotic and abiotic components of the local environment may promote the opportunity to favor key biological adaptations that require the presence and, most importantly, the expression of the alleles determining the breakthrough traits. Therefore, the polymorphism for genes encoding enzymes involved in the metabolic pathways for the transport of soil-derived elements, the synthesis of phytochemicals, or new phenologies, should be studied. Finding alleles and haplotypes for phenotypic traits that better suit the plant to the climatic altered local environment or to long-range dispersal in less temperature-stressed northern or altitudinal environments should be important.

Elemental profiles reflect plant adaptations to the environment because mineral elements found in plant tissues come exclusively from the soil, and the soil-derived elements are required for plant structure, metabolism, protein function, signaling, and proper osmotic and electrochemical potential, which in turn contribute to plant adaptation to highly variable soil compositions to survive and thrive (Baxter and Dilkes 2012). A threshold preference to nitrogen content represents one key ecological adaptation of evergreen and deciduous trees, the former striving in nitrogen-poor soils and the latter preferring nitrogen-rich soil.

Several genes are known to determine response to mineral element fertilization and transport within cell compartments. *Pi*-responsive genes were categorized into different functional groups. For example a *P* response gene *PAP23*, encodes a purple acid phosphatase in *Arabidopsis* (Baxter et al. 2008); *Pht3-2* encodes a mitochondrial ATP/ADP antiporter, during medium- and long-term *Pi* deprivation; *PHO1-H1* is involved in the loading of *Pi* into the xylem vessels (Misson et al. 2005); among the genes coding the *Pht1* family of *Pi* transporters (Mudge et al. 2002), *Pht1-4* is induced during short-, medium-, and long-term treatments, indicating a rapid and sustained induction of this gene in response to *Pi* deprivation. There are *Fe* response genes (*IRT1* and *FRO2*) and iron transporter *IRT1* in the roots (Misson et al. 2005). During a period of Pi deficiency there might be induction of genes coding sulfate transporters (*SULTR 1;3, SULTR 3;4*) that may facilitate the higher uptake of S.

It is likely that soil-derived elements and phytochemicals shape the interdependencies and diversity of plant ecosystems forming the base of the global food chain and biological diversity in the ecoregion (Weng et al. 2012). Genetically controlled flexibility in metabolism allows a dynamic response to developmental and environmental changes while maintaining the homeostasis required by a living cell, organ, or whole organism (Milo and Last 2012). The genetic systems related to the functionality of the ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCO) is an example of such flexibility and homeostasis to external changes.

In fact, changes in temperature and aridity led to dozens of independently evolved variants of C4 metabolism for carbon fixation, even as the core process of the Calvin–Benson–Bassham pathway—which uses RuBisCO for carbon fixation—remained conserved (Milo and Last 2012). Other specialized metabolites fulfill a diversity of adaptive roles for plants (Bednarek et al. 2009; Clay et al. 2009) and serve as a source of resistance to selection pressure exerted by insects and microorganisms on biological diversity.

3.3.5 Gauging Climate Change Effects and Adaptive Measure to Increase Crop and Forest Plant Resilience in Ecoregions

In crop species, the impact of global warming on crop yield loss has been ascertained. Data sets recorded since 1980 on crop production, crop locations, growing seasons, and monthly temperature (T) and precipitation, were analyzed for four crops (maize, wheat, rice, and soybeans) for all countries in the world (these four crops constitute roughly 75 % of the calories that humans directly or indirectly consume) (Lobell et al. 2011). Overall, 65 % of countries experienced T trends in growing regions of at least 1 s for maize and rice, with the number of countries slightly higher (75 %) for wheat (T trend of 1 s means that temperatures at the end of the period were 1 standard deviation higher than at the beginning of the period). At the global scale, maize and wheat exhibited negative impacts for several major countries and global net loss of 3.8 % and 5.5 %, respectively, relative to what would have been achieved without the climate trends observed in 1980–2008 (Lobell et al. 2011). For forest species the causal link between global warming and reduced or increased timber yield, agroforestry tree products, or nontimber forest products, has not been quantitatively established yet at the global scale. However, at local scale indigenous people at the forefront of climate change, are directly faced with melting Alpine glaciers, desertification, or inundated islands (Salik 2012). Alpine plant diversity and endemism in the Himalayas is higher than the global average and will be greatly affected by climate changes, which will threaten endemic medicinal plants by demising slow-growing plants and pushing fast-reproducing species towards higher altitude where people need to strive against the effects of oxygen rarefaction. However, Tibetans are adapting their current communities, forests, fruit tree plantations producing wine and olive oil, and agroforestry, to global warming (Leakey 2012; Salik 2012).

Glacial retreat, treeline and shrub advance in Alpine environments has been documented photographically (Salik 2012) and by temporal assessment of flora altitudinal range shift. Records on the presence of vascular plant species and their maximum altitude in the alpine to nival ecosystems of the Rhaetian Alps, in northern Italy, taken in two periods 50 years apart (1954–1958 and 2003–2005) (Parolo and Rossi 2007), revealed an increase in species richness from 153 to

166 species along a continuous altitudinal transect of 730 m. Fifty-two species, including *Tussilago farfara* (+405 m), *Gentiana bavarica* (+230 m), *Salix helvetica* (+85 m), were found at altitudes 30–430 m higher than their 1950s limits, which corresponds to a median migration rate of 23.9 m per decade. Species with more pronounced altitudinal shifts possessed lighter diaspores. The highest increase in species richness was found between 2,800 and 3,100 m.a.s.l., which was related to (a) an estimated shift of the permafrost limit by +240 m during the last 50 years and (b) to an increase of the mean air temperature in the region by +1.6 °C in summer and by +1.1 °C in winter.

Global meta-analyses documented significant range shifts averaging 6.1 km per decade towards the poles (or 6.1 m of altitude per decade), and significant mean advancement of spring events by 2.3 days per decade (Parmesan and Yohe 2003).

Further demonstrations of significant impact of global warming on forest ecosystems as already discernible in the alteration of wild animal and plant populations, have been given by Root et al. (2003).

The causal link between global warming, consumption of goods (phytochemicals with pharmaceutical properties, coffee, cocoa, etc.), and biodiversity loss has been described (Hertwich 2012). Evidence is increasing that the effects of species loss on productivity and decomposition processes are of comparable magnitude to the effects of other global environmental changes (water pollution, salinization of soils, etc.).

Deforestation, forest degradation caused by climatic unsuitability, and altered land use induce habitat alteration and contractive effects on the distributional area available to each wild animal and plant species in forests, as predicted by the power-law relationship mentioned in Sect. 3.3.1. In experiments, reducing the number of species by about 21–40 % caused a reduced plant production of 5–10 %, comparable to documented and expected effects of ultraviolet radiation and climate warming (Hooper et al. 2012). On the basis of mid-range climate-warming scenarios for 2050, it is expected that 15–37 % of species in 20 % of the examined Earth's terrestrial surface, will be "committed to extinction" if their dispersal ability and reproductive system slow the range shift migration (Thomas et al. 2004).

The increased interest in using forest trees for bioenergy conversion mean that deforestation and forest degradation may become a concern, but it may also become an opportunity if sustainable agroforestry projects are promoted. Agroforestry, fruit tree plantation, and horticulture practiced in the ecotone of the main forest using genetic resources from the nearby forest, will surrogate the natural trend to dispersal under climate change and will provide (a) multifunctionality to the used land around the forested areas, (b) monitored access to phytochemical goods, wood for bioenergy, and other rural services (Hvistendahl 2012), (c) increased carbon sequestration in the soil, and (d) lower impact on species biodiversity by reduced deforestation. This activity has strong similarity to domestication processes at the single species level and will greatly benefit from the mentioned information on the available genetic diversity within as well as between populations (Rehfeldt et al. 1999), and on other aspects such as genetic diversity for phenotypic plasticity.

3.3.6 *Phenotypic Plasticity and Norm of Reaction*

Before any directed effort of domestication through genetic selection for adaptation to environmental changes, it will be necessary to perform common garden studies to explore if the phenotypic response of an accession is under strict genetic control or the response to the environment has an apparent nongenetic component. Phenotypic plasticity is the ability of individuals to alter their physiology, morphology, and/or behavior in response to a change in environmental conditions. An ideal genotype would perform optimally under each environmental condition. However, plastic generalist genotypes do not always evolve, and as for any trait evolution, genetic diversity of plasticity is necessary for selecting more plastic genotypes (Auld et al. 2010). The common way to detect genetic variation for phenotypic plasticity is through a genotype-by-environment (G×E) analysis. Significant G×E effects have been found in a very large number of studies, indicating that genetic diversity for phenotypic plasticity does exist. Graphically, G×E is detected by plotting the norm of reaction. A norm of reaction is an array of phenotypes that will be developed by a genotype over an array of environments. The quantification of a norm of reaction is conceptually quite simple: one obtains a number of different genotypes (i.e., clones) and grows each one in a variety of different environments (e.g., different nutrient, light, water conditions). After a period of growth one measures the desired trait(s) from each individual and plots the data. Each line represents the data for a different genotype. If all lines are perfectly horizontal and on top of one another there is no effect of environment (E) or genotype (G). If all lines are not horizontal but on top of each other there is an environmental effect, but no genotype effect. If all lines are horizontal but at different positions there is no effect of environment but there is an effect of genotype. If lines are not horizontal but are parallel, there is an effect of environment and genotype, but there is no genotype × environment interaction. If the lines are neither horizontal nor parallel there is an effect of (1) environment (nonhorizontality), (2) genotype (lines not on top of each other) and (3) genotype × environment interaction (not parallel).

Properly designed field studies to detect simultaneously (a) the genotype-by-environment interactions at phenotypic level, and (b) the modification of gene expressions of eQTL through cDNA-microarray scanning, quantitative RT-PCR, and two-dimensional gel electrophoresis to quantify protein expression, will adequately assess relation of phenotypic plasticity to gene expression (Van Kleunen and Fischer 2005). Studying plasticity along the pathway from gene expression to the phenotype and its relationship with fitness will help to better understand why adaptive plasticity is not more universal, and to more realistically predict the evolution of plastic responses to environmental change (Van Kleunen and Fischer 2005).

The concept of plasticity is interwoven with the notion of canalization (Waddington 1942). Environmental canalization is defined as the insensitivity of a genotype's phenotype to variation in environments. There are cases in which genes restrict development to a small number of outcomes (i.e., flower organs).

A highly canalized organism (or developmental program) would have low plasticity. There are multiple pathways of development, but in some circumstances, genes may limit the extent to which environments can influence development, such as the genes for phenology. Another variant form of the plasticity issue is that some organisms may exhibit threshold effects where there is not a clear gradual transition between forms, but a stepwise change of phenotype in response to a gradual environmental change. One example of this is plants that have distinctly different growth forms in different environments.

Some studies have linked plasticity response to the variable expression of a single gene. It has been reported that, in *Arabidopsis* accessions and recombinant inbred lines, reducing heat-shock protein 90 (Hsp90) function produces an array of morphological phenotypes, which are dependent on underlying genetic expression which in turn affect the expression of other genes (Queitsch et al. 2002). The strength of Hsp90's effects on the buffering and release of genetic variation suggests that it may have an impact on evolutionary processes.

Assessing the extent of phenotypic plasticity for the selected genotypes for agroforestry or forest management under climate change, will be useful information for the prediction of adaptation events. Once small founder populations are established, the longevity and phenotypic plasticity of most tree species may allow for the persistence of these small populations until sufficient genetic variation is input via long-distance pollen flow for the establishment of populations sufficiently large and genetically variable to be viable and become locally adapted (Mimura and Aitken 2007). This suggests it may be worthwhile to initiate new populations through facilitated migration as new habitat becomes available and help seedlings to grow and become reproductively capable under climate warming, as was done for black spruce (*Picea mariana*) (Gamache and Payette 2005). Such colonies that have historically tolerated harsh conditions may provide a nucleus for further range expansion via seed dispersal to more hospitable climates.

3.3.7 Domestication of Crop and Forest Species

Plant domestication is an evolutionary process operating under the influence of human activities. For millions of years, wild crop relatives have experienced oscillation in climate events and since the time of the Last Glacial Maximum, about 20 millennia ago, several plant species have been exposed to the new selection pressure enacted by humans during plant domestication. Domestication and plant breeding are ongoing 10,000-year-old evolutionary experiments that in some instances have radically altered wild species to meet human needs (Hufford et al. 2012). All the modern crop species have undergone a particularly striking transformation compared to the wild ancestors to facilitate plant adaptation to the edaphic environment near the "domus" and far away from its natural habitat, and respond properly and provide food under the prevailing local climate conditions. Some examples of trait modification through domestication for broad adaptation to

the environment are the reduced seed dormancy for earlier and uniform seed germination after fall sowing, attenuation of the fruit bitter taste, increased fruit size, earlier budflush and flowering-time occurrence for earlier fruit set, and preference of progenies from winter-enduring plants or low-rainfall requirement during summer for planting the next-generation seed crop. Other traits such as soft awns, awnless, and rigid spike rachis in cereals or improved awakening and energizing properties of leaves of some tree species (i.e., tea) have been fixed in some varieties as specific adaptations for improved crop management (cereal harvest) or biomass use (leaf infusions for tonic and aromatic beverages). As human societies have moved away from their ancestral roots, they have also moved down the path of intensifying domestication and land use management rather than relying strictly on reproducing the natural environment. Those practices produced the innovations that form the basis of modern agriculture and forestry.

Researchers have sought for decades to identify the genes underlying crop evolution, especially for new, more complex drugs and pathways within the vast library of plant biodiversity (Hines and Zahn 2012).

Artificial selection causes cultivated populations to diverge morphologically and genetically from their ancestral populations. The dramatic and complex plant changes that occurred during domestication are well illustrated by the genetic and morphological events that link the teosinte (wild *Zea mays*) to corn (cultivated *Z. mays*), although reproductive isolating barriers between the two gene pools have not risen (Studer et al. 2011; Studer and Doebley 2011, 2012; Tsiantis 2012; Lin et al. 2012). In other cereals (e.g.,. wheat), predomestication hybridization and polyploidization events induced diversity bottlenecks but they were compensated by capturing part of the genetic diversity of its progenitors and by generating new diversity at a relatively fast pace, although reproductive isolation barriers were widened in domesticated forms compared to the parental species (Dubcovsky and Dvorak 2007).

Domesticated polyploid (wheats, cotton, tobacco, potato, etc.) and natural polyploid species (representing more than 70 % of plant species) tend to have more extended geographic distributions than those of their close diploid relatives (Zohary 1965). Their larger intrinsic adaptability may be related to the biochemical versatility underlying metabolic pathways contributing to genome and phenotypic plasticity. Newly synthesized allopolyploids usually show the expected additive pattern of the enzymes expressed by the parental species (De Pace et al. 1988) but often chromosomal rearrangements and changes in the number and distribution of repeated DNA sequences within heterochromatin have been observed. In other cases experimental allopolyploids display some form of regulatory abnormalities such as rapid gene silencing (Comai et al. 2000) changes including sequence elimination (Shaked et al. 2001), or activation of dormant retrotransposons determining widespread perturbation of gene expression attributable to intergenomic incompatibilities (Wang et al. 2005). Qualitatively similar gene expression responses may be present in hybrids of any type and may even elucidate positive interactions, such as those responsible for hybrid vigor (Comai et al. 2000), which also have positive effects on adaptation to the agricultural agroecosystems

explaining the large distribution of hybrid varieties. Therefore, hybridity may be the best genome constitution to increase biomass production while at the same time having the biochemical and phenotypic flexibility to respond to changing environmental conditions.

Selection for few traits whose genes are prone to mutations (i.e., awnlessness, nonshattering spike and panicles, fertility of all the floret in the spikelet), occur frequently and increased crop harvesting efficiency, setting the main paths of domestication of cereals for 1,000 years (Lin et al. 2007, 2012; Ishikawa et al. 2010). Seed-propagated crops can make unique contributions to evolutionary studies for other species in terms of time and genetic mechanisms underlying diversity useful for domestication.

Divergence from its wild relatives in seedling morphology and germination type has been the most striking evolutionary change under domestication for outcrossers and clonally propagated species such as *Manihot esculenta* compared to its closest wild relative *M. esculenta* subsp. *flabellifolia*. This duo is the product of evolutionary ecology and domestication occurring in the forest/savanna ecotones distributed around the drier seasonal rim of Amazonia (McKey et al. 2012), and may become a model for domestication of long-lived perennial and outcrossed forest species. The ongoing domestication of *Dioscorea cayennensis/D. rotunda* species complex under gene flow from the wild relatives *D. abyssinica* in savanna environments and *D. praehensilis* in forest environments, and molecular genetic analyses of the progenies will provide information on the selection for extraordinary phenotypic plasticity (McKey et al. 2012) for agroforestry using domesticates in ecotonal zones.

The concept of domesticating trees for timber and nontimber products in forestry emerged only in recent years (Leakey 2012) and is part of the agroforestry practices. Agroforestry addresses several issues related to soil fertility and rehabilitation, loss of biodiversity above and below ground, carbon sequestration, useful marketable production, carbon-neutral fuel, nutritional security, and enhancement of local livelihood (Leakey 2012). Agroforestry is an adequate response to mitigate future climate changes and demand for forestry timber and nontimber product and services. The genetic diversity within forest species, as mentioned, is larger than in annuals, and offers greater opportunities for capturing existing genotypes and those with new combinations of genetic traits after hybridization. Techniques to speed selections of genotypes from the main forests, especially genomic selection (Harfouche et al. 2012), coupled with innovation for the fast vegetative propagation of the best genotypes, will benefit the enlargement of agroforestry practices for adaptations to the ecotonal zones of forests, which will likely be the best places for cooperative agroforestry to increase water resource use (Lansing and Miller 2005), carbon-sink capacity of the ecoregion, and sustainable production of forest goods (edible fruits, edible nuts, nuts for food oil, nuts for cosmetic oils, fruits and nuts for medicinal products, and wood biomass for carbon-neutral conversion into bioenergy), and services despite climate changes.

Genes from the core gene pool of the endemic forest species that have evolved over several thousands of years in the same environment under year-to-year

climatic fluctuation, may contribute to the selection of genotypes expressing plastic, rustic, and resource-conserving phenotypes for agroforestry under abiotic stresses (McKey et al. 2012).

A further motivation for forest tree domestication is related to new scenarios concerning the diffusion range or the biocycle of many native and exotic forest plant parasites, resulting from global climate change (Santini et al. 2012). Less abundant annual precipitation and mild winter temperatures are increasing pressure on forest resilience by stimulating pathogen and pest biotrophic activities. Emblematic is the case of the expansion of the distributional range of *Phytophthora cambivora* in chestnut and of the processionary moth in pine trees, and the more frequent infestations of bark beetles in European conifer forests. Those disturbances if not monitored properly and counteracted by forest ecosystem restoration efforts, may lead to massive loss of biodiversity and decline of ecosystem services. As consequences, forest loss and degradation can have further local impacts, such as floods and landslides, or broader impacts such as reduced capture of tons of GHG such as CO_2 which will be added to the GHG from other sources, contributing to air temperature forcing and related global climate change. Plant and animal wild populations can respond to ongoing disturbance and acceleration of climate change through migration and adaptation to minimize genetic drift, restriction of genetic diversity, and local extinction. Anthropogenic intervention along the natural biological path of plant adaptation to disturbance may increase forest resilience, restoration, and afforestation success. The wise use of wild forest genetic resources and genomics for selective breeding, hybridization and clonal propagation of the highly performing genotypes, are pivotal in those endeavors.

In light of the gene pool concept developed by Harlan and de Wet (1971), the undisturbed stands and patches of native forests can be viewed as a large or scattered reservoir of genes representing the wild "primary gene pool" ($GP1_w$) of each species composing the local tree and shrub community. The establishment of nurseries from more than one cycle of selection within $GP1_w$, causes the managed populations to diverge morphologically and genetically from their wild progenitors, leading to the founding of a domesticated subgene pool ($GP1_d$) for breeding purposes. $GP1_d$ is enriched in those target alleles underlying the adaptive performance of the extracted genotypes from $GP1_w$, when selection criteria such as "increased fitness," "tolerance to stresses," and "success in the intergenotypic competition" have been applied. Current understanding of the genetic basis of adaptation in long-lived populations is based largely on temperate forest trees that have been evolving in response to selection pressures such as disease, drought, and cold; examples include $GP1_w$ management, domestication, and breeding in genera from the northern hemisphere forests (*Populus*, *Castanea*, *Picea, Pinus, Alnus*, *Ulmus*) and some from the southern hemisphere (*Eucalyptus*, *Podocarpus* pines, evergreen southern beech *Nothofagus*, etc.) (Kole 2011).

Genome sequencing, genome-wide association studies, and genome selection (GS) (see Sects. 3.4.4.4, 3.4.4.5 and 3.4.5) projects will play a crucial role in supporting rapid decisions regarding which WFGR germplasm to deploy and the crosses to make to obtain families that are productive and/or adapted to a changing

environment. Several forestry genera (*Populus* sp., *Alnus* sp., *Ulmus* sp., *Eucalyptus* sp., *Castanea* sp., *Quercus* sp.) have expressed hybrid vigor after interspecific hybridization. The genomic toolbox developed for species of those genera, will enhance the understanding of the hybrid vigor phenomena and its use for producing a domesticated gene pool to implement a forest community expressing resilience to external perturbation. Breeding to convey the new genetic information in a domesticated gene pool will help adaptive and improved agroforestry practices for GHG emissions reduction (through selection of clones with increased genetic potential to accumulate tree biomass for CO_2 sequestration and recycling as food, feed, and bioenergy), and ecosystem resilience by reducing the pressure on native forests and their biodiversity.

The number of sexual cycles separating domesticated individuals from their wild progenitors is usually low which helps in keeping a high proportion of genes for adaptation in newly selected genotypes. The conservation of the genetic diversity in the dispersed forest trees towards milder climate is likely to depend on genetic restoration efforts for increasing gene flow and active range dispersal. Also the introduction of plants from other populations beyond the distributional range size (vicariance) will help to increase a community of genotypes with new alleles for adaptation.

Other than domestication through agroforestry in the ecotonal interface of forest and agricultural land, forest tree improvement can be achieved by hybridization between endemic species and accessions of vicariant forms introduced from other geographic areas. During the past 10,000 years, gatherers, nomadic people, and farmers, have been using introduced germplasm for food or to develop the locally adapted varieties that were responsive to improved cultural practices, had acceptable quality for food, feed, or fiber uses, and displayed resistance to disease and insect pests and to environmental stresses. Explorers, colonists, scientists, and other travelers have transported genetic resources from one geographical region to another. The Columbian exchange involved not only a transfer of germplasm between two hemispheres, but also the readaptation of the Mediterranean agrosystem to the New World. Its success varied greatly depending on environmental compatibility or cultural competition with indigenous alternatives, but there is no evidence of colonial agricultural devastation (Butzer 1995). Recurrent and global introductions started with the Columbian exchange helped in diversifying diets, reduced subsistence risk, and improved the quality of human life. Since 1492, few exceptional disasters such as the Irish potato famine or the destruction of the American chestnut have been linked to plant introductions. Plant, pathogen, and pest invasiveness is now becoming a serious problem worldwide. However, advances in genetics, genomics, and ecology prepared the scientific tools to cope with those problems, and opened new perspectives to face global warming with plant introductions from geographical areas where warming temperature is the norm.

Modern agroforestry can then be based on hybridization of plant introductions and local population of the same forest species, followed by selection using the

highly efficient tools offered by genetics and genomics (see Sect. 3.4) to speed a new round of germplasm development for new locally adapted trees.

Hybrid zones have been the basis for the evolutionary processes that have accompanied the dispersal of several tree species from Asia towards Europe (chestnut, hazelnut, etc.). Hybrid zones exist where the ranges of two interbreeding species meet and hybridize and may remain stable for thousands of years (Barton and Hewitt 1985). Several forestry genera (*Populus* sp., *Eucalyptus* sp., *Castanea* sp., *Quercus* sp.) have expressed hybrid vigor after interspecific hybridization. Therefore, experimental hybrid zones should be implemented by planting genotyped trees (after proper phytosanitary controls) from species evolved by vicariance, hybridization by outcrossing, seedling establishment, allele combination monitoring, fitness analysis over the years, and finally clonal propagation of the locally fittest trees as shaped by the climate.

"Domestication is stimulated when demand exceeds supply. The latter would explain the relatively recent need to domesticate tree crops from wild forest species in the tropics, as deforestation has increased in proportion to population growth" (Leakey 2012). Reduction of impacts from stressors generated by global climate change will require further crop, tree, and human adaptations in a scenario of human population increase, and domestication through agroforestry practices will be pivotal for reduction of GHG emissions (through CO_2 sequestration and recycling as food, feed, and bioenergy) and ecosystem resilience by reducing the pressure on forests and their biodiversity.

In summary, it is necessary to avoid that in the face of growing human population and increased levels of consumers' income worldwide, and particularly in emerging economies, the trends in consumption of goods and services remain unchanged, otherwise an accelerated increase in aggregate GHG emissions levels is to be expected, reducing the likelihood of keeping global average temperature increase below 2 °C with respect to preindustrial levels. Therefore, it is necessary to quantify global emissions related to consumption of goods and services, in order to move towards a low-carbon future. It is expected that increased consumption of products (fruits, wood, honey, biomass) from agroforestry and orchard tree plantations using varieties from genetic and genomic selection for increased productivity, phenotypic plasticity, tolerance to biotic and abiotic stresses, will underpin the transition phase to resilient, sustainable and resource efficient agriculture-, forest- and human-related activities for resilience to pressure from climate change stressors. The challenge is to improve the knowledge base on which tree should be domesticated, which traits should be enhanced, and which agroforestry practices to promote to address the interrelated concepts of adaptation to climate extremes (heat waves, drought, flooding), improved plant sink capacity to carbon sequestration, carbon recycling through consumption of goods, reduction of biodiversity offsets and no net loss of biodiversity, and ecosystem resilience. In the following section, the current genomic tool and genetic knowledge from molecular genetic approaches will be examined with reference to major model plants for food and feed annual and perennial crop and forest species.

3.4 Exploring the Genomes to Discover Plant Traits and Genes Related to Environmental Resilience

A large proportion of crop yield is lost due to biotic stresses such as insects or diseases (Kou and Wang 2010) or due to abiotic stresses such as drought, submergence, salinity, and high temperature (Hirt 2004). The occurrence of these stresses is often correlated resulting in a complex dynamics leading to increased crop loss. Recent trends in climate change suggest a further increase in frequency and severity of abiotic stresses (Wassmann et al. 2009). On the one hand the stresses lead to heavy crop losses for major crops such as rice, wheat, and maize across different parts of the world, while on the other hand a constant exposure to these stresses has led to the development of valuable genetic material through the process of natural selection to serve as potential sources of tolerance. In the post green evolution era, the major focus of plant breeding was towards development of high-yielding, input-responsive cultivars. As a result valuable genes controlling resilience to abiotic stresses were unknowingly bred out of the newly developed crop varieties. A large proportion of crop areas constantly affected by one or the other abiotic stresses are covered by these high-yielding varieties. These varieties suffer heavy yield losses under stress situations. For example, in the case of rice, large rainfed areas are covered by high-yielding varieties suitable for irrigated conditions, which are preferred by farmers and consumers but suffer heavy yield losses in the case of severe drought. Conversely, valuable landraces, which grow in these rainfed areas, are highly drought tolerant but are not preferred by farmers and consumers due to their low-yield potential and poor grain quality.

Prevailing present as well as predicted situations demand a thorough scan of large populations to identify the genetic variability available in crop species for tolerance to biotic and abiotic stresses. In order to identify suitable germplasm with tolerance to these stresses, it is important that a clearly defined trait of interest is selected. Traits conferring tolerance to stresses with a high heritability and direct correlation with yield can be considered suitable as selection criteria. For abiotic stresses screening of germplasm for yield under stress conditions is an efficient way of identifying superior germplasm. Use of yield as a selection criterion in precise experiments that can minimize the G×E interaction can be a suitable strategy for improving crop yield under abiotic stresses. The crop genome can then be scanned in various ways such as use of molecular markers (linkage and association mapping), gene expression studies, and through microarray or GBS technologies to find gene(s) or QTL(s) affecting the identified traits of interest. Recent advances in genotyping efficiency have led to the development of high-throughput technologies that provide deep coverage of chromosomes to precisely pin point the locations of desirable genes.

3.4.1 Genetic Markers

Genetic markers can be defined as genetic differences at morphological, biochemical or DNA (molecular) level, which can be used to differentiate individuals or species. Genetic markers were originally used to determine the order of genes along the chromosomes (Andersen and Lübberstedt 2003). The first genetic map was developed by Alfred H. Sturtevant where he used six morphological traits (factors) as genetic markers in Drosophila (Sturtevant 1913). Later, Karl Sax reported genetic linkage between qualitative and quantitative trait locus (seed color and seed size) in common bean (Sax 1923). Since these studies, genetic markers have evolved a great deal. On the basis of expression levels at which the differences are observed, genetic markers can be classified into three main types (1) morphological markers, (2) biochemical markers, and (3) DNA (molecular) markers (Collard et al. 2005).

3.4.2 Morphological Genetic Markers

Morphological markers are visible plant traits controlled by Mendelian genes which cosegregate with genes determining the expression of the trait of interest to allow selection for suitable individuals from a population. These may include characters such as flower color, seed shape, growth habits, or pigmentation (Collard et al. 2005). While these markers can be easily monitored across a population through visual screening, they are more prone to environmental effects (Andersen and Lübberstedt 2003). Apart from this, these markers are limited in number, sometimes appear late in plant development and may have pleiotropic gene action leading to effects on other genetic markers or traits of interest (Andersen and Lübberstedt 2003).

3.4.3 Biochemical Genetic Markers

Biochemical genetic markers detect variation at the gene product level such as changes in proteins and amino acids, and include polymorphic proteins such as isozymes and seed storage proteins (Collard et al. 2005) associated with the trait of interest in a population. Isozymes refer to multiple molecular forms of an enzyme encoded by different alleles at the same locus, and sharing a catalytic activity in a tissue of a single organism (Markert and Moller 1959). Allelic variations in the genes coding for subunits of proteins that function as enzymes, lead to slight variation in the enzymes (allozymes) exerting the same catalytic activity. These variations can be detected through various electrophoresis techniques such as starch gel electrophoresis, polyacrylamide gel electrophoresis (PAGE), sodium dodecyl

sulfate (SDS)-PAGE, etc. (Abdullah 2001). The major limitation with biochemical (isozyme) markers is that they are restricted in number and may be influenced by environmental factors (Collard et al. 2005). Apart from this, isozymes are the translated products of genes; their expression is specific to growth stage and tissues of the plant. Despite these limitations, morphological and biochemical markers have been extremely useful to plant breeders (Weeden et al. 1994; Eagles et al. 2001; Collard et al. 2005).

3.4.4 Molecular Genetic Markers

Molecular genetic markers detect variation at the DNA level due to nucleotide changes such as deletion, duplication, inversion and/or insertion. The nucleotide sequence difference for homologous segments of DNA at the same locus can be readily detected (i.e., as "bands" with different mobility during electrophoresis) and their inheritance can be monitored from one generation to the next (Kumar et al. 2009b).

Molecular genetic markers can be classified into different groups based on:

1. Mode of transmission: biparental nuclear inheritance, maternal nuclear inheritance, maternal organelle inheritance, or paternal organelle inheritance.
2. Mode of genotype resolution: for dominant markers usually one allele is "null" because the corresponding DNA fragment remains undetected, while the other allele at the same locus reveals the "presence" of the corresponding DNA fragment; in this case the homozygote for the "presence" allele is indistinguishable from the heterozygote; for codominant markers the different alleles at the same locus reveal the differences for the corresponding DNA fragments and homozygotes are distinguishable from heterozygotes.
3. Method of analysis: hybridization- or DNA cloning-based, PCR-based, and DNA sequence-based markers.

Molecular (DNA) markers are widely used for mapping, detection of quantitative trait loci (QTLs), and tagging of genes. This is predominantly due to their abundance (Collard et al. 2005) and their stability against changing environments and relative ease of sampling for DNA irrespective of plant part or its growth stage.

A wide range of molecular markers have been developed in the past decades, which have made it possible to have a thorough coverage of plant genomes and thus have led to numerous linkage and association mapping studies related to biotic and abiotic stress tolerance (Table 3.1).

Table 3.1 Molecular markers used for mapping studies for tolerance to various biotic and abiotic studies in several crops

Crop	Trait	Marker	References
Rice	Rice blast resistance	RAPD, SSR, SNP	Naqvi et al. (1995), Wu et al. (2004), Hayashi et al. (2006), Chen et al. (2005), Liu et al. (2007)
	Bacterial leaf blight resistance	RFLP, RAPD, SSR	Yoshimura et al. (1995), Blair et al. (2003)
	Brown plant hopper resistance	RAPD, RFLP, SSR	Huang et al. (1997), Chen et al. (2006)
	Gall midge resistance	RAPD, SSR	Nair et al. (1995, 1996), Jain et al. (2004), Sardesai et al. (2002)
	Green leaf hopper resistance	SSR	Fujita et al. (2006)
	High grain yield under drought	SSR	Bernier et al. (2007), Venuprasad et al. (2009), Vikram et al. (2011), Dixit et al. (2012)
	Tolerance to submergence	SSR, CAPs, INDELs	Septiningsih et al. (2009), Neeraja et al. (2007)
	Salinity tolerance	SSR	Alam et al. (2011), Zheng et al. (2008)
	Cold tolerance	SSR	Lou et al. (2007), Andaya and Mackill (2003a, b)
Wheat	Aluminum tolerance	RFLP	Luo and Dvorak (1996)
	Tolerance to salt stress	Protein polymorphism	Gao et al. (1998)
	Powdery mildew resistance	RFLP, AFLP, STS	Ma et al. (1994), Jahoor (1998), Hartl et al. (1995), Nelson et al. (1995b); Hartl et al. (1998), Liu et al. (1998)
	Cyst nematode resistance	RFLP, RAPD	Williams et al. (1994), Eastwood et al. (1994)
	Hessian fly resistance	RFLP, RAPD	Ma et al. (1993), Dweikat et al. (1997)
	Common bunt resistance	RAPD	Demeke et al. (1996), Lintott et al. (1998)
	Kernal bunt resistance	RFLP	Nelson et al. (1998)
	Durable stem rust resistance	RFLP, STS	Nelson et al. (1995a), Bariana et al. (1998)
	Leaf rust resistance	RFLP, RAPD, STS, SCAR	Autrique et al. (1995), Dedryver et al. (1996); Schachermayr et al. (1995, 1997)
	Stripe rust resistance	RFLP, RAPD, STMS	Sun et al. (2001), Fahima et al. (1997), Peng et al. (1999)
	Loose smut resistance	RAPD, RFLP	Procunier et al. (1997)
Maize	Drought tolerance	RFLP, SSR, AFLP, SNP	Frova et al. (1999), Setter et al. (2011)
	Leaf blight resistance	RFLP	Zaitlin et al. (1993)
	Northern corn blight resistance	RFLP	Simcox and Bennetzen (1993)
Sorghum	Head smut resistance	RFLP, RAPD	Oh et al. (1994)
	Drought resistance	RFLP	Crasta et al. (1999)

(continued)

Table 3.1 (continued)

Crop	Trait	Marker	References
Barley	Stem rust resistance	RFLP, RAPD	Kilian et al. (1994), Borovkova et al. (1995)
	Resistance to *Rhynchosporium secalis*	RFLP	Graner and Tekauz (1996)
	Resistance to salt stress	RFLP, AFLP, SSR, SNP	Mano and Takeda (1997), Rostocks et al. (2005)
	Covered smut resistance	HRM	Lehmensiek et al. (2008)
Soybean	Cyst Nematode resistance	RFLP	Skorupska et al. (1994)
Tomato	Insect resistance mediated by 2-tridecanone (2-TD)	RFLP	Nienhuis et al. (1987)
	Resistance to *Fusarium oxysporum*	RFLP	Sarfatti et al. (1989)
	Nematode resistance *Mi* gene	RFLP	Klein-Lankhorst et al. (1991)
	Resistance to *Oidium neolycopersici*	RAPD, AFLP, SCAR, CAPS	De Giovanni et al. (2004), Ricciardi et al. (2007), Pavan et al. (2008)
Beet	Cyst nematode resistance	RAPD	Salentijn et al. (1995)
Potato	Race-specific resistance to *Phytophthora infestans* R1 allele	RFLP, AFLP	MeKsem et al. (1995)
	Cyst nematode resistance H1 gene	RFLP	Pineda et al. (1993)
	Cyst nematode *GroV1* locus in *Solanum vernei*	RFLP	Jacobs et al. (1996)

Source: Jena and Mackill (2008); Gupta et al. (1999); Mohan et al. (1997)

3.4.4.1 Hybridization- or DNA Cloning-Based Molecular Markers

Restriction Fragment Length Polymorphism

Restriction fragment length polymorphism (RFLP) is a technique in which organisms may be differentiated by analysis of patterns derived from cleavage of their DNA.

RFLP markers were first used in 1975 to identify DNA sequence polymorphisms for genetic mapping of a temperature-sensitive mutation of adeno-virus serotypes (Grodzicker et al. 1975). It was then used for human genome mapping (Botstein et al. 1980), and later adopted for plant genomes (Helentjaris et al. 1986; Weber and Helentjaris 1989). The technique is based on the use of (a) cloned and labeled DNA fragments as a probe, and (b) four- or six-DNA-cutter restriction enzymes that fragment genomic DNA to reveal, upon electrophoretic size separation, the pattern of DNA fragment sizes derived from the DNA rearrangements that occur due to evolutionary processes, point mutations within the restriction enzyme recognition

site sequences, insertions or deletions within the fragments, and unequal crossing over (Schlötterer and Tautz 1992). After gel electrophoresis and the separated DNA fragments are transferred to a membrane by Southern blotting; DNA targets of interest are identified by hybridization to the cloned and labeled (usually with incorporation of a ^{32}P-nucleotide) homologous DNA fragment.

The RFLP markers are relatively highly polymorphic, codominantly inherited and highly reproducible. Because of their presence throughout the plant genome, high heritability and locus specificity the RFLP markers are considered superior. The method also provides the opportunity to simultaneously screen numerous samples (Mohan et al. 1997; White et al. 2007).

Variable Number of Tandem Repeats

Variable number of tandem repeats (VNTRs), also defined as minisatellites (Jeffreys et al. 1985) or hypervariable regions (HVRs), are chromosomal regions containing tandem repeats varying in the number of repeat units between genotypes. Usually, they consist of tandem repeat units of a 10–50 bases motif, flanked by conserved DNA restriction sites. The VNTR profile, consisting of many bands usually within a 4–20 kb size range, is generated by using common multilocus probes that are able to hybridize to minisatellite sequences in different species. *Locus*-specific probes can be developed by molecular cloning of DNA restriction fragments, subsequent screening with a multilocus minisatellite probe, and isolation of specific fragments. Variation in the number of repeat units, because of unequal crossing over or gene conversion, is considered to be the main cause of length polymorphisms. Because of the high mutation rate of minisatellites, the level of polymorphism is substantial, generally resulting in unique multilocus profiles for different individuals within a population.

DNA cloning-based molecular markers (RFLP and VNTR) provide a reliable way to identify desired genotypes. However, the method is labor intensive and time consuming (Mohan et al. 1997). Alternatively, as described below, PCR-based approaches such as random amplified polymorphic DNAs (RAPD), sequence-characterized amplified regions (SCARs), simple sequence repeats (SSRs) or microsatellites, intersimple sequence repeats (ISSRs), sequence tagged sites (STS), amplified fragment length polymorphism (AFLPs), and cleaved amplified polymorphic sequences (CAPS) provide a relatively simpler and quicker approach to the generation of genetic maps of crop plants.

3.4.4.2 PCR-Based Molecular Markers

PCR (polymerase chain reaction) is a technique based on enzymatic replication of small quantities of DNA. It is used to amplify a short (usually up to 10 kb) well-defined region of a DNA, representing a single gene or just a part of a gene.

The basic protocol for PCR is based on the following steps:

1. Denaturation: double-strands are separated at high temperature (92–95 °C) to form single strands
2. Annealing: short single strands of DNA (known as primers) bind the 3′ ends of the single-stranded templates
3. Extension: DNA polymerase enzyme catalyzes (at 72 °C) the template-directed syntheses of new double-stranded DNA molecules

Random Amplified Polymorphic DNA

Random amplified polymorphic DNA (RAPD) is a PCR-based molecular marker technique, which was developed independently by Welsh and McClelland (1990) and Williams et al. (1990). In the RAPD marker system, a PCR reaction is performed using template DNA and a single RAPD primer, which is usually just 10 bp long (10-mers) and of random sequence (White et al. 2007). Several thousand 10-mers primers are commercially available, which in theory will all amplify different regions of the target genome to identify polymorphisms (White et al. 2007). RAPD markers can be converted to a form suitable for PCR assays to reduce the unwanted background signals produced in RAPD gels, obtaining the "sequence-characterized amplified regions" (SCAR) markers (Gupta et al. 1999). SCAR markers reveal polymorphism for both alleles at the original RAPD locus (Mohan et al. 1997). When restriction digestion of SCAR markers is performed before or after amplification, it reveals further interindividual polymorphism. These markers are named "cleaved amplified polymorphic sequences" (CAPS; Gupta et al. 1999).

Amplified Fragment Length Polymorphism

This technique developed by Vos et al. (1995) is based on the detection of the variation among genomic restriction fragments by PCR amplification. The procedure consists of a double digestion of genomic DNA with two restriction enzymes. The fragments generated are ligated with adaptors (short double-stranded oligonucleotide with a known sequence) and then reduced in number by a selective amplification with arbitrary primers containing a core sequence that is a part of the adapter. Since this technique does not require any prior knowledge of the sequence, is very useful in the detection of polymorphisms between closely related genotypes (Belaj et al. 2004).

Polymorphisms are detected from differences in the length of the amplified fragments by polyacrylamide gel electrophoresis (PAGE) (Matthes et al. 1997) or by capillary electrophoresis.

AFLP markers have been demonstrated to be more efficient as compared to RAPD and RFLP markers in detecting polymorphisms (Linn et al. 1996; Powell et al. 1996; Ma and Lapitan 1998).

Sequence-Tagged Sites

A sequence-tagged site (STS) is a short, unique sequence characterized by a pair of PCR primers designed by sequencing an RFLP probe representing a mapped low-copy number sequence that identifies a specific locus (Gupta et al. 1999). The STS concept was introduced by Olson et al. (1989). In assessing the likely impact of the PCR on human genome research, they recognized that single-copy DNA sequences of known map location could serve as markers for genetic and physical mapping of genes along the chromosomes (http://www.ncbi.nlm.nih.gov/projects/genome/probe/doc/TechSTS.shtml). In most cases STS markers are codominant. Another advantage of STS markers is that they are simple and easily reproducible on agarose or polyacrylamide gel.

Microsatellites or Simple Sequence Repeats

Microsatellites or simple sequence repeats (SSR) are regions of DNA consisting of tandemly repeating mono-, di-, tri-, tetra-, or pentanucleotide units that are arranged throughout the genomes of most eukaryotic species (Powell et al. 1996). Microsatellite polymorphisms can be detected by Southern hybridization or PCR. If nucleotide sequences in the flanking regions of the microsatellite are known, specific primers (generally 20–25 bp) can be designed to amplify the microsatellite by PCR (Kumar et al. 2009b). With the availability of genome sequences of a large number of crops, microsatellites may be identified by screening these sequence databases for sequence motifs from which adjacent primers can be designed. Although microsatellite analysis is in principle a single-locus technique, multiplexing of multiple microsatellites with different size ranges of the alleles may be carried out during PCR or gel electrophoresis which reduces the analytical cost to a considerable extent (Ghislain et al. 2004). The high degree of variability of these markers also makes them especially suitable for detecting polymorphism between closely related individuals and makes them highly suitable for linkage mapping studies (Smith and Devey 1994). Other advantages of these markers are the codominance of the SSR alleles and their abundance and random distribution across most of the eukaryotic genomes (Morgante et al. 2002). These advantages have led to the extensive use of microsatellite markers for QTL mapping studies in almost all crop species.

Intersimple Sequence Repeats

SSRs are ubiquitous in eukaryotic genomes and have been developed and utilized for mapping studies in a number of species, although the major bottleneck in developing SSR markers is that the flanking sequences must be known to design 5′-anchors for PCR primers (Godwin et al. 1997). Inter-SSR (ISSR) fingerprinting was developed such that no sequence knowledge was required. ISSRs are DNA fragments of about 100–3,000 bp located between adjacent, oppositely oriented microsatellite regions (Kumar et al. 2009b). Primers based on a repeat sequence, such as $(C_A)_n$, can be made with a degenerate 3′-anchor, such as $(C_A)_8RG$ or $(AG_C)_6TY$ leading to the amplification of sequence between two SSRs. PCR products are radiolabeled with ^{32}P or ^{33}P via end-labeling or PCR incorporation, and separated on a polyacrylamide sequencing gel prior to autoradiographic visualization (Godwin et al. 1997). A typical reaction yields 20–100 bands per lane depending on the species and primer (Godwin et al. 1997). Because of the multilocus fingerprinting profiles obtained, ISSR analysis can be applied in studies involving genetic identity, parentage, clone and strain identification, and taxonomic studies of closely related species (Kumar et al. 2009b). In addition, ISSRs are useful in gene mapping studies (Gupta et al. 1994; Zietkiewicz et al. 1994; Godwin et al. 1997; Kumar et al. 2009b).

3.4.4.3 Sequence-Based Molecular Genetic Markers

Sequence-Related Amplified Polymorphism

Sequence-related amplified polymorphism (SRAP) is another type of molecular marker system advantageous for mapping and tagging genes useful to explore open reading frame (ORFs) polymorphisms (Li et al. 2001). The genotyping approach with these markers is based on a two-primer amplification system. The primers are 17 or 18 nucleotides long, and consist of: core elements, which are 13–14 bases long, where the first 10 or 11 bases starting at the 5′ end are sequences of no specific constitution (“filler” sequences), followed by the sequence CCGG in the forward primer and AATT in the reverse primer. The core element is followed by three selective nucleotides at the 3′ end. The filler sequences of the forward and reverse primers must be different from each other.

Single Nucleotide Polymorphism

Single nucleotide polymorphism (SNP) has recently become highly preferred in genomic studies. The fact that in many organisms most polymorphisms result from changes in a single nucleotide position (point mutations), has led to the development of techniques to study SNPs (Kumar et al. 2009b). The availability of sequence information of a variety of crop species has made it possible to obtain

the positions of SNPs on the genome and their flanking sequences. Once the location of SNPs is identified and appropriate primers designed, these primers can be used to differentiate between individuals based on their polymorphism. One of the advantages of SNPs is that they offer the possibility of high-throughput automation (Kumar et al. 2009b). For this reason, several high-speed genotyping platforms are often used to achieve high sample throughput with SNPs. SNP markers may also be combined with other markers, such as SSRs, to saturate linkage maps or identified QTL regions where limited polymorphism is available in order to fine-map identified QTLs.

Among the simple SNP genotyping methods, CAPS and derived CAPS (dCAPS) are widely applied (Neff et al. 1998). Generally, the CAPS marker is a PCR-based marker in which a restriction site is present only in one of the two amplified sequences and can be used in conjunction with restriction site-associated DNA (RAD-tag) sequencing (Barchi et al. 2011; Scaglione et al. 2012).

High-Resolution Melting Markers

Recently, a next-generation mutation scanning technology called High-resolution melting™ (HRM) has been developed (Wittwer et al. 2003), that appears to offer especially time savings over other methods for detection of DNA polymorphisms, even though it requires considerable cost. In this method amplicons from genotypes carrying wild type and variant alleles are produced by PCR in the presence of a dsDNA-intercalating saturating fluorescent dye and are then transferred to a HRM instrument for melting analyses. The dye does not interact with ssDNA but intercalates with dsDNA and fluoresces brightly in this state. HRM instruments slowly heat the samples while simultaneously monitoring the progressive reduction in fluorescence caused by the release of a fluorescent dye from dsDNA amplicons as the molecules are denatured (Wittwer et al. 2003). The data output from a HRM instrument appears as so-called melting profiles or melting curves that plot the reduction in fluorescence against the increased temperature. Amplicons that contain a sequence variant yield altered shapes of melting curves compared with wild-type control samples.

Since its invention in 2003, HRM has been used widely in clinical chemistry and human pathology for efficient molecular diagnostics of diseases and patient genotyping (Reed et al. 2007). However, until very recently, there have been no reports on using HRM in the plant. During the last 12 months, several groups more or less simultaneously reported the application of HRM for mapping SNPs or microsatellite markers in various crop species (Chagne et al. 2008; Croxford et al. 2008; Lehmensiek et al. 2008; Mackay et al. 2008; Mader et al. 2008; Wu et al. 2008) and one group reported the use of HRM for detection and quantification of RNA editing in *A. thaliana* (Chateigner-Boutin and Small 2007).

EST for Differential Display Studies of Gene Expression During Experimentally Induced Environmental Stresses (Microarray)

Plants are constantly challenged with biotic and abiotic stresses and have evolved a diversity of constitutive and inducible responses in order to adapt and survive in the environment. Several genes are induced when the plant is attacked by diverse aggressors, like microbial pathogens, viruses, or insects and studies regarding the mechanism of changes in gene expression have been performed in several species, starting from model species such as *A. thaliana* (Dong 1998; Glazebrook 1999).

Because of the complexity of plant defense responses, DNA microarray technology can provide wide information on number and, then function, of involved genes. Microarrays containing a representation of the whole plant genome will serve to identify the expression pattern of genes of unknown function, to define specific sets of genes responding to various stresses or stimuli, to provide a global view on metabolic processes, and to assist in comparing wild-type and mutant plants.

DNA microarray technology was first set up in Stanford (Schena et al. 1995) using a small set of *Arabidopsis* EST and then applied to several model organisms including *Escherichia coli*, yeast, *Drosophila melanogaster*, mouse, and human (Schena et al. 1996; Chu et al. 1998; Richmond et al. 1999; White et al. 1999; Tanaka et al. 2000).

Currently, two complementary techniques are available: fragment-based DNA microarrays and oligonucleotide-based chips, also referred to as Affymetrix chips (Cheung et al. 1999; Eisen and Brown 1999; Lipshutz et al. 1999; Lockhart and Winzeler 2000; van Hal et al. 2000). DNA microarrays allow the simultaneous hybridization of two fluorescently labeled probes to an array of immobilized DNA fragments (usually PCR-amplified DNA sequences), each corresponding to a specific gene. The microarray is analyzed with a laser scanner, obtaining a signal for each DNA fragment that reflects the abundance of the corresponding messenger RNA in the sample. The use of two differently labeled samples allows the quantitative comparison of gene expression between a control and a test experiment. Affymetrix chips consist of an array of oligonucleotides (usually 20–25 bp), which have been synthesized in situ on a glass surface. Each gene to be analyzed is typically represented by 20 specific probes on the chip. Different methods for labeling RNA are available and allow a quantitative measurement of transcript abundance. As opposed to fragment-based microarrays, oligonucleotide arrays require prior knowledge of DNA sequence information and permit single base change analysis. Usually information on oligonucleotide sequences to be placed on the chips is available from ESTs obtained from a diverse set of cDNA libraries and provides information on transcript abundance, tissue location, and developmental expression of genes. Oligos from full length cDNAs are necessary to identify intron–exon boundaries, alternative splice sites, and to clearly define gene-coding regions within the genomic sequence, as well as for comprehensive gene function analyses at the transcriptional and translational levels.

3.4.4.4 Genotyping-by-Sequencing Approaches

Modern techniques of genome sequencing have become more cost effective in the recent past and several crop species and fruit tree species (i.e., apple, grape, and peach), and timber species (e.g., poplar) have been sequenced while sequencing of many others is in the pipeline (olive). Next-generation sequencing (NGS) is widening the application of genetic and genomic studies also to nonmodel organisms (Nawy 2012). Much of the genetic and breeding research attempts to gain insight into sequence variation that leads to functional phenotypic variation. The key to finding these associations is to assess multiple markers in many individuals, which can be achieved through next-generation sequencing in one experiment.

Complexity reduction of polymorphic sequences (CroPS; van Orsouw et al. 2007) and restriction-site associated DNA (RAD-tag) sequencing are examples of the last class of GBS approach that generates markers en masse without the need for a reference sequence (Baird et al. 2008). Markers generated in this way can be used for preparing linkage maps, quantitative trait locus mapping, haplotype determination linked to phenotypes, comparative genomics, examining phylogeography, tracking domestication, evolution in natural populations, etc. Targeted or candidate gene sequencing can similarly be used to identify allelic variation driving processes such as beginning of anthesis and bud sprouting, metabolite content in seeds, seed size, and genome-wide genetic variation for many compounds important for human health such as molecules with anti-inflammatory properties or for regulating the cell cycle in humans (i.e., taxanes in *T. baccata* and *Corylus avellana*; β-sitosterol in *P. africana*).

The RAD-tag paired-end sequencing (Etter et al. 2011) allows de novo transcriptome assembly tools and high-throughput tagging approaches (i.e., allow studies of changes in coding sequences and gene expression in the absence of a reference sequence, as is the case for many forest species).

Fine mapping of the RAD-tags on the linkage map can be achieved by using 6-base-cutter restriction enzymes and bar-coded adapters to track the genotype of each individual in the F_2 population. While pooling the tagged DNA from different F_2 individuals, Illumina sequencing, and tracking by barcodes eases isolation of RAD-tags from many samples and reduces preparation variation between samples (Baird et al. 2008). RAD-tagged mapping of populations in combination with bulked segregant analysis (BSA) allowed the rapid identification of many candidate genes for the contrasting phenotypes of the trait used for bulking (Baird et al. 2008).

The increase of linkage map information on coding genes, the RNA-seq of the transcriptome of tissue extracts has been performed in teleosts (Pan et al. 2008), and sequenced reads have been successfully placed on the RAD-tag sequenced sites. These are promising NGS methods for forest tree wide genomics.

3.4.4.5 Genome-Wide Association of Molecular Genetic Markers

Linkage and Association Mapping

The main goal of linkage and association mapping is to detect molecular genetic markers in close proximity to the genes controlling the complex quantitative traits (Abdurakhmonov and Abdukarimov 2008).

A quantitative trait is affected by both multiple genetic and environmental factors. Mather (1949) developed biometrical methods that were used up to 1980 to study the genetic component of quantitative trait variation. Those methods were based on the assumption that (a) independent segregation of plus and minus alleles at many polymeric loci underlined quantitative genetic variation, and (b) the effects on genotype of the plus (or minus) alleles were equal at all the loci underlying the trait. Those assumptions were difficult to test until advances in molecular techniques occurred after 1980, which allowed mapping and cloning of the loci affecting quantitative traits, which were defined as quantitative trait loci (QTL).

Two approaches have recently been applied to complex trait analysis in plants, both of which allow QTL identification in samples containing diverse genotypes (1) biparental mapping populations (also referred to as linkage mapping) and (2) linkage disequilibrium (LD)-mapping or association mapping using the diverse lines from the natural populations or germplasm collections. The coming sections deal with the techniques of linkage and association mapping in crop plants.

Biparental or Linkage Mapping

Genetic linkage can be defined as the tendency of genes located in close proximity to each other on a chromosome to be inherited together during meiosis. Genes, whose loci are nearer to each other are less likely to be separated onto different chromatids during chromosomal crossover, and are therefore said to be genetically linked. This concept has been widely used in linkage mapping studies where genetic markers with known chromosomal positions are used to construct a genetic linkage map and then used to locate the position of markers with significant effect on traits of interest. There are four basic steps to perform linkage mapping (1) development of a mapping population, (2) phenotyping of the mapping population, (3) association of markers and trait data, and (4) construction of the linkage map.

A wide range of population structures can be used for QTL mapping. Plants that tolerate inbreeding have especially high latitude for different population structures (Peterson 2002). Moreover, the relative ease of maintaining purity of the lines due to a high percentage of self-pollination in the subsequent generations after crossing also makes population development easier. This makes self-pollinated species such as rice highly suitable for linkage mapping. The major types of population structures being used for linkage mapping include recombinant inbred lines

(RILs), backcross inbred lines (BILs), advanced backcross lines (ABLs), and doubled haploid (DH) lines.

RILs are developed by crossing two parents contrasting for the trait of interest followed by subsequent selfing and advancement through the single-seed decent (SSD) method to achieve nearly homozygous lines.

The development of BILs involve backcrossing (*n* number of times) of F_1 developed by crossing two parents contrasting for the target trait to one of the parents to develop a BC_nF_1 population. Most often, the parent used for backcrossing is the recipient inferior parent into which the QTL has to be finally introgressed. These BC_1F_1 plants are then selfed through the SSD method to a BC_nF_3 generation and then bulked to develop $BC_nF_{3:4}$ lines, which can be used for linkage mapping studies. These populations are especially suitable for introgression of exotic germplasm from wild species to domestic varieties (Peterson 2002).

Advanced backcross populations developed through this procedure are also suitable for fine mapping purposes where large NILs (near-isogenic lines) populations with different segments of the target QTL and similar genetic backgrounds are needed. An advanced backcross QTL (AB-QTL) provides the opportunity to simultaneously identify and introgress QTLs in the recurrent parent, and saves time involved in the varietal development (Tanksley et al. 1996).

In DH lines an attempt has been carried out to combine the advantages of homozygosity with the speed at which an early generation population can be made (Peterson 2002). These populations may be produced by regenerating plants through induction of chromosome doubling from pollen grains; however, their production is only possible in species that are amenable to tissue culture (Collard et al. 2005).

Uniform phenotyping of mapping populations and the development of genetic maps (genotyping) are the next steps for linkage mapping. While most of the linkage mapping studies require the use of large populations for higher precision, the appropriate use of molecular tools becomes an important aspect of QTL mapping. The challenge of identifying a gene or QTL within a plant genome is like finding the proverbial needle in a haystack (Collard et al. 2005). However, genetic markers can be used to develop a systematic linkage map of the chromosome that divides the chromosome into smaller sections in which it is easier to search for a putative QTL. Markers with codominant inheritance, abundance across the genome and suitability for quick and easy PCR amplification and electrophoresis are highly suitable for mapping studies. Several genotyping strategies can be undertaken according to objectives and traits under study. These include (1) whole genome scanning (WGS), (2) bulked segregant analysis (BSA), and (3) selective genotyping (SG).

WGS has been used as a genotyping strategy in many studies. It includes the identification of polymorphic markers between the parents and development of a complete linkage map through genotyping of all individuals in a mapping population with either all polymorphic markers or markers spread evenly across the genome. WGS, although costly, provides a complete linkage map and hence

provides the opportunity to identify major- as well as minor-effect QTLs and at the same time assess the interaction between different loci.

BSA is a DNA pooling technique, which utilizes the phenotypic data of the mapping population to develop bulks of phenotypic extremes for genotyping. This technique was first proposed by Michelmore et al. (1991) where markers linked to disease-resistant genes were identified. BSA involves the pooling of DNA of phenotypic extremes so as to develop high and low bulks, which are genotyped along with the parents with all polymorphic markers. The markers having bulk bands corresponding clearly to the parents are considered as candidates and a full population is genotyped with these markers for identification of QTLs. Apart from the fact that this strategy is highly cost-effective and time-saving, it also eliminates the possibility of identification of any small-effect QTLs. The only drawback of this strategy is that it concentrates on just few segments of the genome and hence provides limited amounts of information.

An SG strategy was suggested by Lebowitz et al. (1987). In this approach 10–15 % lines from the phenotypic extremes are selected from both tails for genotyping. This leads to the generation of genotypic data for a subset of the whole population, which in turn can be used to develop a linkage map for mapping and interaction studies. The markers found to be significant in the subset of the population can be used to genotype the whole population for a more precise estimation of the QTL effect. This strategy is cost-effective, time-saving, and provides almost similar amounts of information as WGS. The success of both BSA and SG depends on the precision of phenotyping and a proper identification strategy of phenotypic extremes. These methods can reliably detect large-effect QTLs, with minimum genotyping and thus allow screening of larger numbers of mapping populations to identify useful QTLs that are effective across genetic backgrounds, or multiple QTLs from different donors that are effective in the same genetic background.

The genotypic and phenotypic data generated through the above-mentioned techniques can be used for the development of linkage maps followed by pinpointing of the location of QTLs based on significance of effect of markers on the trait of interest. Several programs are available online that can be used for constructing linkage maps such as Map-maker/EXP (Lander et al. 1987; Lincoln et al. 1993), MapManager QTX (Manly et al. 2001), JoinMap (Stam 1993), and Mapdisto (Lorieux 2007). Similarly, several software solutions for associating map data with phenotypic data are also available which can perform mapping techniques such as single-marker regression, interval mapping (IM), composite interval mapping (CIM), multiple interval mapping (MIM), Bayesian interval mapping (BIM), etc. Some of the software solutions that help to carry out the above-mentioned analysis are Q gene 4.3.10 (Joehanes and Nelson 2008), Windows QTL cartographer 2.5.009 (Wang et al. 2011a, b), and QTL network 2.1 (Yang et al. 2008). The precision of QTL-mapping largely depends on the genetic variation covered by a mapping population, the size of the mapping population, and the number of marker loci used (Abdurakhmonov and Abdukarimov 2008). Once accurately tagged, marker tags for QTLs can be effectively used to improve

breeding material through marker-assisted selection (MAS). Although the traditional QTL-mapping approach still continues to be an important tool for gene tagging of crops, it is a "now classical approach" and overall is very costly (Stich et al. 2006; Ibarra et al. 2007). Apart from this, the resolution is low with simultaneous evaluation of only a few alleles (Garcia et al. 2003) in a longer research time scale. Another limitation of linkage mapping is the hampering of fine mapping due to the availability of limited number of meiotic events that occurred due to experimental hybridization (Jannink and Walsh 2002). These limitations can be reduced with the use of association mapping, which is discussed in the section ahead.

Linkage Disequilibrium for Association Mapping

The term LD refers to a historically reduced (nonequilibrium) level of the recombination of specific alleles at different loci controlling particular genetic variations in a population (Abdurakhmonov and Abdukarimov 2008). Genetic linkage generally refers to the tendency of different loci within a certain distance on the chromosome to be inherited together. In population genetics, linkage equilibrium (LE) and linkage disequilibrium (LD) are used to describe linkage relationships of alleles at different loci in a population. LE in simple terms can be defined as the random association of alleles at different loci such that at random combination of alleles at each locus, its haplotype (combination of alleles) frequency has equal value in a population. In contrast, LD is a nonrandom association of alleles at different loci, describing the condition with nonequal (increased or reduced) frequency of the haplotypes in a population at random combination of alleles at different loci (Abdurakhmonov and Abdukarimov 2008). The fact that LD can be detected statistically has led to its wide use to map and clone a number of genes underlying complex genetic traits in humans (Risch and Merikangas 1996; Weiss and Clark 2002; Chapman et al. 2003; Taniguchi et al. 2006). There are several advantages of population-based association study conducted on individuals from a diverse germplasm collection or a natural population, over traditional QTL-mapping involving biparental mapping population. These advantages are primarily due to (1) availability of broader genetic variations with wider background for marker-trait correlations (i.e., many alleles evaluated simultaneously); (2) likelihood for a higher resolution mapping because of the utilization of majority recombination events from a large number of meiosis throughout the germplasm development history; (3) possibility of exploiting historically measured trait data for association; and (4) no need for the development of expensive and tedious biparental populations makes this approach time saving and cost-effective (Hansen et al. 2001; Kraakman et al. 2004, 2006).

Although the overall approach of population-based association mapping in plants varies based on the methodology chosen, Abdurakhmonov and Abdukarimov (2008) have outlined six basic steps of conducting association mapping studies in plants. These include (1) selection of a group of individuals from a natural

population or germplasm collection with wide coverage of genetic diversity; (2) recording or measuring the phenotypic characteristics (yield, quality, tolerance, or resistance) of selected population groups, preferably, in multilocation trials with multiple replications; (3) generation of genotypic data of individuals in the population with available molecular markers; (4) estimation of extent of LD of a chosen population genome using molecular marker data; (5) assessment of population structure (the level of genetic differentiation among groups within sampled population individuals) and kinship (coefficient of relatedness between pairs of each individual within a sample); and (6) based on information gained through quantification of LD and population structure, correlation of phenotypic and genotypic/haplotypic data with the application of an appropriate statistical approach that reveals "marker tags" positioned within close proximity to the targeted trait of interest.

The gene(s)/alleles so identified can be cloned and annotated for specific biological function. Several software solutions are freely available to detect the structure and pattern of LD in a specific population. These include: graphical overview of linkage disequilibrium (GOLD) (Abecasis and Cookson 2000), Trait Analysis by association, Evolution and Linkage (TASSEL) (http://www.maizegenetics.net/tassel/), and Power Marker (Liu and Muse 2005).

Several classes of molecular markers were used in association studies to broadly characterize the genetic composition of individuals. RAPD and AFLP markers can serve as background markers but, due to their dominant inheritance, data analyses require special statistical methods if used to estimate population genetic parameters (Ritland 2005; Falush et al. 2007). Conversely, SSRs and SNPs are more powerful in estimating population structure and the relative kinship matrix (Agarwal et al. 2008).

The association mapping approach can generally be subdivided into two broad categories (1) candidate-gene association mapping (which relates polymorphisms in selected candidate genes that have putative roles in controlling phenotypic variation for specific traits), and (2) genome-wide association mapping also named genome scan [which surveys genetic variation in the whole genome to find signals of association for various complex traits (Risch and Merikangas 1996)].

Candidate-Gene Association Mapping

Genes with known functions in the trait of interest may enrich the number of meaningful trait associations, based on the available knowledge from genetic linkage studies and the biochemical or physiological effects of the candidate genes in model and nonmodel plant species (Risch and Merikangas 1996; Mackay 2001). Genetic markers are genotyped at a locus thought to be involved in some phenotype, and one tests for an association between these genetic markers and the phenotype.

In plants, this approach has been successful for candidate genes in relatively simple pathways (Harjes et al. 2008; Zheng et al. 2008) and for candidate genes

with extensive prior evidence of a role in the phenotype of interest (Werner et al. 2005).

Candidate-gene association mapping requires the identification of SNPs between lines and within specific genes, because they offer the highest resolution for mapping QTL and are potentially in LD with the causative polymorphism (Rafalski 2002). The most straightforward method of identifying candidate gene SNPs relies on the resequencing of amplicons from several genetically distinct individuals of a larger association population. SNPs can be identified in coding and non-coding regions (such as promoter, intron, exon, and 5′/3′-untranslated regions), with noncoding regions expected to have higher levels of nucleotide diversity than coding regions. The rate of LD decay for a specific candidate gene locus dictates the number of SNPs per unit length (e.g., kb) needed to identify significant associations (Whitt and Buckler 2003). Therefore, the number and base-pair length of amplicons required to sufficiently sample a candidate gene locus is almost entirely dependent on LD and SNP distribution, with a higher density of SNP markers needed in regions of relatively low LD and high nucleotide diversity. It is not essential to score every candidate gene SNP, because a key objective of this approach is to identify SNPs that are causal of phenotypic variation, those with a higher likelihood to alter protein function (coding SNPs) or gene expression (regulatory SNPs) should be a top priority for genotyping (Tabor et al. 2002).

The biological function of SNPs, if any, for the most part is unknown or not easily recognized. Whereas several SNPs were found in significant LD, an alternative strategy is to select and score a small fraction of SNPs (tag SNPs) that capture most of the haplotype block structure in candidate gene regions (Johnson et al. 2001). Genotyping tag SNPs is more cost effective and, if properly designed, does not result in a significant loss of statistical testing power (Kui et al. 2002). In most cases, allele resequencing in diploid inbred lines (homozygous loci) allows for the direct determination of haplotypes. Reconstructing haplotypes from SNP data in heterozygous and polyploid (ancient or modern) individuals is more challenging, as statistical algorithms are needed to resolve phase ambiguities (Stephens et al. 2001; Simko 2004) and transmission tests are needed to confirm orthologous relationships (Cogan et al. 2007).

Candidate gene selection is straightforward for relatively simple biochemical pathways (e.g., starch synthesis in maize) or well-characterized pathways (e.g., flowering time in *Arabidopsis*) that have been resolved mainly through genetic analysis of mutant loci (natural or induced). But for complex traits such as grain or biomass yield, the entire genome could potentially serve as a candidate (Yu and Buckler 2006).

Genome-wide Association Mapping

Genome-wide association mapping allows one to identify association between phenotypes and genotypes using unrelated individuals that have been simultaneously genotyped and phenotyped (Hirschhorn and Daly 2005; Weigel and

Nordborg 2005; Nordborg and Weigel 2008). The strategy of a genome-wide association mapping is to genotype enough markers across the genome so that functional alleles will likely be in LD with at least one of the genotyped markers. The first step in this process is the discovery of a large number of genetic markers, typically SNPs, as a reference resource. The number of markers and their density are defined by genome size and LD decay and will therefore vary considerably among species. Most genome-wide association studies proceed first by identifying a set of reference SNPs that segregate at intermediate frequency in a small panel of individuals. These SNPs are then genotyped in large samples for which phenotype data are available. The motivation for this strategy is the assumption that common phenotypic variation will be caused by common genetic variation.

This approach requires one to use high-capacity DNA sequencing instruments (i.e., 454-GS FLX or Illumina Genome Analyzer) or high-density oligonucleotide arrays to efficiently identify SNPs at a density that accurately reflects genome-wide LD structure and haplotype diversity (Myles et al. 2009).

Linkage Mapping Versus Association Mapping

Linkage and association mapping, although based on different experimental strategies, are both aimed at identifying recombination events that occurred in the genome which can be correlated with phenotypic variation (Lander and Schork 1994; Risch and Merikangas 1996; Mackay 2001; Thornsberry et al. 2001; Doerge 2002; Shifman and Darvasi 2005; Hirschhorn and Daly 2005). However, while linkage mapping is a highly controlled experiment, instead association mapping can be defined as a natural experiment. In the former approach, two selected genotypes are crossed to generate a mapping population, in which relatedness is known and using a small number of genetic markers it is possible to localize the relatively few recombination breakpoints, also determining if a chromosomal fragment between two specific breakpoints is associated with a phenotype. In the latter strategy, genotype and phenotype data are collected from a population in which relatedness is not controlled and correlations between genetic markers and phenotypes are detected. Association mapping ensures higher mapping resolution in respect to a linkage strategy, but it is difficult to infer when and where recombination has occurred. Moreover, the uncontrolled relatedness among individuals can result in spurious signals of association in downstream analyses (Myles et al. 2009). This approach offers several advantages for QTL identification allowing utilization of all of the recombination events that occurred in the evolutionary history of the analyzed population and, thus, provides a higher mapping resolution. Conversely, the linkage mapping approach, because of the limited recombination events occurring during the establishment of the mapping population, allows one to localize QTLs on large chromosomal regions (10–20 cM), thus they may often represent only a small fraction of the phenotypically relevant variation in a species (Holland 2007).

Association mapping is particularly useful in plant breeding where alleles associated with desirable phenotypes can be introduced efficiently into selected lines (Zhu et al. 2008) and, moreover, because of its ease and cost-effectiveness compared to the laborious and often expensive process of establishing mapping families, especially in the case of analysis performed on organisms that cannot be crossed, cloned, or have long generation times (Nordborg and Weigel 2008).

Recently an approach, called nested association mapping (NAM), has been proposed (Nordborg and Weigel 2008; Yu et al. 2008), which combines the power of linkage mapping with the resolution of association mapping. NAM is usually based on the use of RIL mapping populations in which parental inbred lines are re-sequenced or array-genotyped and the obtained information is coupled with low-cost genotyping of their segregating progenies (Guo and Beavis 2011).

3.4.4.6 Quantitative Trait Loci and Marker-Assisted Selection

The genetics of quantitative traits, such as resistance to biotic and abiotic stresses, is difficult to study, as the effect of each gene associated to the trait phenotype, named quantitative trait locus (QTL), is small and often influenced by the environment or by the interaction with other genes (epistasis). For genetic studies, progenies are obtained by crossing genotypes that contrast for the phenotypic value (i.e., the level of quantitative resistance also known as "field resistance," "horizontal resistance," "tolerance," "partial resistance," etc.; Van der Plank 1963). The segregation of quantitative traits in these progenies enables one to estimate the number of genes involved in the phenotypic expression. These estimations are based on the assumptions that each genotype has the same environmental variation (experimental error) and that the quantitative effects of each gene are similar and additive. However, these assumptions are debatable and often not supported by statistical evidence.

QTLs for tolerance to different biotic and abiotic stresses have been favored by the development of breakthrough technologies for genome sequencing and genome-wide easy-to-use DNA markers. Identification of such QTLs and their precise introgression into varieties is considered as a rapid approach to developing stress-tolerant versions of popular susceptible varieties. The first step of this approach is the development of a mapping population by crossing two or more parents contrasting for the traits of interest followed by a series of selfing for development of a mapping population. This population is then genotyped with markers and screened for the traits of interest. The data so generated is used for the identification of QTLs. Once the position of a QTL is located, line(s) carrying the QTL can be used to perform a series of backcrosses with the recipient parent. At each step, the QTL is monitored with the use of tightly linked markers and markers spread evenly throughout the genome are used to ensure adequate background recovery. The key in this procedure is development of adequate population size and availability of suitable markers for foreground and background selection. The method leads to quick and precise development of stress-tolerant versions of

desired individuals. The advancement in genotyping technology has now made it possible to pyramid several QTLs/genes affecting the same or different biotic and abiotic stresses to achieve wider adaptability and tolerance to a wide range of stresses.

In general terms, a QTL may also be described as "major" or "minor." This definition is based on the proportion of the phenotypic variance explained by a QTL (based on the R^2 value): major QTLs will account for a relatively large amount (e.g., >10 %) and minor QTLs will usually account for <10 %. Sometimes, major QTLs may refer to QTLs that are stable across environments whereas minor QTLs may refer to QTLs that may be environmentally sensitive, especially for QTLs that are associated with disease resistance (Li et al. 2001; Lindhout 2002; Pilet et al. 2001).

The mapping of "major" QTLs for biotic disease can be independent of environment, season, year, or race of the challenging pathogen, whereas, the detection of minor QTLs may be essentially dependent on the "environment." As QTLs are defined by the position on the genome and the quantitative effect on resistance, they do not provide information on the mechanism of resistance. By comparing QTLs with the loci that are involved in race-specific resistance, the coincidence of these loci may suggest a common mechanism. However, the histological characterization of the resistance is more informative about the resistance mechanism.

The relative effect and function of QTLs can be best studied when the responsible gene(s) is (are) isolated. QTL mapping studies should be independently confirmed or verified (Lander and Kruglyak 1995). Such confirmation studies may involve independent populations constructed from the same parental genotypes or closely related genotypes used in the primary QTL mapping study. QTL confirmation can be performed using larger population sizes. However, some studies have proposed that QTL positions and effects should be evaluated in independent populations, because QTL mapping based on typical population sizes results in a low power of QTL detection and a large bias of QTL effects (Melchinger et al. 1998; Utz et al. 2000). Furthermore, NILs appear to be the optimal materials for QTL identification and confirmation as to the influence of possibly other interfering genetic factors is minimal and hence the phenotypes are most discriminative. By genotyping NILs with important markers, and comparing mean trait values of particular NIL lines with the recurrent parent, the effects of QTLs can be confirmed. Examples of studies utilizing NILs to confirm QTLs include leaf rust resistance in barley (Van Berloo et al. 2001), nematode resistance in soybean (Glover et al. 2004).

The germplasm-regression-combined (GRC) molecular marker-trait association identification in plants is a type of QTL identification procedure based on a regression technique that analyzes the association of molecular marker and trait phenotype scored in different germplasm resources available for starting a plant breeding project (Ruan 2010). The method has been developed to overcome difficulties raised by the use of biparental QTL mapping such as: modest repeatability of QTL effects, absence of tight linkage between trait phenotype effect and molecular markers, nonavailability of mapping populations, and lack of substantial

time needed to develop such populations. Traits used for GRC studies include yield and quality traits, disease and pest resistance, and drought tolerance. Molecular markers used in these studies involve RAPD, RFLP, AFLP, ISSR, selective amplification of microsatellite polymorphic loci (SAMPL) and SSR markers. The regression methods used in the GRC association to molecular markers have been linear and multiple regression, and general and mixed linear models. These methods provide the necessary criteria for selecting accessions from a species growing in the same or different region(s) with the genetic endowment for adaptation. They have been suggested for MAS breeding programs of woody plants with a long juvenile phase for which no other genetic information such as on linkage maps and QTLs are available (Ruan et al. 2010).

Information on the relative contribution of each QTL to the total variation, the interactions with other QTLs, the stability in different environments, linkage to undesirable traits, pleiotropic effects, race specificity, etc., may expedite the choice of the better QTL combination to obtain highly resistant cultivars with minimal deleterious effects (Qi et al. 2000; Van Berloo and Lindhout 2000).

MAS increases efficiency and effectiveness in plant breeding. However, despite the development of more high-density maps that incorporate SNPs, EST-derived markers, and STSs, QTL mapping alone is not directly useful in MAS. An important study concerning the more effective integration of MAS and plant breeding suggested the use of advanced backcross QTL analysis, to simultaneously combine QTL detection with variety development (Tanksley and Nelson 1996; Tanksley et al. 1996). The marker-assisted backcrossing (MAB) strategy was used to introgress the *Sub1* locus, for tolerance to submergence, from the landrace FR13A of *Oryza sativa* ssp. *indica* into several widely grown Asian rice cultivars (Septiningsih et al. 2009).

Furthermore, the availability of high-density consensus maps greatly facilitates the construction of new maps and mapping specific chromosomal regions (Chalmers et al. 2001; Harker et al. 2001; Lombard and Delourme 2001; Lefebvre et al. 2002; Karakousis et al. 2003). Comparative mapping could be used to make considerable progress in QTL mapping between related species.

It is expected that the development of high-resolution maps using GBS (i.e., RAD-tag coupled to RNA-seq in Sect. 3.4.4.4) will also facilitate the isolation of genes via map-based cloning (syn. positional cloning).

3.4.5 Genomic Selection or Genome-Wide Selection

One of the earliest forms of genome-wide selection (GWS) was developed by Meuwissen et al. (2001). The method was based on the assumption that when a dense marker map is available, some markers will be very close to a QTL and probably in linkage disequilibrium with it. Therefore, some marker alleles will be correlated with positive effects on the quantitative trait across all individuals in the segregating reference population and can be used for selection without the need to

establish the linkage phase in each individual. Close markers can be combined into a haplotype, the marker haplotypes will be in linkage disequilibrium with the QTL located between the markers, and the haplotype with the biggest effects could be used for predicting genetic values of the offspring. The haplotype effects are predicted using generalized linear models (GLM) or Bayesian statistical models, and selection on genomic breeding values predicted from the estimated effects of haplotype markers substantially increase (up to 83 %) the rate of genetic gain in plants (Meuwissen et al. 2001).

Currently, genomic selection is one of the analytical approaches that is being proposed for genome-based prediction of genomic breeding values for either oligogenic (for which the estimated genomic value of each individual predicts almost 100 % of the phenotypic values using the molecular haplotypes for few markers) or polygenic traits (for which the estimated genomic value of each individual predict less than 100 % of the phenotypic value even using haplotypes for hundreds of molecular markers), in substitution of MAS. Contrary to MAS, GS is based on the available molecular information for all the markers segregating in a reference population. Each marker is included as an independent variable, sometimes in a stepwise manner, into a GLM to find the best fit to the detected phenotypic values of the same individuals for which the intensive genome-wide genotyping for the molecular marker loci have been performed. The fitted values represent the predicted genomic breeding value of the progeny in the present and future generations as long as each plant considered has the same molecular combination for the markers as that used in the GLM regression model that generated the fitted values to the phenotypes in the reference population. Once the relationship between marker allele composition and genomic breeding value has been established in a reference population for a given trait, then the progenies are selected at the seedling stage for the molecular marker combinations corresponding to the highest genomic breeding value, even though the phenotype of the trait is expressed late in the life cycle. Therefore, the procedure is particularly well suited for species with long generation times, for characteristics that display low heritability, for traits that are expensive to measure, and for selection of traits expressed late in the life cycle, as is the case for most traits for horizontal resistance to biotic and biotic stresses in forestry.

3.4.6 Molecular Genetic Markers Used for Major Crop Species

A wide range of molecular markers have been developed in the past decades, which have made it possible to have a thorough coverage of plant genomes and thus have led to numerous linkage and association mapping studies (Table 3.1). Markers such as RFLPs, RAPDs, AFLPs, and SSRs have been widely used in almost all major crop species such as rice, wheat, maize, sorghum, etc., to pinpoint the location of

QTLs/genes controlling biotic and abiotic stress tolerance. In the case of rice, several QTLs/genes conferring resistance to major diseases, such as rice blast and bacterial blight, insect pests such as brown plant hopper, gall midge and green leaf hopper, and to abiotic stresses such as drought, heat, cold, salinity, and submergence have been mapped using molecular markers. In the past decade, rice SSRs have widely been used for linkage mapping studies and MAS. Similarly, in wheat RFLP and RAPD markers have been used widely to map genes conferring tolerance to abiotic stresses such as aluminum toxicity and salinity, diseases such as rusts, bunt, and powdery mildew and nematode and Hessian fly resistance. RFLP and RAPD markers have also been used in crops like maize, sorghum, and barley for identification of QTLs conferring tolerance to drought and diseases. In the case of legumes and vegetables, molecular markers have also proven to be useful for mapping QTLs/genes for tolerance to nematodes and other diseases.

Recent advancements in genome sequencing technology have led to the development of high-throughput genotyping platforms for SNP markers useful for a variety of crops. These markers not only provide elaborate coverage of the genome in order to conduct both linkage and association mapping but are also useful for dissecting the identified QTL regions to fine map and pinpoint genes of interest.

3.4.7 Traits, Genes, Proteins, Metabolites, and Pathways Involved in Resistance to Biotic and Abiotic Stresses in Various Species

3.4.7.1 Traits and Genes Involved in Pathogen–Host Recognition Events

Plants are continuously exposed to a plethora of pathogens and herbivores. Nonetheless, the occurrence of biotic stresses is most of the times prevented by innate mechanisms of resistance, acting either constitutively or upon the recognition of nonself/danger signals. Constitutive barriers include physical elements, such as bark, thorns, trichomes, waxy cuticle layers, and pre-formed antimicrobial secondary metabolites, collectively referred to as phytoanticipins (van Etten et al. 1994). It is generally accepted that constitutive defense mechanisms account for many cases of nonhost resistance, i.e., the immunity exhibited by an entire plant species towards pathogens and pests of other plant species (Nurnberger and Lipka 2005; An and Mou 2012).

Organisms overcoming constitutive barriers may still be recognized by a battery of plant receptors, leading to the induction of a vast array of defense responses. Commonly induced plant reactions to pathogens include cell wall reinforcement, the accumulation of pathogen-related proteins and secondary metabolites with antimicrobial activity (phytoalexins), and the onset of a form of programmed cell death (hypersensitive response or HR), which is thought to deny nutrients to

biotrophic pathogens (Hong et al. 2008). Induced responses to herbivores might be direct, such as the production of compounds, which affect insect host preference or performance, or indirect, such as the production of volatiles that attract insect predators (Bodenhausen and Reymond 2007).

In plant–microbe interactions, a first kind of recognition event occurs between plant transmembrane pattern recognition receptors (PRRs) and conserved molecular signatures associated with pathogenesis. These can be either slowly evolving pathogen-associated molecular patterns (PAMPs), like bacterial flagellin and fungal chitin, or damage-associated molecular patterns (DAMPs), such as oligogalacturonides produced by the pathogen-mediated degradation of the plant cell wall (Zipfel and Robatzek 2010). Corresponding induced immunities, referred to as PAMP-triggered immunity (PTI) and DAMP-triggered immunity (DTI), have been associated to both nonhost and host resistance, the latter being the immunity exhibited, towards a given pathogen species, by particular genotypes of an otherwise susceptible plant species (Jones and Dangl 2006).

It is now clear that the process of adaptation of pathogens to cognate host species involves the differentiation of proteins, known as effectors, which establish susceptibility through the suppression of PTI and DTI. Subsequently, plant–pathogen coevolution might proceed through a zig-zag model, in which plants differentiate receptors (R) enabling the recognition of effectors and the onset of a second kind of induced immunity (referred to as effector-triggered immunity or ETI), and pathogens evolve new effectors which re-establish susceptibility (Jones and Dangl 2006). ETI has been associated with most cases of gene-for-gene resistance, described in detail in the following section.

Recognition events associated with induced immunity towards insects are less understood. However, they are thought to derive from the recognition of DAMPs, ovoposition-related compounds, or putative herbivore-associated molecular patterns (Erb et al. 2012).

After the local activation of defense responses at the interaction sites, systemic defense can be induced or primed, thus protecting undamaged tissues by subsequent invasion. Systemic acquired resistance (SAR) may follow PTI or ETI and is characterized by the activation, dependent on the hormone salicylic acid (SA), of pathogenesis-related proteins. Induced systemic resistance (ISR) commonly follows plant interactions with beneficial microorganisms like plant-growth-promoting rhizobacteria and consists in a systemic primed state for the activation of defense responses, mediated by the hormones jasmonic acid (JA) and ethylene (ET) (Pieterse et al. 2009).

Understanding the molecular mechanisms underlying plant resistance to biotic stresses is essential in order to elaborate efficient breeding strategies, and to cope with sudden urgencies predicted to derive from climate changes. In the last few years, several genes, proteins, metabolites, and pathways involved in these processes have been characterized, which will be described in the next paragraphs. In addition, literature indicating their actual or possible exploitation in breeding will also be reviewed.

R-Genes

H.H. Flor (1955) first demonstrated that the outcome of a plant–pathogen interaction—the one between flax and the rust pathogen *Melampsora lini*—could be explained by a gene-for-gene model, in which resistance occurs upon copresence of a dominant allele by the plant (referred to as R-gene) and a dominant allele by the pathogen (referred to as the Avr-gene). Over the years, gene-for-gene relationships have been documented in many interactions involving crop species and biotrophic organisms, the latter being viruses, bacteria, oomycetes, fungi, and parasitic plants.

It is now clear that gene-for-gene resistance can be assimilated to ETI. Indeed, Avr-genes encode for pathogen effectors, whereas R-genes encode for Avr-recognizing receptors, mostly characterized by nucleotide binding (NB) and leucine-reach repeat (LRR) domains. Recognition between R- and Avr-gene products occurs most of the time indirectly, since R-proteins monitor the molecular state of plant proteins, referred to as virulence targets, with which effectors interact in order to interfere with plant defense mechanisms and promote pathogenesis.

As shown by the example reported in Table 3.2, concerning tomato, breeding for resistance heavily relies on (NB)-LRR R-genes for cultivar development, and thus is still mostly referable to the pioneering work of Flor. However, the high selective pressure originating from ETI typically prompts the onset of pathogen variants harboring new alleles at the Avr locus, which escape the recognition process. As a consequence, although there are notable exceptions (e.g., wheat powdery mildew associated to the R-gene *Pm21*), R-gene mediated resistance is generally not durable (Cao et al. 2011). Another complication to the exploitation of R-genes derives from the progressive loss of biodiversity available for breeders, which makes their identification more and more difficult.

In order to increase the spectrum and durability of R-gene-mediated resistance, many authors have suggested the possibility of pyramiding several R-genes in the same cultivar. Indeed, this kind of resistance would in principle require multiple mutation events at Avr loci to be broken. Successful R-gene pyramiding has been achieved aiming to cope with several plant diseases, including rice blight and blast, wheat powdery mildew and rust, barley yellow mosaic viruses (Joshi and Nayak 2010), and potato late blight (Tan et al. 2010). However, it has been anticipated that, in order to effectively hamper the onset of new virulent pathogenic variants, R-gene pyramiding would require that cultivars carrying single R-genes are not cultivated in the same area (Niks et al. 2011). An alternative to pyramiding is the simultaneous cultivation of genotypes (isogenic lines or cultivars) carrying different R-genes. However, this approach did not have success in practice, in relation to the time and efforts necessary to develop isogenic lines, or because cultivars with different R-gene repertoires usually differ in terms of other important agronomic traits, for which uniformity is highly desirable.

Table 3.2 Resistance and susceptibility genes cloned in tomato and biochemical properties of the correspondent proteins

Resistance source	Encoded protein	Pathogen	Reference
Asc	Homologous to the yeast longevity assurance protein LAG1	*Alternaria alternata*	Brandwagt et al. (2000)
Cf-2; Cf-4, Cf-4E, Cf-5, Cf-9	Extracellular LRR proteins	*Cladosporium fulvum*	Rooney et al. (2005)
Hero	NB-LRR protein	*Globodera rostochiensis*	Ernst et al. (2002)
I-2	NB-LRR protein	*Fusarium oxysporum*	Takken and Rep (2010)
Mi	NB-LRR protein	Aphids and nematodes	Rossi et al. (1998)
ol-2	Nonfunctional seven transmembrane domain protein SlMLO1	*Oidium neolycopersici*	Bai et al. (2008)
pot-1	Nonfunctional translation initiation factor 4E	Potato virus Y and tobacco etch virus	Ruffel et al. (2005)
Sw-5	NB-LRR protein	Tospovirus	Brommonschenkel et al. (2000)
Tm1	Peptide inhibiting viral RNA replication	Tomato mosaic virus	Ishibashi et al. (2007)
Tm2	NB-LRR protein	Tomato mosaic virus	Lanfermeijer et al. (2003)
Ve	LRR protein	*Verticillium dahlie*	Kawchuk et al. (2001)

S-Genes

In breeding, recessive resistance to diseases have been known for many years. When characterized at the molecular level, they have been proven to originate from loss-of-function mutations of genes, which are thus required for pathogenesis, referred to as susceptibility or S-genes (Pavan et al. 2010; Tables 3.2, 3.3 and 3.4).

One well-characterized S-gene is barley *MLO*, which is required for susceptibility to the powdery mildew fungus *Blumeria graminis* f. sp. *hordei* (Buschges et al. 1997; Piffanelli et al. 2004). Barley *MLO* encodes for the prototype of plant specific family of seven transmembrane domain proteins (Piffanelli et al. 2004). It negatively regulates secretory defense pathways at the cell periphery, involving the cell actin cytoskeleton and the PEN proteins PEN1, PEN2, and PEN3 (Miklis et al. 2007). Recently, loss-of-function mutations of *MLO* gene orthologs have been associated with recessive powdery mildew resistance in tomato and pea (Bai et al. 2008; Pavan et al. 2011).

Many S-genes have been characterized in the model plant species *A. thaliana* (Table 3.4), following experimental approaches of induced mutagenesis or gene

Table 3.3 Susceptibility genes cloned in cultivated species and features of resistance derived by their loss-of-function mutations

Gene	Plant species	Encoded protein	Pathogen	Reported pleiotropic phenotype	Reference
MLO orthologs	Barley	Transmembrane protein	*Blumeria graminis* f.sp. *hordei*	Early senescence and axenic cell death	Buschges et al. (1997)
	Tomato		*Oidium neolycopersici*	Not reported	Bai et al. (2008)
	Pea		*Erysiphe pisi*	Not reported	Pavan et al. (2011), Humphry et al. (2011)
eIF4E orthologs	Several species	Translation initiation factor	*Potyviridae*	Not reported	Diaz-Pendon et al. (2004), Robaglia and Caranta (2006)
Xa5	Rice	Transcription factor IIA-γ	*Xanthomonas oryzae*	Not reported	Iyer-Pascuzzi and McCouch (2007)
Xa13	Rice	Membrane-localized protein	*X. oryzae*	Pollen abortion	Chu et al. (2006), Yang et al. (2006), Sugio et al. (2007)

silencing. Some of them, like *TOM1* and *TOM3*, have been predicted to encode for virulence targets (Tsujimoto et al. 2003). Others have been shown to act as negative regulators of plant defense pathways, whose inactivation leads to resistance through enhanced defense responses. Examples of this kind are *EDR1*, *MPK4*, and *PMR4*, which negatively regulate defense pathways dependent on salicylic acid, and *CEV1*, negatively regulating defense pathways dependent on jasmonic acid (Petersen et al. 2000; Ellis and Turner 2001; Frye et al. 2001; Nishimura et al. 2003).

Data reported in the literature strongly suggest that resistance associated with the loss of susceptibility genes resembles nonhost resistance in terms of range of action, durability, and defense mechanisms involved. Indeed, it has often proven to be effective towards different genetic variants of a pathogenic species, and even towards different pathogens (Table 3.4). In addition, immunities associated with loss-of-function alleles of *eIF4E* in pepper and *MLO* in barley and pea are still

Table 3.4 Susceptibility genes cloned in *Arabidopsis* and features of resistance derived by their loss of-function mutations or silencing

Gene	Encoded protein	Pathogen	Reported pleiotropic phenotype	Reference
BIK1	Membrane-anchored protein kinase	*P. syringae*	Enhanced susceptibility to *Botrytis cinerea* and *Alternaria brassicicola;* altered root growth	Veronese et al. (2006)
CEV1	Cellulose synthase	*G. orontii* *G. cichoracearum* *Oidium neolycopersic*i	Reduced size, darker green leaf color, anthocyanin accumulation	Ellis and Turner (2001) Ellis et al. (2002)
CPR5	Transmembrane protein	*Hyaloperonospora parasitica* *P. syringae*	Reduced growth; chlorotic lesions	Bowling et al. (1997) Kirik et al. (2001)
DMR1	Homoserine kinase	*H. parasitica*	No effect or slightly smaller size, depending on the *dmr1* allele	van Damme et al. (2005, 2009)
DMR6	2-oxoglutarate-Fe(II) oxygenase	*H. parasitica* *Colletotrichum higginsianum*	Slightly rounded leaves	van Damme et al. (2005) van Damme et al. (2008)
DND1	Cyclic nucleotide-gated ion channel	*P. syringae* *Xanthomonas campestris* Tobacco ringspot virus	Dwarf	Yu et al. (1998) Clough et al. (2000)
DND2	Cyclic nucleotide-gated ion channel	*P. syringae*	Dwarf	Jurkowski et al. (2004)
EDR1	Mitogen activated protein kinase kinase kinase	*P. syringae* *G. cichoracearum*	Stunted plants with spontaneous lesions under drought conditions	Asai et al. (2002) Frye et al. (2001) Tang et al. (2005)
LSD1	Zinc finger protein	*H. parasitica* *P. syringae*	Lesion formation	Dietrich et al. (1997) Kaminaka et al. (2006)
MAP65-3	Microtubule-associated protein	*Meloidogyne incognita*	Dwarf phenotype and reduced fertility	Caillaud et al. (2008)
MLO2	Transmembrane protein	*G. orontii* *G. cichoracearum* *O. neolycopersici*	Early senescence and axenic cell death	Consonni et al. (2006) Bai et al. (2008)
MPK4	Mitogen-activated protein kinase	*H. parasitica* *P. syringae*	Dwarf, curled leaves and reduced fertility	Petersen et al. (2000)

(continued)

Table 3.4 (continued)

Gene	Encoded protein	Pathogen	Reported pleiotropic phenotype	Reference
PMR4	Callose synthase	*G. orontii* *G. cichoracearum* *H. parasitica*	Epinastic leaves	Vogel and Somerville (2000) Nishimura et al. (2003)
PMR5	Unknown function protein	*Golovinomyces orontii* *G. cichoracearum*	Reduced growth, microlesions	Vogel et al. (2004)
PMR6	Pectate lyase-like protein	*G. orontii* *G. cichoracearum*	Reduced growth, microlesions	Vogel et al. (2002)
RAR1	Zinc-binding protein	*P. syringae*	Not reported	Shang et al. (2006)
SNI1	Leucine-rich nuclear protein	*H. parasitica* *P. syringae*	Reduced growth and fertility	Li et al. (1999); Mosher et al. (2006)
SON1	F-box protein	*H. parasitica* *P. syringae*	Not reported	Kim and Delaney (2002)
SR1	Ca^{2+}/calmodulin-binding transcription factor	*Pseudomonas syringae*	Chlorosis and autonomous lesions	Du et al. (2009)
SSI2	Stearoyl-acyl carrier protein desaturase	*H. parasitica* *P. syringae*	Small rosette, curled leaves, lesions	Kachroo et al. (2001) Shah et al. (2001)
TOM and TOM3	Transmembrane proteins	Tobacco mosaic virus	Not reported	Diaz-Pendon et al. (2004)
TOM2A	Transmembrane protein interacting with TOM1	Tobacco mosaic virus	Not reported	Tsujimoto et al. (2003)

effective and widely used in breeding several decades after their introduction in agriculture (Pavan et al. 2010). However, in order to be useful in breeding, S-gene inactivation must be devoid of severe pleiotropic effects, such as dwarfism or lesion-mimic phenotypes, which derive from constitutive defense activation (Table 3.4).

A valuable strategy to identify new S-genes to be exploited in breeding is to mine functional orthologs of those already characterized in other species, for example through the research of phylogenetically related sequences or the design of conserved primers for PCR amplification. Such an approach, which has been successfully followed for the identification of *MLO* susceptibility genes in tomato

and pea (Bai et al. 2008; Pavan et al. 2011), will be prompted by the availability of large sequence databases for cultivated species. The selective inactivation of S-genes of interest can now be achieved by means of different nontransgenic reverse genetic approaches, such as RNAi and targeted mutagenesis by targeting induced local lesions in genomes (TILLING) (McCallum et al. 2000) or zinc-finger nucleases (Colbert et al. 2001; Marton et al. 2010).

Phytoanticipins

The term "phytoanticipins" was first proposed by van Etten et al. (1994) to refer to "low molecular weight antimicrobial compounds that are present in plants before challenge by microorganisms or are produced after infection from preexisting constituents." Therefore, the definition also includes (a) glycosylated compounds converted in biologically active forms by preexisting hydrolyzing enzymes, following cell decompartmentalization; (b) compounds which are constitutively present in a given tissue and whose synthesis is upregulated upon pathogen challenge. There are a few reports in which metabolic engineering of phytoanticipins has been associated with a substantial increase in resistance towards pathogens and pests. However, breeding approaches focused on phytoanticipins might increase their importance in the future, in relation to (a) new insights on genes and pathways involved in their biosynthesis and regulation; (b) growing evidence for the positive effect of many phytoanticipins, including glucosinolates and tomatine, on human diet.

Saponins

Saponins are glycosilated triterpenoids, steroids, or steroidal glycoalkaloids, whose antimicrobial activity is due to the capacity of forming complexes with sterols of fungal membranes. This is thought to cause the loss of membrane integrity (Morrissey et al. 2000). The defensive role of the root-localized saponin avenacin has been proven by studies associating saponin-deficient (*sad*) mutants with compromised resistance to a wide range of fungal pathogens (Papadopoulou et al. 1999). In addition, mutants of the fungus *Gaeumannomyces graminis* var. *avenae,* impaired in the synthesis of the avenacin-detoxifying β-glucosyl hydrolase avenacinase, are unable to cause disease (Bowyer et al. 1995; Osbourn et al. 1995).

The antimicrobial activity of the tomato saponin α-tomatine has been proven in vitro for a number of nonadapted pathogens. However, it has been shown that α-tomatine does not play a major role in the interaction with many adapted tomato pathogens, since they have evolved detoxifying enzymes. For example, tomatinase from the fungus *Septoria lycopersici* has been shown to convert α-tomatine in the inactive compound β2-tomatine (Morrissey et al. 2000), and mutants impaired in its synthesis are unable to colonize the normally susceptible host *Nicotiana benthamiana* (Bouarab et al. 2002).

Glucosinolates

Glucosinolates, sulfur-containing glucosides specifically produced by the botanic order of Capparales (which includes the family of Brassicaceae), are other well-known examples of phytoanticipins. Depending on the amino acid from which they are formed, glucosinolates can be further divided into aliphatic (from alanine, isoleucine, leucine, methionine, and valine), aromatic (from phenylalanine and tyrosine), and indolic (from tryptophan). Glucosinolates are normally compartmentalized in inactive forms which, upon cell damage, are hydrolyzed by plant myrosinases and release unstable aglycones. These have been shown to have toxic activity toward a wide range of bacteria, fungi, and oomycetes in vitro (Schlaeppi et al. 2010).

Several genes involved in the biosynthesis of glucosinolates have been characterized (Wang et al. 2011a, b). Genetic analysis with Arabidopsis mutants has shown that the combined action of indole glucosinolates and camalexin (described in the following paragraph) plays a major role in defense against the oomycete *Phytophthora brassicae* (Schlaeppi et al. 2010) and in resistance to the powdery mildew fungus *Golovinomyces orontii* associated with the loss-of-function mutation of the *MLO* ortholog *AtMLO2* (Consonni et al. 2010). Intriguingly, indole glucosinolates have been recently shown to act as signaling molecules for the plant response to PAMPs, thus pointing to an additional role for them in defense besides direct antimicrobial activity (Clay et al. 2009).

Cyanogenic Glucosides

Cyanogenic glucosides are a class of phytoantycipins that are widely distributed in the plant kingdom, including ferns, gymnospermae, and angiospermae. They are glucosides of α-hydroxynitrile derived from aliphatic (L-isoleucine, L-leucine, and L-valine) or aromatic (L-phenylalanine and L-tyrosine) protein amino acids, or from the nonprotein amino acid cyclopentenyl-glycine (Zagrobelny et al. 2004). Their biosynthetic pathway involves two P450 cytochromes, responsible for the formation of an aldoxime and an α-hydroxynitrile, respectively, and a glycosyl transferase.

Similarly to glucosinolates, cyanogenic glucosides are stored in the vacuole and, upon cell damage, enter into contact with the enzymes responsible for the release of HCN and an equimolar amount of keto-compounds or aldehydes, i.e., β-glucosidase and α-hydroxynitrile lyase. The defensive role of cyanogenic glucosides has been related, besides to the direct toxicity of HCN, to feeding deterrent activities exerted by cheto-compounds and aldehydes, and to the effects of compounds released during HCN detoxification, β-cyanoalanine, thiocyanate, and sulfite (Zagrobelny et al. 2004).

The introduction of sorghum genes necessary for the synthesis of the cyanogenic glucoside dhurrin (encoding the P450 cytochromes CYP79A1, CYP71E1 and the glucosyl transferase UGT85B1) in the noncyanogenic species *A. thaliana* resulted in resistance to the flea beetle *Phyllotreta nemorum* (Tattersall et al. 2001). This,

besides providing final evidence for the important role of cyanogenic glucosides in plant defense, clearly suggests the possibility of breeding approaches based on metabolic engineering (Morant et al. 2007). However, success of such strategies will largely vary according to the specific interaction, as many herbivores use feeding mechanisms, which limit cell damage and thus the hydrolysis of cyanogenic glucosides (e.g., aphids), or have evolved the enzymatic repertoire necessary for detoxification (Moller 2010).

Phenolic Phytoanticipins

Pre-existing phenolic compounds, including simple phenols, phenolic acids, flavonols, and dihydrochalcones, have been found to have an important antimicrobial activity in planta or postharvest (Lattanzio et al. 2006). Well-known phenolic phytoanticipins are onion catechol and protocatechuic acid, which prevent the germination of most spores of the smudge disease-causing fungus *Colletotrichum circinans*. Other examples include chlorogenic acid, which in potato has been shown to possess antibiotic activity towards *Streptomyces scabies* and *Verticillium albo-atrum*, dihydroquercetin, associated with barley resistance towards different *Fusarium* species (Skadhauge et al. 1997), and the bitter orange compounds naringin and tangeretin, active against *Penicillium digitatum* (Lattanzio et al. 2006).

Phytoalexins

Phytoalexins are low molecular weight secondary metabolites with antimicrobial activity, whose synthesis is induced in response to pathogens (Ahuja et al. 2012). Similarly to PR-proteins, phytoalexins can also be produced in response to stimuli mimicking a pathogenic attack, such as the exogenous application of PAMPs and methyl jasmonate (Millet et al. 2010).

Phytoalexins have been characterized in both monocots and dicots, and their biochemical structure varies considerably according to the botanic family in which they are produced (Harborne 1999). For example, phytoalexins from Leguminosae are mostly flavonoids (e.g., medicarpin), those from Vitaceae are stilbenes (e.g., resveratrol), whereas sulfur-containing indolic phytoalexins (e.g., camalexin) seem to be only produced by members of the order Capparales (Grayer and Kokubun 2001).

The production of camalexin, the main *Arabidopsis* phytoalexin, is regulated by a phosphorylation cascade involving the kinases MPK3 and MPK6 (Ren et al. 2008). The first biosynthetic step is the conversion of the amino acid tryptophan in indole-3-acetaldoxime, which is mediated by the two functionally redundant cytochrome P450 homologs CYP79B2 and CYP79B3. The cytochrome P450 homolog CYP71A15 (also known as PAD3) catalyzes the last steps of camalexin biosynthesis. Genetic studies using *Arabidopsis pad3* mutants, impaired in the accumulation of camalexin, have been addressed to establish the actual effect of these metabolites, whose toxic activity has been related to cell membrane damage, on

plant–pathogen interactions. An important role of camalexin was shown in relation to resistance against the necrotrophic pathogens *Alternaria brassicicola* and *Botrytis cinerea*, the powdery mildew biotrophic fungus *G. orontii* and the hemibiotroph *P. brassicae* (Glazebrook 2005; Consonni et al. 2010; Schlaeppi et al. 2010).

The transgenic overexpression of phytoalexins has been associated with enhanced resistance in a number of cultivated species. Many examples regard the heterologous expression of stilbene synthase genes, responsible for the biosynthesis of resveratrol from malonyl-CoA and *p*-coumaroyl-CoA (Delaunois et al. 2009). For instance, the expression of grapevine resveratrol in tomato and papaya has been associated with a reduction of 65 % and 35 %, respectively, of the disease level caused by *Phytophthora* species (Zhu et al. 2004). Overexpression of the enzyme isoflavone O-methyltransferase in transgenic alfalfa resulted in decreased symptoms caused by *Phoma medicaginis* (Dixon 2001). In general, success of metabolic engineering for enhanced phytoalexin production is thought to be largely dependent on the particular phytoalexin–pathogen combination and the number of genes that need to be transferred (Dixon 2001).

Pathogenesis-Related Proteins

Pathogenesis-related (PR) proteins are defined as plant proteins which, in a given tissue, become readily detectable when challenged by pathogenic microorganisms (viruses, bacteria, oomycetes, fungi), parasitic organisms (insects, nematodes, and herbivores), and stimuli such as the exogenous application of effectors or salicylic acid or wounding. This definition excludes a large number of proteins, which are constitutively expressed and whose synthesis is upregulated following exposure to the conditions mentioned above (van Loon et al. 2006). The first five PR proteins were distinguished in tobacco based on their different electrophoretic mobility; afterward, a classification method of PR protein families based on amino acid sequence, serological properties, and/or enzymatic/biological activity was adopted (van Loon et al. 1994), resulting in an additional 12 PR families (Table 3.5).

The induction of a protein in a situation of biotic stress does not necessarily imply its role in defense. However, strong evidence for the involvement of many PR proteins in the plant immune response derives from the characterization of their biochemical features, which has been in some cases tested in vitro on plant pathogens and pests, and from studies relating overexpression/silencing of PR-proteins with enhanced resistance/susceptibility. Moreover, many PR proteins have been shown to accumulate in response to plant cell-wall appositions (papillae) formed at the interaction sites with pathogens and into the fungal structures penetrating plant tissues (van Loon et al. 2006).

PR-proteins are usually characterized by a low molecular weight (<50 kDa). Most of them evolved to be stable to the harsh conditions of extracellular spaces, cell walls and vacuoles, and thus are resistant to extremely acid pH conditions and proteases (Stintzi et al. 1993). Defense-associated hydrolytic actions have been associated with pathogen-related proteins belonging to the families of glucanases

Table 3.5 Pathogen-related (PR) protein families and correspondent biochemical properties

Pathogen-related protein family	Property
PR-1	Unknown
PR-2	β-1,3-glucanase
PR-3	Chitinase
PR-4	Chitinase
PR-5	Thaumatin-like
PR-6	Protease-inhibitor
PR-7	Endoproteinase
PR-8	Chitinase type III
PR-9	Peroxidase
PR-10	Ribonuclease-like
PR-11	Citinase
PR-12	Defensin
PR-13	Thionin
PR-14	Lipid-transfer protein
PR-15	Oxalate oxidase
PR-16	Oxalate oxidase-like
PR-17	Unknown

(PR-2), proteinases (PR-7), and chitinases (PR-3, -4, -8 and -11). Glucanases and proteinases have been related to the degradation of fungal and oomycetes cell walls. All chitinase families might mediate the lysis of the main constituents of fungal cell wall and insect exoskeleton. Notably, type III chitinases, included in the PR-8 family, also show substantial lysozyme activity, which has been implicated in the hydrolysis of the cell wall of Gram-positive bacteria. Thaumatin-like proteins (PR-5), defensins (PR-12), thionins (PR-13), and lipid-transfer proteins (PR-14) have been associated with defense mechanisms involving the permeabilization of microbial plasma membranes (Regente et al. 2005; van der Weerden et al. 2010; Oard 2011). Proteinase inhibitors of the PR-6 family form complexes with the active site of target proteases, and have therefore been implicated in the limitation of amino acid availability to the parasite. Peroxidases of the PR-9 family have been implicated in reinforcing plant cell walls by catalyzing lignification (Taheri and Tarighi 2012). Finally, ribonuclease-like proteins of the PR-10 family have been associated with defense against viruses, and oxalate oxidase and oxalate oxidase-like proteins (PR-15 and PR-16) generate hydrogen peroxide, which can be directly toxic or might activate other defense responses (Park et al. 2004; Vandenbroucke et al. 2008).

Several studies have been carried out aiming to test the effect of transgenic overexpression of single PR proteins on plant–microbe interactions. But, these have rarely led to highly resistant phenotypes (van Loon et al. 2006). However, notable exceptions have been reported. For example, the overexpression of heterologous defensins has been associated with a substantial decrease of symptoms caused by *Fusarium oxysporum* sp. *lycopersici* and *B. cinerea* in tomato (Kostov et al. 2009) and by *Magnaporthe oryzae* and *Rhizoctonia solani* in rice (Jha et al. 2009).

A promising breeding strategy seems to be the simultaneous overexpression of more PR-proteins. The synergic action of glucanases and chitinases in conferring resistance to fungi and oomycetes has been demonstrated in several crops, including pea, bean, tomato, tobacco, maize, soybean, potato, and wheat (Ebrahim et al. 2011). One must applay caution, however, before the release of PRs transgenic crops, because of the assumption that some PRs members display allergenic properties. In this respect, plant-derived allergens have been identified with sequence similarities to PR-protein families 2, 3, 4, 5, 8, 10, and 14 (Hoffmann-Sommergruber 2002).

3.4.7.2 Traits and Genes Involved in the Biotic Stress Defense Signaling Network

Following the recognition event, a signaling network is triggered, ultimately leading to the activation of a set of induced defense responses, among which, for example, is the biosynthesis of the above-mentioned phytoalexins and PR proteins. It is now clear that such a network involves a complex interplay between several partners, including calcium, protein kinases, reactive oxygen species (ROS), reactive nitrogen species (RNS), phytohormones, and transcription factors (Fig. 3.1).

Calcium

Calcium ions act as second messengers in several signaling pathways, among which are those in response to pathogens and herbivores (Dodd et al. 2010; Arimura et al. 2011). Proteins transducing calcium signaling have been classified into (a) sensor relays, such as calmodulin (CaM), which, upon Ca^{2+} binding, undergo a conformational change that results in the interaction and the modification of the activity of target proteins; and (b) sensor responders, such as Ca^{2+}-dependent protein kinases (CDPKs), which, upon Ca^{2+} binding, undergo a conformational change that alters their own activity (Lecourieux et al. 2006).

In plant–pathogen interactions, increased levels of cytoplasmic calcium occur in response to both PAMPs and effectors, and are essential for defense signaling (Lecourieux et al. 2006). A subgroup of Arabidopsis CDPKs is a convergent point of signaling triggered by different PAMPs, leading to early transcriptional reprogramming and the production of ROS, and two potato CDPKs mediate ROS production through the phosphorylation of a plasma membrane NADPH oxidase (Kobayashi et al. 2007; Boudsocq et al. 2010). In addition, evidence has been reported for the Ca^{2+}/CaM-dependent generation of the RNS nitric oxide (NO) and ROS, and the Ca^{2+}/CaM-dependent activation of SA and SAR, through the transcription factor CBP60g (Choi et al. 2009; Tena et al. 2011). A role for calcium in defense downregulation has also been established, since the activity of barley MLO and the one of the Arabidopsis transcription factor AtSR1, repressing EDS1,

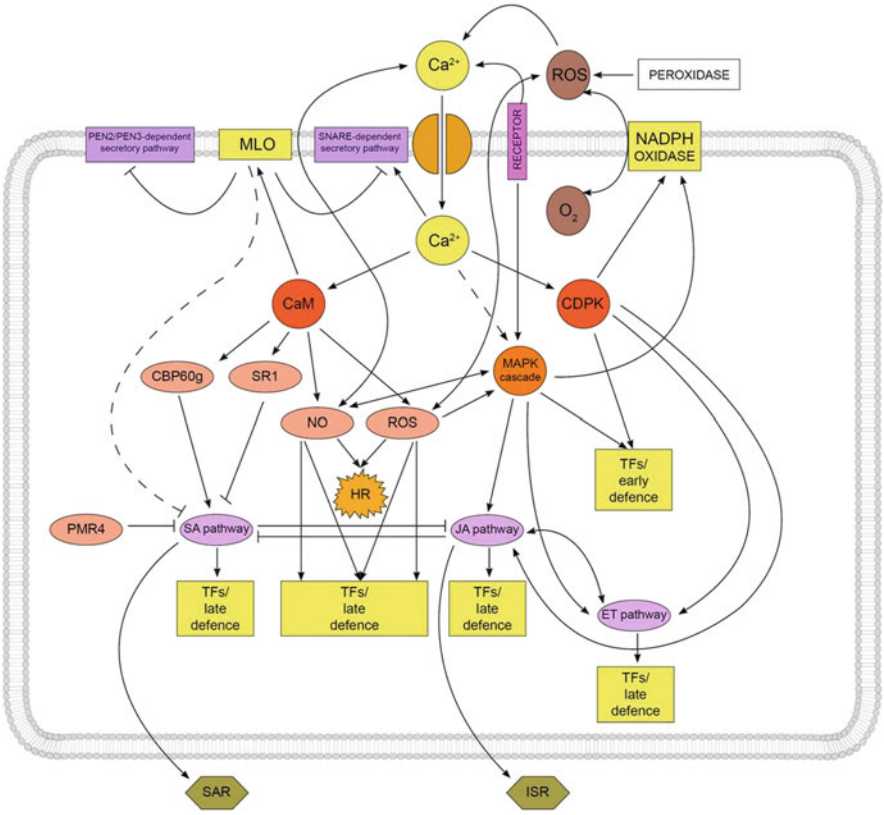

Fig. 3.1 Signaling network in plant defense. *Dashed connections* point to hypothetical links. *Abbreviations*: *CaM* calmodulin, *CBP60g* calmodulin-binding protein 60g, *CDPK* calcium-dependent protein kinases, *ET* ethylene, *HR* hypersensitive response, *ISR* induced systemic resistance, *JA* jasmonic acid, *MAPK* mitogen-activated protein kinase, *MLO* mildew locus O, *NO* nitric oxide, *PEN2* and *PEN3* penetration proteins 2 and 3, *PMR4* powdery mildew-resistant protein 4, *ROS* reactive oxygen species, *SA* salicylic acid, *SAR* systemic acquired resistance, *SNARE* soluble N-ethylmaleimide-sensitive factor attachment protein receptor, *SR1* signal responsive 1, *Fs* transcription factors

an activator of SA-dependent defense responses, are both dependent on Ca^{2+}/CaM binding (Kim et al. 2002; Du et al. 2009).

In plant–insect interactions, CDPKs have been involved in transcriptional reprogramming through the phosphorylation of the transcription factor HsfB2a (Tena et al. 2011). In addition, Ca^{2+}/CaM binds to the mitogen-activated protein kinase MAPK8, which in turn limits the oxidative burst by controlling the NAPH oxidase (Takahashi et al. 2011).

Phosphorylation

Phosphorylation events have a key role in defense signaling during both PTI and ETI. Ca^{2+} influx following the perception of the PAMP cryptogein is prevented by the serine/threonine protein kinase inhibitor staurosporine, thus indicating that phosphorylation is responsible for the increase of cytoplasmic Ca^{2+} (Lecourieux et al. 2006). Phosphorylation might also take place downstream of the elicitor-induced Ca^{2+} influx. For example, this is the case for CDPKs-mediated phosphorylations, or the Ca^{2+}-dependent phosphorylation of the synthaxin PEN1 (Lecourieux et al. 2006).

Phosphorylation cascades mediated by the family of mitogen-activated protein kinases (MAPKs) are key players in defense signaling, and are likely to be at least partially dependent on Ca^{2+}-influxes (Boudsocq et al. 2010). A minimal MAPK cascade module consists of a MAPK kinase kinase (MAPKK or MEKK), a MAPK kinase (MAPKK or MKK) and a MAPK. MAPKs are then responsible for the activation, through phosphorylation, of target transcription factors (Galletti et al. 2011). In Arabidopsis, a complete MAPK cascade, involving MEKK1, MKK4/MKK5, and MPK3/MPK6 has been shown to be triggered by the perception of the bacterial PAMP flg22. This, in turn, activates downstream targets, among which are the WRKY family transcription factors WRKY22 and WRKY29, which regulate genes associated with early defense responses (Asai et al. 2002). MAPKs have been positioned in many other nodes of the plant defense signaling network against pathogens, including: the activation of the biosynthesis of the *Arabidopsis* phytoalexin camalexin, through a cascade involving MPK3/MPK6 (Pitzschke et al. 2009); the positive regulation of ROS production, through a cascade, identified in *N. benthamiana*, made up of the closest homologs of *Arabidopsis* MKK6 and MPK4, and acting on an ROS-generating plasma membrane NADPH oxidase (Pitzschke and Hirt 2009); the positive regulation of NO production, through a cascade, identified in *N. benthamiana*, made up of the closest homologs of Arabidopsis MKK4/MKK5 and MPK3/MPK6 and acting on the NO biosynthetic enzyme NOA1 (Pitzschke and Hirt 2009); the ROS-dependent activation of transcription factors, through oxidative-stress-dependent MAPK cascades (Pitzschke and Hirt 2009); the negative regulation of ROS, SA and SAR signaling, through *Arabidopsis* MEKK1, MKK1/2, and MPK4 (Pitzschke and Hirt 2009).

In plant–insect interactions, a MAPK cascade involving MKK3 and MKK8 has been shown to limit the oxidative burst following wounding. In addition, other MAPK cascades are thought to activate signaling dependent on JA (Tena et al. 2011).

Reactive Oxygen and Nitrogen Species

The production of ROS like hydrogen peroxide (H_2O_2) and superoxide (O_2^-), a phenomenon known as oxidative burst (Miller et al. 2008), and that of reactive nitrogen species (RNS), like nitric oxide (NO), are other early responses to

pathogen recognition, occurring during both PTI and ETI. A second prolonged peak of ROS and RNS production is only observed during ETI (Lamb and Dixon 1997; Delledonne et al. 1998; Tsuda and Katagiri 2010).

Plasma membrane NADPH oxidases (also known as respiratory oxidative burst oxidases) and cell wall peroxidases have been indicated as primary sources of ROS, which can also be accumulated by the downregulation of scavenging/antioxidant systems such as ascorbate and glutathione peroxidase and catalase (Geisler 2010; Torres 2010; Daudi et al. 2012). As mentioned earlier, the biosynthetic route of NO is Ca^{2+}/CaM dependent and involves nitric oxide associated (NOA) enzyme(s), which are thought to convert L-arginine in citrulline and NO (Courtois et al. 2008; Hong et al. 2008).

Because of their reactive nature, ROS and RNS could be directly toxic to the invading organism. In addition, ROS may contribute to establishing a physical barrier to the invading organism by cross-linking of cell wall glycoproteins. However, an emerging body of evidence points to the importance of ROS and RNS as signaling molecules in plant defense pathways. Transcriptomic studies have revealed sets of stress-responsive genes, which are modulated by either NO or H_2O_2, and by the combined action of the two molecules (Zago et al. 2006). Both ROS and RNS might act on gene expression through the modification of redox potential (Hong et al. 2008; Lindermayr et al. 2010; Torres 2010). In addition, oxidative-stress activated MAPK cascades have been identified (Kovtun et al. 2000). A balanced level of ROS and RNS is thought to regulate, together with SA, cell death associated with the HR (Hong et al. 2008). ROS and RNS have been suggested to establish cross-talks with calcium signaling, through the activation of plasma membrane Ca^{2+} channels (Lecourieux et al. 2006; Besson-Bard et al. 2008; Ranf et al. 2011), and with SA signaling through a self-amplifying feedback loop (Vlot et al. 2009). A link between NO and SA signaling has been provided by the recent finding that S-nitrosilation involves proteins involved in SA biosynthesis and accumulation (Hong et al. 2008).

Hormonal Signaling

The phytohormones salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) are major players in defense signal transduction against pathogens (Glazebrook 2005). A recent study with Arabidopsis mutants impaired in individual and multiple hormonal signaling pathways has shown that SA, JA, and ET play a major role in both PTI and ETI (Tsuda et al. 2009). The same authors also showed that, while synergistic interactions between individual hormonal signaling sectors occur during PTI, compensatory interactions occur during ETI. This might explain why pathogen effectors are typically able to suppress PTI by dampening part of the signaling machinery, but seem to be unable to suppress ETI. Additional hormones, namely abscisic acid, auxin, gibberellic acid, cytokinin, brassinosteroids, and peptide hormones have recently been implicated in a complex signaling network, in which extensive synergic and antagonistic cross-talks take place (Pieterse et al. 2009).

Variation in the amount, composition, and timing of the phytohormonal blend produced by the plant might account for the kind of defense response activated, which may vary considerably in relation to the attacking pathogen and the kind of immunity (PTI to ETI) (Pieterse et al. 2009). Several genetic studies have shown that SA signaling prevails in cases of defense against biotrophic pathogens, whereas JA/ET signaling pathways play a major role in defense against necrotrophic pathogens and herbivores (Glazebrook 2005).

Many genes and proteins involved in phytohormone biosynthesis and individual defense signaling networks have been characterized. SA production causes a change in cell redox potential, which ultimately causes the reduction of oligomers of the ankirin repeat protein NPR1 in active monomers. These are translocated into the nucleus where they interact with TGA transcription factors and regulate SA-responsive defense genes, among which is the pathogenesis-related protein PR1 (Glazebrook 2005). It is likely the existence of a NPR1-independent branch of the SA signaling pathway leads to the accumulation of PR proteins (Desveaux et al. 2004).

JA-responsive genes are normally repressed from the interaction between the ubiquitin ligase SCFCOI1 complex and jasmonate ZIM-domain (JAZ) proteins. Upon accumulation of JA, JA-isoleucine (JA-Ile) binds to the F-box protein COI1 in the SCFCOI1 complex, causing the ubiquination and degradation of JAZ proteins and the activation of the JA-responsive transcription factors MYC2, ERF1, and ORA59 (Pieterse et al. 2009).

ET signaling occurs through plasma membrane receptors like ETR1 which, upon ET binding, cause the Raf-like protein kinase CTR to stop its repressing activity on the ET-signaling positive regulator EIN2. Through a still unknown mechanism, EIN2 activates the transcription factors EIN3 and EIL, which in turn activate the ERF1 transcription factor and cause the expression of ET-responsive genes (Kendrick and Chang 2008).

Recently, many genes and proteins determining cross-talk nodes between hormonal pathways referred to biotic stresses have been characterized (reviewed by Pieterse et al. 2009). For example, the Arabidopsis transcription factor WRKY70, which is activated by NPR1, downregulates the JA signaling pathway, thus contributing to the well-documented negative cross-talk between SA and JA signaling pathways (Li et al. 2004). In addition, many genes having a positive or negative role on individual signaling pathways have been reported. Many S-genes reported in Table 3.3 are indeed, as previously mentioned, negative regulators of main hormonal pathways.

3.4.7.3 Traits Related to Abiotic Stress Response in Plants

Abiotic stresses are the major sources of crop loss around the world (Wang et al. 2003). These stresses lead to a series of morphological, physiological, biochemical, and molecular changes that adversely affect plant growth and productivity (Wang et al. 2001). A strong interconnection between stresses such as drought, salinity,

extreme temperatures, and oxidative stress is often observed, which may induce similar cellular damage (Wang et al. 2003). For example, drought and/or salinization are considered primarily as osmotic stress and all result in the disruption of homeostasis and ion distribution in the cell (Serrano et al. 1999; Zhu 2001a). Oxidative stress, which is frequently observed with abiotic stresses such as high temperature, salinity, or drought stress, may cause denaturation of functional and structural proteins (Smirnoff 1998).

Traits for Drought Tolerance

Drought tolerance of a plant can be defined as its ability to complete vital processes and reproduce under limited water availability. Understanding the degree of drought tolerance of different genotypes is the first step of any breeding program. In case of drought, many studies have based the assessment of degree of tolerance of different genotypes on a few drought-response-related physiological traits (Cattivelli et al. 2008). Blum (2002) has classified plant traits controlling water status and productivity under drought into (1) phenology, (2) root traits, (3) plant and organ size, (4) leaf surface properties, (5) nonsenescence, and (6) stem reserve.

Phenology affects plant performance under drought in two ways (a) short growth duration enables escape from severe end-of-season (terminal) stress; and (b) plants of short growth duration generally tend to use less water because of their shorter growing period and smaller leaf area (Blum 2002).The expression of genes that control flowering is relatively independent of plant water status (Blum 2002). However, drought may advance or delay flowering depending on crops and varieties.

Senescence in plants is caused due to breakdown of chlorophyll and leads to reduction in cellular capacity and vital functions (Blum 2002). Several QTLs and genes have been identified in a variety of crops for delayed senescence under drought (Crasta et al. 1999). While these genes do not require drought for their expression, their effect is more pronounced under drought stress conditions. Nonsenescence or "stay-green" can be defined as the delayed or reduced rate of normal plant senescence as it approaches maturity (Blum 2002). The stay-green character is considered important for tolerance to drought in a wide range of crops, however it is most extensively researched in sorghum. It is regarded as a postflowering drought resistance trait, which leads to resistance of plants to premature senescence under drought during the grain-filling stage (Crasta et al. 1999).

Although, plants with shorter growth period generally tend to show more tolerance to drought, they are also smaller in size with lower leaf area index (LAI), which leads to their low yield potential. The trade-off between water economy and reduced yield potential is an important consideration in designing a drought-resistant crop ideotype (Blum 2002).

Root traits are the key components of plant adaptation to drought environments (Blum 2002). Several studies have been conducted on the effect of different root traits on drought tolerance of crop plants. For the crops growing in aerobic environments such as maize, sorghum, etc, it has been seen that deep roots play an important part in maintaining plant water status when the soil moisture decreases in the top soil layers. Apart from this several root traits such as root volume, root mass, soil penetration, root thickness, etc. have been researched in the past. Recent studies in rice have also shown that the percentage of lateral roots play an important role in increasing water uptake. It has also been seen that increased lateral roots in rice can be a drought-induced phenomenon. Conversely, traits such as maximum rooting depth are expressed irrespective of stress conditions (Blum 2002).

Leaf surface properties including the form, shape, composition of cuticular and epicuticular wax (EW), leaf pubescence, and leaf color affect the radiation load on the leaf canopy and subsequently leaf temperature and transpiration (Blum 2002). Although most of these traits are affected by water stress, their expression is still more dependent on the genotype of the plant.

Stem reserve is a major resource providing carbohydrates and nitrogen for grain filling when the transient photosynthetic source is inhibited by stress (Blum 2002). The accumulation of reserves before stress and its effective transport and utilization for grain filling are two important processes affecting response to drought tolerance. The accumulation of reserves before grain filling is not in response to stress, although the signal for reserve conversion into soluble fractions that can be transported from stems to grain can be stress responsive (Blum 2002).

In the case of crop plants, it is important that the tolerance to drought in terms of physiological processes is translated in form of yielding ability of the plant under limited water availability. The complexity of grain yield under drought and the involvement of a large number of physiological processes regulating yield response under drought make it difficult to screen large amounts of genotypes for many physiological traits simultaneously. Apart from this, it is difficult to identify one/few physiological parameters, which can serve as a reliable indicator of drought tolerance in terms of high yield (Cattivelli et al. 2008). In such cases, it has been suggested that yield performance over a range of environments should be used as the main indicator of tolerance to drought (Voltas et al. 2005). Rice is perhaps the best example of utilization of grain yield as a selection criterion for assessing the relative tolerance of genotypes. Several studies conducted on breeding populations in rice have successfully utilized yield as a selection criterion for identification of superior breeding lines (Venuprasad et al. 2007, 2008; Kumar et al. 2009a, b). Moreover, it has also been seen in the case of rice that selection for yield under varying degrees of stress and nonstress conditions can be successfully utilized for identification of genotypes suited to a wide range of environmental conditions (Mandal et al. 2010; Verulkar et al. 2010). The molecular studies have also led to the identification of QTLs with large effects on grain yield under drought (Bernier et al. 2007; Venuprasad et al. 2009; Vikram et al. 2011; Ghimire et al. 2012).

Traits for Salinity Tolerance

Soil salinity is an important abiotic stress that affects crop yields around the world. Several attempts have been made in the past to develop salt-tolerant varieties through conventional and molecular breeding approaches. In these studies, several traits were taken into account for the identification of tolerant genotypes from a breeding or mapping population. These traits can broadly be grouped into three major classes (1) growth and yield, (2) damage or tolerance to very high salinity levels, and (3) physiological mechanisms (Munns and James 2003).

Screening large numbers of genotypes for salinity tolerance in the field is difficult due to variability in soil properties and seasonal fluctuations in rainfall (Munns and James 2003). In such cases, screening of populations under controlled conditions for effect of various levels of salinity at various growth stages and its effect on yield and yield attributing traits can be a criterion of selection for superior genotypes. In general, screening for traits such as germination percentage, coleoptiles and radicle lengths, survival percentage, robustness of root system, leaf elongation and biomass under saline conditions provide an effective screening criterion at seedling stage, which is, together with the reproductive stage, the life-cycle phase when plants are more vulnerable to salinity. An assessment of final yield along with these measurements can provide an effective idea about tolerance and yielding ability of a genotype under saline conditions.

Apart from measurements of growth, traits, and yielding ability of crop plants under saline environments, screening of genotypes for injury caused due to salinity at different crop growth stages also provides an effective estimation of the level of tolerance of a genotype. Traits such as injury to the leaf, leakage from leaf discs, desiccation, and deformation of plant parts can be visually scored for an assessment of extent of effect of salinity on different genotypes. Apart from this, traits such as chlorophyll content and chlorophyll fluorescence can also be estimated (Munns and James 2003). At the reproductive stage, genotypes can also be screened for the effect on reproductive systems. Therefore, traits such as spikelet and pollen sterility can be taken into account. Standard evaluation systems (SES) have been developed in crops like rice (Gregorio et al. 1997), which provide an effective selection criterion based on overall survival and vigor of the plant. However, a major limitation to scoring for injuries and survival in crop plants under field conditions arises due to injury caused to plants by factors other than salinity (Greenway and Munns 1980). Detailed assessment of traits such as nutrient uptake, Na^+ uptake, K^+ uptake, Na^+/K^+ uptake ratio, and Cl^- exclusion provide an effective estimation of the ability of plants to maintain low ion concentration under saline conditions. Screening for traits related to ion exclusion along with traits related to survival and injury can aid in the estimation of tolerance levels of genotypes.

Traits for Submergence Tolerance

Submergence (flooding) in field conditions can mainly be of two types (1) short-term flash flooding, and (2) stagnant or deep water flooding. Crop plants respond in different ways to these two kinds of flooding with several morphophysiological mechanisms. Carbohydrate reserves before and during the submergence period is considered one of the most important factors affecting tolerance of a plant. Yamada (1959) reported rapid loss of starch and total carbohydrates in rice leaves, leaf sheath and roots due to submergence and higher starch content prior to submergence favored tolerance. There is, however, hardly any evidence available that tolerant plants may have higher underwater photosynthesis levels (Mazaredo and Vergara 1982). It can be concluded that higher concentrations of starch and total carbohydrates prior to submergence and their slow rate of depletion are adaptive traits for tolerance to submergence (Mallik et al. 1995; Chaturvedi et al. 1996).

It is also seen that limited stem elongation is associated with higher degrees of tolerance to flash flooding. The theory behind this is that metabolic costs associated with rapid elongation of stem under submerged conditions shorten the period of survival (Jackson et al. 1987; Greenway and Settler 1996; Sarker et al. 1996; Setter et al. 1997). Conversely, in the case of deep water, rice is able to survive continuous flooding because of its rapid stem elongation with rising water levels (Catling 1992). Thus, on the one hand limited stem elongation allows the plant to conserve its energy under short-term flood conditions and allows it to re grow after the water level goes down. However, the plants may die if the flooding is prolonged (Hattori et al. 2011). On the other hand, rapid stem elongation provides plants access to the atmosphere under prolonged flooded conditions (Hattori et al. 2011). Interestingly, it has been reported that the underlying mechanisms for these two opposing behaviors are controlled by ethylene response transcription factors (ERF) (Hattori et al. 2011).

Submergence rapidly depletes the protein reserves through hydrolysis to amino acids and other soluble nitrogen-containing compounds (Yamada 1959). Nitrogen and phosphorus availability and assimilation can therefore influence submergence tolerance among cultivars (Jackson and Ram 2003). Several anatomical features in plants also affect tolerance to submergence. One major feature is the presence of aerenchyma. Aerenchyma comprises gas-filled spaces within plant tissues and is considered an essential anatomical adaptive trait for survival under flooded conditions. Crops such as rice, which are adapted to growth under puddled/flooded conditions, possess aerenchyma in plant parts such as roots and shoots, which remain under water. Several reports have shown the role of aerenchyma in submergence tolerance. These mainly help in providing a diffusion path of low resistance for the transport of oxygen from shoot to roots in waterlogged soils. Aerenchyma also help to diffuse volatile compounds produced in anaerobic soils and plant tissue.

It is widely believed that plants suffer damage by oxidative stress initiated by re-entry of atmospheric oxygen in the postsubmergence period (Wollenweber-Ratze and Crawford 1994). The mechanisms behind this injury are related to postanoxic formation of reactive oxygen species and possible loss of protection

against these molecules. It is believed that the swing to highly reducing conditions favors the transfer of electrons to O_2 and leads to the development of superoxide radicals (O_2^-) (Hendry and Crawford 1994). The free radicals so produced promote an auto-oxidative chain reaction, which in turn damages the lipid membrane by peroxidation (Monk et al. 1989). The basic assumption is that the extent to which the free radicals can be detoxified using enzymatic and chemical means, determines the level of injury (Jackson and Ram 2003) and thus the level of tolerance.

Several scavenging enzymes present in the plant cell such as superoxide dismutase complex (converts H_2O_2 into H_2O and O_2 in microbodies), ascorbate peroxidase (converts H_2O_2 to H_2O to monodehydroascorbate, which can dismutate back to ascorbate and dehydroascorbate), dehydro ascorbate reductase (regenerates ascorbate from dehydro ascorbate by reacting it with reduced glutathione) and monohydroascorbate reductase and glutathione reductase enzymes (recycle ascorbate or use NADPH to chemically reduce oxidized glutathione back to reduced glutathione) play a role in scavenging the antioxidants present in the plant cells (Asada and Takahashi 1987; Jackson and Ram 2003).

Traits for Temperature Stress Tolerance

An average rise of 2–4 °C in the temperature of the Earth is expected by the end of the twenty-first century (IPCC 2007a). This change will expose most of the world's crops to heat stress during various crop growth stages (Shah et al. 2011). Most of the areas for crops such as rice are already close to optimum for crop production and further increase in temperatures or short phases of high temperature stress at sensitive crop growth stages may reduce yield (Shah et al. 2011). While high day and night temperatures affect yield for crops like rice in some parts of the world, there are some regions where low temperature affects growth and yield for crops. Crop plants are most sensitive to temperature stress during the reproductive phase of their life cycle. Indeed, temperature extremes have several major effects on the reproductive system of plants which lead to poor seed set. Some of the effects include (1) early or delayed flowering, (2) asynchrony of male and female reproductive development, (3) defects in parental tissue, and (4) defects to male and female gametes (Zinn et al. 2010). Some of the traits that affect tolerance to temperature stress are discussed below.

Plant architecture can play an important role in high temperature tolerance in crops such as rice (Shah et al. 2011). Morphology of plants that supports the covering of panicles with leaves can save the male and female reproductive organs from the effect of high temperature through increased transpirational cooling and by preventing evaporation from the anther due to its shading by the leaves leading to improved pollen viability and dehiscence (Shah et al. 2011). Varieties with improved canopy architecture and short height not only prove more useful in tolerating temperature stress but are also useful for resistance against lodging. In wheat, the prostate plant type is considered to be more favorable to increased cold tolerance. Plants with this type of growth pattern are less exposed to low temperature and are better protected against snow (Sãulescu and Braun 2001).

Another important factor that affects resistance to high heat stress is the time of flowering during the day. Apart from plant phenology, that is time of flowering during the life cycle, plants may tend to flower early in the morning to escape the effect of rising temperatures during the day. This trait can easily be used for selecting genotypes that avoid heat stress. However, if the tolerance to heat stress is to be determined, it is essential to avoid the "escape effect" of earlier-hour flowering and make sure that the high temperature treatment coincides with the daytime period in which the peak of flower opening occurs (Jagadish et al. 2007). In addition to time of flowering, avoidance mechanisms in rice also include (1) cooling of the spikelet at flowering, (2) asynchronous tiller and panicle development, (3) asynchronous flowering time of spikelets within each panicle, and (4) anthesis and pollination taking place within the same spikelet (Shah et al. 2011).

In addition to these traits, characters such as length of anthers and size of basal pore also affect tolerance to heat stress at flowering (Shah et al. 2011). It is believed that since heat stress leads to pollen sterility, genotypes with larger anthers, which are likely to contain more pollen grain, tend to be more tolerant (Matsui and Omasa 2002). Similarly, anthers with a larger basal pore can release pollen more rapidly compared to those with smaller basal pores in which most of the pollen grains remain in the anthers during the time of anthesis (Matsui and Kagata 2003). Experiments have also shown that a well-developed lacuna may weaken the septum and promote dehiscence, thus increasing pollination at high temperatures (Matsui and Omasa 2002).

Similar to high temperature stress, tolerance to cold also results due to complex physiological mechanisms involving many cell and plant traits. For example, it has been reported that as many as 15 out of 21 chromosomes in wheat influence tolerance to cold (Stushnoff et al. 1984). In the case of winter wheat, a high level of hardening can only be achieved in "dormant," not rapidly developing plant types; therefore, a strong association exists between the degree of vernalization and the degree of freezing tolerance (Roberts 1990a). Conversely, several wheat cultivars with very long vernalization requirements have only moderate freezing tolerance (Gusta et al. 1997). Apart from this, wheat cultivars with low vernalization requirements and high photoperiod sensitivity are reported to be only moderately winter hardy. However, in barley some of the most cold-tolerant cultivars are known to be day length sensitive with low vernalization requirements (Sãulescu and Braun 2001). Length of stomatal guard cells along with leaf length and height of hardened plants is also reported to be associated with cold hardiness (Roberts 1990b; Limin and Fowler 1994). Although, the association of these traits is not very strong, they can be easily determined on individual plants (Sãulescu and Braun 2001).

3.4.7.4 Gene Classes Related to Abiotic Stress Responses in Crop Plants

The response to abiotic stresses involves many genes and biochemical–molecular mechanisms (Wang et al. 2003). These authors have classified genes affecting crop response to abiotic stresses into three major classes (1) genes involved in signaling

cascades and in transcriptional control, such as MYC, MAP kinases, and SOS kinase (Shinozaki and Yamaguchi-Shinozaki 1997; Munnik et al. 1999; Zhu 2001b), phospholipases (Chapman 1998; Frank et al. 2000), and transcriptional factors such as the transactive heat-shock factors (HSF) and the CBF/DREB and ABF/ABAE families (Stockinger et al. 1997; Schöffl et al. 1998; Choi et al. 2000; Shinozaki and Yamaguchi-Shinozaki 2000); (2) genes that function directly in the protection of membranes and proteins, such as heat-shock proteins (Hsps) and chaperones, late embryogenesis abundant (LEA) proteins (Vierling 1991; Ingram and Bartels 1996; Thomashow 1998, 1999; Bray et al. 2000), osmoprotectants, and free-radical scavengers (Bohnert and Sheveleva 1998); (3) genes that are involved in water and ion uptake and transport such as aquaporins and ion transporters (Maurel 1997; Serrano et al. 1999; Tyerman et al. 1999; Zimmermann and Sentenac 1999; Blumwald 2000).

To maintain growth and productivity, plants must adapt to stress conditions through specific tolerance mechanisms (Wang et al. 2003). Stress tolerance mechanisms in plants are activated through a series of signaling processes, which are initiated from stress signals such as osmotic and ionic effects, or temperature, membrane fluidity changes leading to the activation of downstream signaling process and transcription controls, which activate stress-responsive mechanisms to re-establish homeostasis and protect and repair damaged proteins and membranes (Wang et al. 2003). Inadequate response at one or several steps in the signaling and gene activation may ultimately result in irreversible changes of cellular homeostasis and in the destruction of functional and structural proteins and membranes, leading to cell death mechanisms (Wang et al. 2003). Table 3.6 presents a summary of mechanisms and genes related to different crop species known to be involved in plant response to abiotic stresses compiled by Wang et al. (2003). The functions of some of these gene classes are discussed in the forthcoming sections.

Genes Regulating Compatible Solutes or Osmolytes

Osmolytes accumulate in organisms in response to osmotic stress. Many of them are often also referred to as compatible solutes, since they do not have toxic effects on cellular compartments. The primary function of compatible solutes is to maintain cell turgor and thus act as the driving gradient for water uptake. Recent studies indicate that compatible solutes can also act as free-radical scavengers or chemical chaperones by directly stabilizing membranes and/or proteins (Lee et al. 1997; Hare et al. 1998; Bohnert and Shen 1999; McNeil et al. 1999; Diamant et al. 2001). Compatible solutes fall into three major groups: amino acids (e.g., proline), quaternary amines (e.g., glycine betaine, dimethylsulfoniopropionate) and polyol/sugars (e.g., mannitol, trehalose) and overexpression of compatible solutes in transgenic plants can result in improved stress tolerance (Wang et al. 2003). Kishor et al. (1995) have reported increased tolerance to salt stress in transgenic tobacco (*Nicotiana tabacum*) through the overexpression of the *p5cs* gene that encodes

Table 3.6 Mechanisms, genes, and genetically modified plant species implicated in plant response to abiotic stress (Source: Wang et al. 2003)

Mechanism	Genes	Species	Reference
Transcription control	CBF1	*Arabidopsis thaliana*	Jaglo-Ottosen et al. (1998)
	DREB1A	*A. thaliana*	Kasuga et al. (1999)
	CBF3	*A. thaliana*	Gilmour et al. (2000)
	CBFs	*Brassica napus*	Jaglo et al. (2001)
	CBF1	*Lycopersicon esculentum*	Hsieh et al. (2002)
	CBF4	*A. thaliana*	Haake et al. (2002)
	AtMYC2 and AtMYB2	*A. thaliana*	Abe et al. (2003)
	ABF3 or ABF4	*A. thaliana*	Kang et al. (2002)
	HSF1 and HSF3	*A. thaliana*	Lee et al. (1995), Prändl et al. (1998)
	HsfA1	*L. esculentum*	Mishra et al. (2002)
	spl7	*Oryza sativa*	Yamanouchi et al. (2002)
Compatible solute			
Proline	P5CS	*Nicotiana tabacum*	Kishor et al. (1995), Konstantinova et al. (2002), Hong et al. (2000)
	ProDH	*A. thaliana*	Nanjo et al. (1999)
Myo-inositol	IMT1	*N. tabacum*	Sheveleva et al. (1997)
Sorbitol	*stpd1*	*N. tabacum*	Sheveleva et al. (1998)
Antioxidants and detoxification	CuZn-SOD	*N. tabacum*	Gupta et al. (1993a, b), Pitcher and Zilinskas (1996)
	Mn-SOD or Fe-SOD	*Medicago sativa, N. tabacum*	McKersie et al. (1996, 1999, 2000), Van Camp et al. (1996)
	GST and GPX	*N. tabacum*	Roxas et al. (1997)
	chyB	*A. thaliana*	Davison et al. (2002)
	Aldose-aldehyde reductase	*N. tabacum*	Oberschall et al. (2000)
Ion transport	AtNHX1	*A. thaliana*	Apse et al. (1999)
		B. napus	Zhang et al. (2001)
		L. esculentum	Zhang and Blumwald (2001)
	SOS1	*A. thaliana*	Shi et al. (2003)
	HAL1	*Cucurbita melo*	Bordas et al. (1997), Rus et al. (2001)
	AVP1	*A. thaliana*	Gaxiola et al. (2001)
Hsps and molecular chaperones	Hsp17.7	*Daucus carota*	Malik et al. (1999)
	Hsp21	*A. thaliana*	Härndahl et al. (1999)
	AtHSP17.6A	*A. thaliana*	Sun et al. (2001)
	DnaK1	*N. tabacum*	Sugino et al. (1999)
	SP1	*Populus tremula*	Wang et al. (2003)
LEA-type proteins	COR15a	*A. thaliana*	Artus et al. (1996), Steponkus et al. (1998), Jaglo-Ottosen et al. (1998)
	HVA1	*Onn sativa*	Xu and Mackill (1996)
		Triticum aestivum	Sivamani et al. (2000)
	WCS19	*ss*	Ndong et al. (2002)

D1-pyrroline-5-carboxylate synthase (P5CS) leading to the production of 10- to 18-fold more proline. Similarly, increased freezing tolerance was achieved by transforming tobacco with the same gene (*p5cs*; Konstantinova et al. 2002). Alternatively, high proline levels can be sustained during stress through downregulation of proline catabolism. Proline dehydrogenase (ProDH) catalyzes the first step of proline degradation (Wang et al. 2003), therefore, as shown in *Arabidopsis*, accumulation of proline can be achieved through repression of proline dehydrogenase RNA in antisense transgenic plants (Nanjo et al. 1999). Such plants show more tolerance to freezing (−7 °C) and to NaCl (600 mM) than wild-type and vector-transformed plants (Wang et al. 2003).

Antioxidants and Detoxification Genes

Almost all major abiotic stresses lead to the formation of ROS in plant cells, which can be damaging to plant cell structure and metabolism. Several defense mechanisms in the plant system work to scavenge these toxic compounds and an enhancement in these mechanisms may lead to improved stress tolerance (Wang et al. 2003). Several enzymes such as catalase, superoxide dismutase (SOD), ascorbate peroxidase (APX), and glutathione reductase, as well as nonenzyme molecules such as ascorbate, glutathione, carotenoids, and anthocyanins and additional compounds, such as osmolytes, proteins (e.g., peroxiredoxin), and amphiphilic molecules (e.g., tocopherol) function as ROS scavengers (Bowler et al. 1992; Noctor and Foyer 1998; Wang et al. 2003). The effect of Cu/Zn-SOD has been reported on resistance to oxidative stress caused by high light, low temperature and ozone-induced foliar necrosis in tobacco (Gupta et al. 1993a, b; Pitcher and Zilinskas 1996). Similarly, an effect of Mn-SOD and Fe-SOD on increased drought and cold tolerance has been reported in alfalfa (McKersie et al. 1996, 1999, 2000). The effect of other enzymes such as glutathione S-transferase (GST) and glutathione peroxidase (GPX) on chilling or salt stress in tobacco (Roxas et al. 1997), β-carotene hydroxylase (controlled by the *chyB* gene) on high light and temperature stress in *Arabidopsis* (Davison et al. 2002), and aldosealdehyde reductase on tolerance to heavy metals, salt and dehydration stress in alfalfa (Oberschall et al. 2000) is also reported.

Genes Affecting Ion Transport

Plant growth is significantly affected by osmotic stress, ion toxicity, and high salt content in the soil and the irrigation water (Wang et al. 2003). Ion transporters play an important role in maintaining the ion levels at physiologically relevant concentrations by selectively transporting ions thus permitting plant survival and growth under saline conditions (Wang et al. 2003). Apart from this, the Na+/H+ antiporters catalyze the exchange of Na+ for H+ across membranes and regulate cytoplasmic pH, sodium levels, and cell turgor (Serrano et al. 1999; Wang et al.

2003). Overexpression of the vacuolar Na+/H+ antiporter *AtNHX1* is reported to promote salinity tolerance in *Arabidopsis* (Apse et al. 1999). Similarly, *Saccharomyces cerevisiae* cation transport systems, such as *HAL1* and *HAL3*, are involved in the regulation of K+ and Na+ transport, respectively (Wang et al. 2003). Bordas et al. (1997) have reported that transgenic melon plants expressing the *HAL1* gene show some level of salt tolerance under in vitro conditions.

Heat-Shock Proteins and Chaperones

Heat-shock proteins (Hsps) and molecular chaperones, as well as late embryogenesis abundant (LEA) protein families, are involved in plant abiotic stress tolerance (Wang et al. 2003, 2004). Abiotic stresses such as high temperature, salinity, and drought stress can cause denaturation and dysfunction of many proteins (Vinocur and Altman 2005) leading to loss of enzyme function and membrane stability. While Hsps act as molecular chaperones associated with protein synthesis, targeting, maturation, and degradation during normal cellular processes (Wang et al. 2003), it is also believed that Hsps along with LEA proteins help to protect against stress by controlling the proper folding and conformation of both structural (i.e., cell membrane) and functional (i.e., enzymes) proteins (Vinocur and Altman 2005). sHsps are the most abundant among the five conserved families of Hsps namely Hsp100, Hsp90, Hsp70, Hsp60, and sHsp (Wang et al. 2003).

The expression of plant sHsps under water, salt, and oxidative stress has been reported in several studies (Almoguera et al. 1993; Alamillo et al. 1995; Sabehat et al. 1998; Härndahl et al. 1999; Hamilton and Heckathorn 2001). Similarly, overexpression of LEA proteins is being related to desiccation tolerance in several cases, although the actual function of these proteins is still unknown (Villalobos et al. 2004). LEA proteins are hydrophilic and heat stable and their expression is mostly regulated by and responsive to ABA (as reviewed by Wang et al. 2003). Overexpression of HVA1, a group three LEA proteins isolated from barley, conferring dehydration tolerance to transgenic rice plants has been reported (Chandra Babu et al. 2004). Similarly, overexpression of *COR15a* encoding a LEA protein results in increased membrane stability leading to increased freezing tolerance of chloroplasts in *Arabidopsis* (Artus et al. 1996; Steponkus et al. 1998).

Genes Involved in the Abiotic Stress Defense Signaling Network

A number of signaling pathways for sensing and signaling abiotic stresses such as drought have been identified (Geisler 2010). Molecules like calcium, phosphoproteins such as protein kinases, phosphatases and MAPK modules, ROS, RNS, the sugar trehalose, the hormones abscisic acid (ABA) (Setter et al. 2011) and methyl jasmonate (Bartels and Sunker 2005; Kaur and Gupta 2005; Ge et al. 2008; Huang et al. 2008; Paul 2008; Sakamoto et al. 2008; Wasilewska et al.

2008), and a number of known transcription factor (TF) families, are involved in these signaling pathways (Geisler 2010).

Ca^{2+}-dependent protein kinases (CDPK) and other calcium-binding proteins such as the calcineurin B-like calcium-binding protein Salt Overlay Sensitive 3 (SOS3) are involved in calcium signaling (Kaur and Gupta 2005). This occurs in repetitive transit bursts (Krishnan et al. 2010). The first round initiates the generation of secondary signaling molecules like ABA and ROS which simulate the second round of Ca^{2+} signaling (Krishnan et al. 2010). Studies have shown that overexpression of OsCDPK7 results in cold and osmotic stress tolerance in rice (Krishnan et al. 2010).

Microarray experiments have shown the activity of many known key drought network genes in experiments involving treatments with the ROS hydrogen peroxide (Geisler 2010). For example hydrogen peroxide activates DREB2A, ZAT 12, and two drought-responding NAC-type transcription factor genes (Geisler 2010). It is also been seen that drought and salt stress lead to an increase in ROS and plants overexpressing scavenging enzymes are tolerant to salt (Geisler 2010).

Abscisic acid (ABA) signaling is pivotal for signaling of many abiotic stresses. However, ABA is involved in so many processes that it is possibly more an integration step for multiple pathways (Wasilewska et al. 2008; Geisler 2010). Two TF families, bZIP and MYB are primarily involved in ABA signaling and its gene activation (Wang et al. 2003). Several *cis*-acting ABA-responsive element (ABRE) binding proteins such as TREB, AREB/ABF, and AB15 have been isolated in rice and *Arabidopsis* (Hobo et al. 1999; Choi et al. 2000; Finkelstein and Lynch 2000; Lopez-Molina and Chua 2000; Uno et al. 2000; Kang et al. 2002; Wang et al. 2003). Similarly, an ABA-dependent pathway for drought-inducible *RD22* gene, which can be activated by TFs AtMYB2 and AtMYC2, has also been reported (Abe et al. 2003). Single nucleotide polymorphisms in genes that affect abscisic acid levels in maize floral tissues during drought have been identified by Setter et al. (2011). Heat-shock response is primarily regulated at the transcriptional level (Wang et al. 2003). Conserved *cis*-regulatory promoter elements (HSEs) that act as binding sites for HSFs play an important role in thermoinducibility (Schöffl et al. 1998). Plant HSFs are a unique family containing a number of members: 21 from *Arabidopsis*, more than 16 from tomato, and 15 from soybean (Nover et al. 2001). While heat-shock proteins (Hsps) are chaperones, which function both under normal and stress conditions, it is not surprising that HSFs provide diverse functions that differentially control the activation of heat-shock genes (Morimoto 1998; Schöffl et al. 1998; Mishra et al. 2002). It is known that overexpression of HSF in transgenic plants leads to the expression of several *hsp* genes, which lead to thermotolerance (Lee et al. 1995; Prändl et al. 1998). For example in tomato plants, overexpression of the *HsfA1* gene results in heat-stress tolerance, while *HsfA1* antisense plants are extremely sensitive to high temperatures (Mishra et al. 2002).

Plant genomes contain a large number of transcription factors (Geisler 2010). For example, it is reported that 5.9 % of the *Arabidopsis* genes (about 1,500 in total) encodes for TFs (Riechmann et al. 2000). Although belonging to a few large multigene families, these TFs may differ in their response to different stress stimuli

(Wang et al. 2003). Conversely, it is also reported that some stress-responsive genes share common TFs (Wang et al. 2003). For example, transcription factors such as dehydration-responsive transcription factors (DREB), and C-repeat binding factors (CBF) are reported to mediate the transcription of several genes (Wang et al. 2003). These include genes such as *rd29A*, *rd17*, *cor6.6*, *cor15a*, *erd10*, *kin1*, *kin2*, and others in response to cold and water stress (Ingram and Bartels 1996; Stockinger et al. 1997; Gilmour et al. 1998; Liu et al. 1998b; Seki et al. 2001; Thomashow et al. 2001; Wang et al. 2003).

3.4.7.5 Genetic and Epigenetic Regulation of Stress Responses

Variation in chromatin states provide a further supply of phenotypic variation for responding to stresses at anatomical, morphological, cellular, biochemical, and molecular level and survive under adverse conditions. These modifications include changes in DNA methylation at the fifth carbon position of a cytosine ring, posttranslational modifications in the histone N-terminal tail (acetylation, methylation, phosphorylation, ubiquitination, ribosylation, and biotinylation) for chromatin remodeling, and regulatory mechanisms activated by small RNAs (Grativol et al. 2012). These types of modifications that alter DNA activity without altering its basic nucleotide structure are referred to as epigenetic changes. Prolonged exposure to stress could convert an epigenetic modification into a permanent (epi)genetic trait of tolerance or resistance (Richards 2006).

Under stress conditions, the mobilization of DNA transposons and retroelements may be promoted, which can induce transcriptional and posttranscriptional epigenetic regulation of gene expression resulting in short-term adaptation as well as heritable phenotypes with increased ability to adapt to environmental challenges. Therefore, transient and permanent epigenetic effects may act alone or together to enhance the stress response, and can contribute to phenotypic plasticity of individual genome, population dynamic by the differential individual phenotypic and fitness response to stresses, and heritable variation across generations (Grativol et al. 2012).

3.4.7.6 Transient Gene Expression Modification

The exposure of plant parts to stresses bring forth processes of systemic acquired acclimation to light, systemic wound signaling, systemic acquired resistance, systemic virus defense, photoperiodic induction of flowering, etc. (Boyko and Kovalchuk 2011). Ample transient epigenetic changes associated with flowering in *A. thaliana* grown under abiotic stress conditions such as cold, drought, and high salinity have been described (Yaish et al. 2011).

Earlier studies on reversible response to osmotic stress (Kovařik et al. 1997) evidenced hypermethylation of heterochromatic loci. van Dijk et al. (2010) assessed that *Arabidopsis* plants exposed to dehydration stress conditions had

about 90 % of annotated genes with H3K4Me3 (histone H3 lysine 4 trimethylation) marks and that H3K4Me3 abundance was directly related to the transcriptional level of dehydration-responsive genes. The involvement of different classes of siRNA has also been reported in plants under stress (Yan et al. 2011; Khraiwesh et al. 2012). A temporary exposure to temperatures of 37–42 °C results in a transient release of suppression of transgene expression due to transcriptional gene silencing in differentiated *Arabidopsis* tissues (but not in meristematic cells) (Pecinka et al. 2010). The activation of the transcription of a particular family of copia retrotransposons, named *Onsen*, was also observed. Cold stress in rice, may alter host gene expression due to activation and copy number increase of cold-responsive and naturally active DNA transposon (e.g., *mPing*), which may influence neighboring gene expression increasing cold-hardiness (Naito et al. 2009). This suggests that (1) transient activation of silenced transgenes and retrotransposons are useful experimental indicators to study epigenetic response of plants to environmental stresses, and (2) induced epigenetic changes may be restricted to tissue not related to germline, although the bursts of retrotransposon mobility may contribute to the acquisition of new intensity of expression for genes affecting adaptive traits (Kalendar et al. 2000).

The TLC1.1 retrotransposon family in *Solanum chilense* can be activated in response to diverse stress conditions such as those related to high salt concentrations (Tapia et al. 2005), and the promoter of the TLC1.1 retrotransposon is activated by multiple stress-related signaling molecules (Salazar et al. 2007).

3.4.7.7 Permanent Gene Expression Modification: Formation of New Epialleles

Methylated cytosines are prone to spontaneous deamination inducing G/C to A/T transition-type mutations. Mutations in genes encoding epigenetic regulators cause changes in chromatin properties and may induce transcription of small RNAs (smRNAs) leading to the formation and inheritance of novel epialleles (Mirouze and Paszkowski 2011). smRNAs spread systemically from the place of stress or infection (i.e., leaf) to nonaffected tissues, including those that form the gametes, and may result in a loss or gain of DNA methylation at the specific loci such as R-gene and R-gene-like loci. The newly established methylation patterns can be transmitted to the progeny (Zhang and Zhou 2010) providing heritable changes or epimutations. Local viral infection and abiotic stresses inducing reactive oxygen species may elicit a mobilization to the noninfected tissue of a systemic recombination signal, which stimulates an increase in the frequency of homologous recombination (Kovalchuk et al. 2003) whose genetic effects can be transmitted to the next generation. The molecules and pathway that lead to systemic recombination signal migration and the links among changes in DNA methylation of specific stress-responsive loci, changes in genome rearrangements, and stress tolerance, have not yet been clearly established.

3.4.8 The Intertwining of the Gene Networks Regulating the Plant Response to Different Environmental Stresses

As a physical phenomenon, stress is defined as a mechanical force per unit area applied to an object that causes a change in the dimensions of the object known as strain. An exact definition of this physical force as a biological phenomenon especially in reference to sessile organisms such as plants is difficult, and hence a practical definition given for plant stress is an adverse force or condition that inhibits normal function and biological well being of a plant system (Jones and Jones 1989). To fully decipher stress as a biological phenomenon, it is best to visualize a plant system in terms of an individual plant cell separated from its environment by a physical barrier that is the plasma membrane. The property of selective permeability of the plasma membrane to molecules such as lipids and hormones, and its impermeability to water-soluble material such as ions, proteins, and other macromolecules can then be utilized to visualize stress as an elicitor-based phenomenon. The extracellular molecules called ligands or elicitors bind and interact with the plasma membrane proteins called receptors to trigger stress through a signal transduction pathway that involves a cascade of plant biomolecules and their accompanying genes (Mahajan and Tuteja 2005; Ni et al. 2009).

A general scheme for the stress signal transduction pathway and the expression of specific genes to environmental stresses has been worked out in detail (Fig. 3.2). Stress elicitors and the consequent signal transduction pathways are classified according to the induced abiotic (cold, heat or temperature, salinity or salt, drought or water deficit, excess water or flood, radiation, chemical/pollutant, oxidative, wind, and nutrient deprivation) and biotic (viral, bacterial and fungal pathogens, insects, herbivores, and rodents) stresses. A stress signal is first perceived by a membrane receptor activating a phospholipase (PLC) hydrolyzing phosphatidylinositol 4, 5-bisphosphate (PIP_2) that acts as a canonical precursor to generate inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG) (Wang 2005). IP_3 mediates the release of Ca^{2+} ions from intracellular sources and a parallel influx of Ca^{2+} ions from apoplast increases the cytoplasmic calcium levels (Liu et al. 2003). This change in the Ca^{2+} ion levels in combination with other secondary biochemical messengers such as inositol phosphates (InsP) and reactive oxygen species (ROS) triggers the calcium-binding protein sensors such as calcineurin B-like molecules (CBLs) and calmodulins (CaM) that in turn interact with downstream signaling molecules such as kinases and phosphatases (Liu et al. 2005a). Some of the major kinases involved in this signal transduction include CBL-interacting protein kinase (CIPK), salt overly sensitive-2 (SOS-2), calcium-dependent protein kinases (CDPKs), and mitogen-activated proteins (MAPKs). These along with other protein phosphatases trigger a phosphorylation cascade targeting the expression of major stress-responsive genes or their related transcription factors. The products of these stress-responsive genes result in the observable physiological plant adaptation phenomena that help the plants to survive in unfavorable environmental conditions (for a detailed list of genes that are triggered by

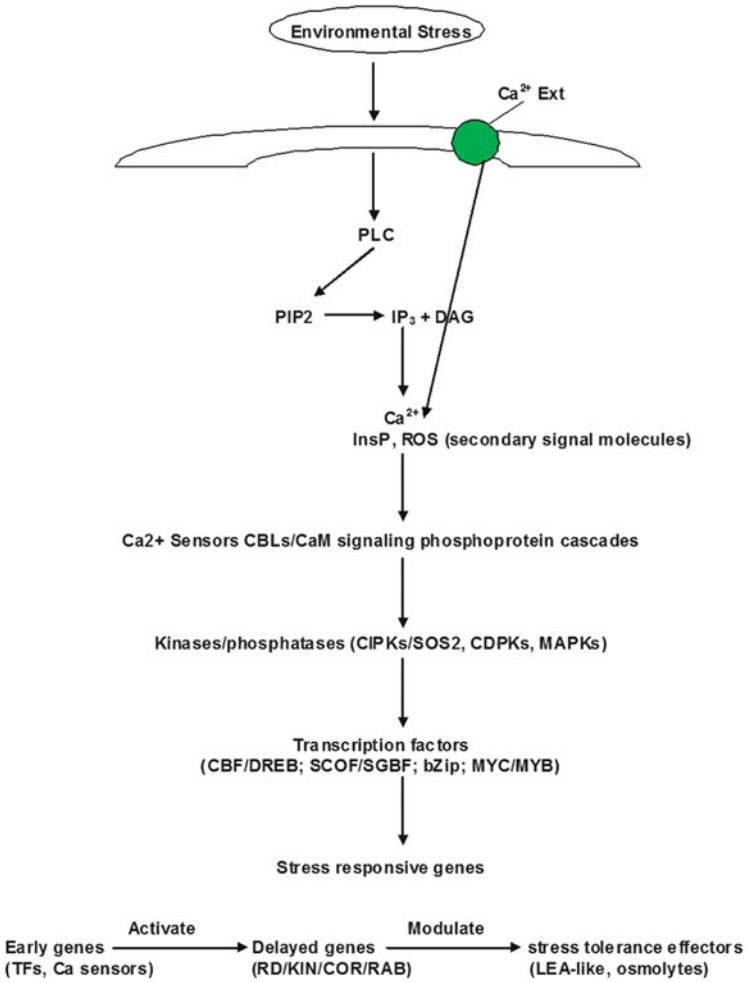

Fig. 3.2 A generic gene network for plant response to environmental stresses (adapted from Mahajan and Tuteja 2005)

stress, the reader is directed to Ni et al. 2009). The altered gene expressions may also trigger increased generation of hormones such as abscisic acid (ABA) (Wilkinson and Davies 2002; Liu et al. 2006), salicylic acid (Liu et al. 2006), jasmonic acid (Wang et al. 2000), and ethylene (Sharp 2002) that trigger a second round of signaling pathways (Liu et al. 2006). Certain other accessory molecules such as protein modifiers participate in translational modifications of enzymes involved in the molecular processes such as myristoylation (Traverso et al. 2008), glycosylation (Schaewen et al. 2008), methylation (Lukens and Zhan 2007), and ubiquitination (Dreher and Callis 2006).

Changes in gene expression during plant stress start within minutes of the plant stress signal perception. The early responsive genes are those that do not require synthesis of new proteins and whose signaling components are already primed. Transcription factors (TFs) fall into this category, and a diverse array of TFs regulate the upstream activity of various stress-responsive promoters through their *cis*-regulatory elements such as the C-repeat dehydration-responsive element (CRT/DRE) that has a conserved core sequence of CCGAC, that imparts responsiveness to low temperature and dehydration (Thomashow 2001), ABA-responsive element (ABRE) that induces abscisic acid expression (Thomashow 1999), and the myelocytomatosis (MYC) and myeloblastosis (MYB) oncogene regulon-recognition sequences (MYCRS/MYBRS) that induce abscisic acid during drought (Abe et al. 2003). Other important TFs that were well characterized include the soybean zinc-finger protein (SCOF-1) that is involved in cold tolerance and ABA-response (Kim et al. 2001), dehydration-responsive (DRE) elements DREB2A and DREB2B that become activated for cold tolerance and help maintain osmotic equilibrium (Liu et al. 1998b), and the basic leucine zipper (bZip) transcription factors that bind to ABRE elements (Uno et al. 2000). Conversely, other genes that are expressed due to a sustained late response include the responsive to dehydration (RD), cold responsive (COR), and kinetin-induced (KIN) genes that are involved in protein synthesis modulation of crucial plant molecular elements such as late embryogenesis abundant (LEA)-like proteins, antioxidants, membrane stabilizing proteins, and osmolyte synthesizers (Vranova et al. 2002).

Striking similarities are shared by the signaling pathways underlying the response to biotic and abiotic stresses (Figs. 3.1 and 3.2). In both cases, a pivotal role is played by intracellular calcium influxes, which act as second messengers once the stress stimulus is perceived. Next, proteins transducing calcium signaling, such as calmodulin, calcium-dependent protein kinases (CDPKs), and other calcium-binding proteins activate cascades of reactions, which primarily involve phosphorylation events (by means of CDPKs of other kinases, mainly MAPKs), the activation of hormonal pathways, and transcriptional reprogramming.

3.4.9 Reverse Genetics: From the Genotype to the Phenotype

There is overwhelming scientific evidence and a broad consensus among the scientific and agricultural communities that unpredictable climate changes will impose severe limitations on crop production in many parts of the world (Wang et al. 2003), which will be coped with by the search for new mutants for adaptive genes or by overexpressing known adaptive genes. This calls for a sustained effort to design strategies for selecting plants containing novel alleles expressing tolerant phenotypes to diverse environmental stresses. In the reverse genetics approach, SNPs or other types of sequence differences (e.g., indels) are sought in candidate genes to identify their phenotypic effects for tolerance to stresses. Known SNPs that correspond to significant phenotypic differences can be used for MAS or screening

germplasm and elite breeding lines. Fine mapping combined with sequence analysis can narrow the chromosomal region containing a SNP associated with a QTL affecting plant tolerance to stresses.

SNP discovery methods have been adapted for TILLING, which is a reverse genetic method that combines random chemical mutagenesis with high-throughput screening for point mutations (McCallum et al. 2000). It is based on the formation of heteroduplexes that assemble when alleles, induced by mutagenic agents on a single nucleotide, are amplified in a PCR (using either labeled or unlabeled primers), heated, and then slowly cooled. A "bubble" forms at the mismatch of the two DNA strands which is then cleaved by single-stranded nuclease such as that in celery juice extract. Capillary as well as slab gel electrophoretic analyses have been used to separate PCR products and compare isogenic genotypes differing in single sequence motifs combined with phenotyping. TILLING provides direct proof of function of both induced and natural polymorphisms without involvement of transgenic modifications and it is attractive not only for functional genomics but also for agricultural applications. However, when SNPs are detected in individuals expressing contrasting phenotypes for stress response in natural populations, the procedure is named "Ecotilling" and becomes a sort of forward genetic approach developed to discover the underlying molecular diversity for multiple types of polymorphisms (Comai et al. 2004). The discovered molecular polymorphisms can be confirmed by sequencing and include base-pair changes, small insertions and deletions, and variation in microsatellite repeat number.

Libraries or populations of mutants that cover all possible genes are increasing in importance as tools in functional genomics. Mutant libraries have been constructed in rice and other cereals and in forest trees (Neale and Kremer 2011; Harfouche et al. 2012) using chemical and physical mutagenesis, T-DNA insertion, and transposon tagging. These libraries are primarily used for functional analysis based on loss-of-function analyses. Gain-of-function approaches such as T-DNA activation tagging and gene overexpression are powerful complements to insertional mutagenesis. A library of enhancer trap lines has been developed, which will facilitate the detection and isolation of regulatory elements (Liu et al. 2005b).

Genetic engineering for stress tolerance is another reverse genetics technology of choice in terms of the speedy transfer of genes in transgenic plants and their field-testing for stress tolerance. However, genetic engineering to develop stress-tolerant plant varieties has to take into account the gene regulatory network as outlined in previous sections.

Single-gene transfers to generate transgenic varieties may confer the needed stress tolerance, but the unbalanced gene interactions may result in stunted, physiologically imbalanced plant phenotypes, thus causing the unwanted limitation of fitness of field transferred novel plants. A model example of the earliest successful transgenic stress tolerant plant was in the model plant *Arabidopsis*, where the engineering of a single gene, namely the overexpression of the cDNA encoding

dehydration-responsive element (DREB1A) resulted in improving plant drought, salt, and freezing tolerance all in a single transgenic plant (Kasuga et al. 1999). However, the use of the strong constitutive 35S cauliflower mosaic virus (CaMV) promoter to drive gene expression resulted in severe growth retardation. This limitation was overcome by using a stress-inducible *rd29A* promoter that gave rise to minimal effects on plant growth while providing an even greater tolerance to stress conditions as compared to the CaMV-derived transgenic plants (Kasuga et al. 1999). The biggest lesson learned from this study is to have a comprehensive genebank listing of well-characterized stress-responsive genes and their related molecular regulatory elements. The most significant effort in this direction was the whole-genome expression profile of *Arabidopsis* that contributed both to the understanding of the complex network of stress-related genes, and also aided in the identification and evaluation of novel transgenes that hold the key to generating commercially viable and sustainable transgenic stress tolerant varieties (Denby and Gehring 2005).

Some of the immediate applications of utilizing the whole-genome transcriptome profile of *Arabidopsis* were evidenced in the generation of transgenic rice plants (Oh et al. 2005) that expressed the *Arabidopsis CBF3*/*DREB1A* and *ABF3* genes. The *CBF3* in transgenic rice elevated the tolerance to both drought and high salinity levels. The *ABF3* and *CBF3* genes induced a total of 12 and 7 target genes, respectively in the transgenic rice, and additional 13 and 27 genes upon exposure to drought stress. Further, the transgenic plants did not show any growth anomalies. Another example of transgenic rice was the engineering of the gene fusion construct of the MADS-glucorticoid receptor, the *OsMADS26:GR* (Lee et al. 2008) where a microarray analysis showed a 1.5-fold increase of 301 genes and a 2-fold increase in 48 genes related to biosynthesis of jasmonate, ethylene, and reactive-oxidative genes along with a host of putative downstream targets related to stress responses.

Other examples of abiotic stress tolerant transgenic plants are the potato plants overexpressing the *StEREBP1* gene that induces expression of several GCC box and DRE-containing stress-response genes that enhance tolerance to both cold and salt stress (Lee et al. 2007), and transgenic *Arabidopsis* overexpressing the DNA topoisomerase 6 genes *OsTOP6A3* and *OsTOP6B*, which conferred reduced sensitivity to stress hormone ABA and tolerance to high salinity and dehydration (Jain et al. 2006).

Research on generating transgenic plants resistant to biotic stresses such as pathogen attack is another important aspect. One of the classic examples is that of the Rockefeller Foundation's bacterial blight (caused by *Xantomonas oryzae*)-resistant *Xa 21* rice (Chen et al. 2000) where classical breeding, molecular breeding, and plant genetic engineering were effectively combined to generate a hybrid rice "Minghui 63"demonstrating field level efficiency in combating bacterial blight by the International Rice Research Institute, Philippines (for a detailed description

and review of the methodology, refer to Emani et al. 2008). The use of environmentally friendly microorganisms as a source of genes engineered in plants to confer disease resistance, is also a novel approach. Genes from mycoparasitic fungi such as *Trichoderma harzianum* encoding the chitinase genes in transgenic tobacco and potato were shown to confer resistance to foliar pathogens *Alternaria alternate*, *A. solani*, *B. cinerea*, and root pathogen *R. solani* (Lorito et al. 1998). Transgenic cotton plants engineered with the *Trichoderma virens* chitinase gene also conferred resistance to foliar pathogen *A. alternata* and root pathogen *R. solani* (Emani et al. 2003). These examples show that engineering a single gene derived from an existing biocontrol agent already in use by farmers may be a novel approach to combating biotic stress in an environmentally friendly way.

Tolerance to abiotic and biotic stresses in a single plant is also a focus of research and there are examples of genes that confer tolerance to both stresses in an individual transgenic plant. Ectopic overexpression of pepper (*C. annum*) pathogenesis induced factor (*CaP1F1*) in transgenic tomato resulted in a massive change of 8,700 genes of which 110 were regulated without any visible morphological abnormalities (Seong et al. 2007). The upregulation of genes conferred tolerance to cold stress and the bacterial pathogen *Pseudomonas syringae*.

3.5 Options Available to Breeders and Foresters to Develop Plant Types Better Adapted to Current and Predicted Climate Change Conditions

3.5.1 Rationale for Preparing Breeding Intervention to Enhance Crop and Forest Resilience to Global Warming

The fact that climate changes will now significantly affect the world's agricultural and forestry output and in turn directly influence biodiversity and world food security, is on the agenda of the scientific, political, and industrial communities (White et al. 2004). Even before climate change was a major factor in influencing research on crop improvement, biodiversity was a major concern for conservation, agroforestry was practiced as an agroecologically acceptable alternative to deforestation, and world food security was the main objective of both conventional breeding as well as biotechnological crop improvement strategies, especially in the area of developing crops with increased tolerance to salinity and drought stress (Wang et al. 2003). Agroforestry is now becoming an activity to convey efforts to domesticate the large number of tree species that can provide timber and nontimber products and increase land use in the ecotonal zone of forests. That practice, if implemented, may release the pressure on the plant and animal community within the core of natural forests and amplify carbon sequestration. Plantations made with

tree genotypes adapted to tolerate global warming effects will prevent future degradation of the animal and plant community and the land where agroforestry is practiced. The diverse array of strategies that were the focus of research in developing crops resistant to biotic stresses and tolerant to abiotic stresses (Munns 2002; Xiong et al. 2002; Ashraf and Harris 2004; Flowers 2004) need now to be complemented with novel approaches that take climate change into consideration (Shao et al. 2007).

Plants are the first molecular link between the atmosphere and biosphere and are directly affected by increased CO_2 in particular (Ahmad et al. 2010). In the event of climate change, it is obvious that crop pests and diseases are as likely to become affected as crop plants themselves. This may not only lead to change in the frequency of appearance of these biotic stresses but may also displace upward the population size of new races of pathogens and pests, which may not be a threat at present. It is expected, for example, that the rise in temperature will allow certain pathogens and pests to spread into novel areas, as a consequence of overwintering, and to increase the duration and the severity of epidemics in other areas.

In general, it will be difficult to predict which particular biotic stress will become predominant in consequence of climate changes. Therefore, it will be of utmost importance to exploit broad-spectrum and durable resistance sources in future breeding for resilience to biotic stresses. In this respect, pyramiding of different resistance sources and the exploitation of defense mechanisms typical of nonhost resistance, like those associated with the loss of function of some S genes, would be highly desirable.

Many of the biotic stresses also tend to be correlated with incidence of abiotic stresses leading to further crop loss. For example in the case of rice, the incidence of diseases such as blast and brown spot is highly correlated to drought. Mitigating these stresses through the development of improved varieties is one of the biggest challenges in present-day agriculture research. It is important that target traits be clearly defined and strategies suitable to a specific stress be applied to achieve maximum tolerance to the stress. It is also important that varieties combine tolerance to stresses with high yield under nonstress conditions as well as farmer/consumer preference in terms of quality.

Concerted efforts are needed between conventional and molecular breeders (van Buerren et al. 2010) to accelerate varietal turnover using breeding materials that incorporate an increased frequency of favorable allele combinations at loci controlling resistance to pest biotypes and fungal strains causing diseases, and tolerance to abiotic stresses, especially those induced by rainfall scarcity during certain growing periods and flooding in others, which are the two most prominent effects elicited by GHG emission pressure on air temperature forcing.

Plant introductions of vicariant species and subspecies (species and subspecies of the same genus that evolved separately due to geographical barriers that divided the gene pool of their last common ancestor) from warmer environments will be an option when short-term breeding efforts on local germplasm experiencing warmer temperature effects do not produce the expected adaptations at the same pace as air temperature increase due to climate processes. In this case, careful prospection will

be needed to avoid the possibility that species that are nonnative and noninvasive become invasive as habitats and dispersers change.

Some of the strategies that can prove useful to breeders and agroforesters aiming at developing genotypes from local germplasm to face the climate change extremes are suggested in the following paragraphs.

3.5.2 Methodologies to Harness Genetic Diversity to Increase Adaptation and Mitigate the Impact of Global Warming

3.5.2.1 Classical Breeding Approaches

Existence of genetic diversity within species is the first requirement of any breeding program, including those aiming at increasing adaptation to mitigate the impact of global warming. Plant gene pools of cultivated and semidomesticated forest species and their wild relatives residing in geographic areas that are affected repeatedly by selection pressure due to biotic and abiotic forces lead to the development of valuable ecotypes and landraces tolerant and "preadapted" to one or multiple stresses. However, while these ecotypes of forest species could be used directly for agroforestry practices, the landraces of crop varieties cannot be used directly for cultivation due to poor yield potential, poor input responsiveness, and poor quality of products. It is therefore suggested that progenies derived from crosses of such tolerant landraces and high-yielding popular varieties be screened for tolerance as well as yield and quality attributes by either classical breeding approaches coupled with MAS, or genomic selection to obtain genotypes expressing highly heritable and enhanced trait performance.

Classical breeding approaches can be divided essentially into two broad categories (1) methods for within-landrace selection (mass selection and pure line breeding for selfers and recurrent selection with and without inbreeding for outcrossers), and (2) methods for selecting progenies after landrace × elite hybridization (selfers) or recurrent selection of progenies after test-cross to a tester that has either a narrow (i.e. an inbred line) or broad (another related or unrelated population) genetic base (outcrossers).

The former group of methods involves phenotypic selection of superior individuals from the landrace, while the latter group of methods is based on selection of the best-performing individuals endowed with new trait combination. The selected progenies applying the first group of methods can be directly used for cultivation in case they have plant and yield conforming to the farmer's ideotype or they can be used as improved donors for other breeding programs. For example, N22, a highly drought- and heat-tolerant rice variety, was selected from landrace Rajbhog. This variety was later used for several breeding programs to develop drought- and heat-tolerant varieties and detection of QTLs. Similarly, the tomato accession LA1230, harboring a loss-of-function mutation of the *SlMLO1* gene

conferring broad spectrum powdery mildew resistance, is being used in backcross breeding programs with elite genotypes (Ciccarese et al. 1998; Bai et al. 2008). Also flooding-tolerant local varieties have been derived from lines selected from the landrace FR13A.

The second group of methods involve creation of genetic variability in a population through crossing of adapted (i.e., tolerant to stresses) and unadapted (i.e., susceptible to stresses) parental lines or populations. In most cases improved breeding materials are developed through such a crossing scheme. Progenies developed in this way can be tested under stress and nonstress conditions to select tolerant high-yielding varieties. In case of narrow genetic variability available for trait improvement, mutagenesis (using treatments with forms of radiation such as X-rays, γ-rays, α-particles, β-particles, etc or chemicals such as ethyl methane sulfonate, methyl methane sulfonate, ethyl ethane sulfonate, diethyl sulfate, ethylene imines, 5-bromo uracil, 2-amino purine, acriflavin, proflavin, nitrous acid, hydroxylamine, sodium azide, etc.) coupled with in vitro selection of somaclonal variation (Novak and Brunner 1992) can be attempted to increase the frequencies of point mutation and their rapid identification in selective medium or under selective environmental conditions (i.e., high temperature; anoxic condition for flooding-resistance breeding). A mutation breeding approach with diethyl sulfate has recently allowed the identification of a mutation in the *PsMLO1* gene in pea, associated with broad-spectrum and durable powdery mildew resistance (Pavan et al. 2011).

3.5.2.2 Genomic Resources from Model Plants to Identify Markers to Accelerate Breeding for Enhancing Biotic and Abiotic Stress Tolerance

Plant breeding can greatly benefit, at different stages of the selection programs, from the availability of genomic databases. Usually genomic data platforms revolve around model plants. However, the identification of an efficient model crop plant to study the genetic, biochemical, and molecular basis of abiotic and biotic stresses is still not fully realized. Though it may be argued that *A. thaliana* with its complete genomic sequence in the public domain, easy transformation protocols, short generation times, ESTs, microarray and proteomics data, and a large set of well-characterized mutants as exemplified by the TAIR database (http://www.arabidopsis.org) seems to meet the requirements of molecular researchers, efforts should be focused on species such as rice, sorghum, maize, and wheat. The availability of the complete genomes of rice (Goff et al. 2002; Yu et al. 2002) and sorghum (Paterson et al. 2009), together with the ones of dicotyledonous crops such as grape and tomato (Jaillon et al. 2007; Sato et al. 2012), provide a rich resource for targeted gene discovery as illustrated by *Arabidopsis* (Denby and Gehring 2005). A comprehensive database for rice and sorghum plants aimed at data related to transformation protocols, ESTs, microarray, experimental mutants, transcriptome and proteome data in line with the TAIR database will be an effective

resource for breeders aiming to develop cultivars adapted to climate extremes. Similar databases are being constructed for tree species amenable to agroforestry (http://genome.jgi-psf.org/Poptr1_1/Poptr1_1.info.html; http://www.phytozome.net/cgi-bin/gbrowse/eucalyptus/; http://www.pinegenome.org/cgp/). Efforts are now conveyed to prepare a comprehensive inventory of the genetic resources available for the forest species (http://dendrome.ucdavis.edu/treegenes/pubdata/summary_count.php) and to facilitate comparative genomics of key tree (http://dendrome.ucdavis.edu/treegenes/) and other plant species (http://www.ncbi.nlm.nih.gov/genomes/PLANTS/PlantList.html#C_SEQ) to speed domestication and breeding through genomic selection methodologies.

3.5.2.3 Databases to Identify the Target Genes for Adaptation to Climate Change Extremes

Databases such as TAIR (http://www.arabidopsis.org), Gene Ontology (http://www.geneontology.org), Plant GO slim (http://www.geneontology.org/GO.slims.shtml), and the more recent TRY (http://www.try-db.org) with over three million trait records for 69,000 plant species with the integrated whole-genome profiling information can be rich resources for identifying a vast number of target genes related to climate change. The extracted information can then be effectively utilized to select target genes with an important function in abiotic stress tolerance and biotic stress resistance, and evaluate their biotechnological potential by genetically engineering them into popular cultivars. The lessons learned in genome-wide expression profiling in *Arabidopsis* (Denby and Gehring 2005) and the success story of the Rockefeller Foundation's blight-resistant *Xa21* rice (Chen et al. 2000) are good examples for future research aimed at developing cultivars suited to meeting the demands of climate changes.

3.5.2.4 Harnessing Molecular Techniques to Deploy the Target Genes for Adaptation to Climate Change Extremes

Genomics-assisted methods may help to reveal complex regulatory networks controlling abiotic stress-tolerance mechanisms by high-throughput expression profiling and gene-inactivation techniques. Apart from exploiting cutting-edge technologies such as microarrays for functional genomics and functional allele identification (as outlined in Sect. 3.4), a more recent development is the discovery of microRNAs involved in abiotic stress in popular cultivars of rice (Li et al. 2010) and sorghum (Zhang et al. 2001)which are useful for designing future breeding strategies. With effective techniques available in designing and silencing miRNAs for various traits across plant species (Schwab et al. 2006; Ossowski et al. 2008; Warthmann et al. 2008), and the availability of dedicated web sites that makes the technology readily accessible (http://wmd3.weigelworld.org/cgi-bin/webapp.cgi), researchers can effectively use this technology to model crop varieties tolerant to various abiotic stresses.

As pointed out by a number of experiments, overexpression or RNAi-based silencing of genes implicated in plant–pathogen and plant–pest interactions might significantly enhance the efficiency of selecting resistant genotypes. In addition, breeders now have the opportunity to exploit other nontransgenic reverse genetics approaches, such as targeted mutagenesis by TILLING, ecoTILLING, and zinc-finger nucleases.

3.5.2.5 Traits and Genes for Immediate Breeding to Face the Most Prominent and Tangible Current Effects of Global Warming

A wide range of techniques have been used to search for any link between the increase of atmosphere GHG and extreme weather and climate around the world.

Global warming is not behind any extreme climatic events. While some events such as the 2011 hottest and driest spring-to-summer growing season on record since 1895 in Texas and the unusual warmth in November 2011 in several European countries, are more likely to occur in the next years compared to 50 years ago, other events such as flooding might not be directly caused by climate warming, although storms with catastrophic consequences are increasing in number and should be attracting the attention of researchers, which can address them in breeding objectives to constitute varieties that endure long periods of adverse climatic conditions (http://www.wired.com/wiredscience/2012/07/extreme-weather-2011/). The most prominent effects of the above-mentioned extreme events consist of altered plant phenologies due to prolonged time to accumulate chilling units for bud flushing, plant suffering due to spring–summer drought, root anoxia due to flooding, or the increased incidence of pathogen and insect stressors, which are perceived to be a consequence of global warming. Therefore, genomic tools and resources should be conveyed to increase the efficiency of germplasm evaluation and screening for alleles and haplotypes affecting phenology, flooding and drought tolerance, and pest and parasite incidence, especially for long-lived perennial crop and forest species that are exposed to multiple and repeated climate-related stressors.

Phenology. Methodologies for studying phenological rhythms have been described for several forest and crop tree species (Bednářová and Merklová 2007 and Hájková et al. 2010, for *Fagus sylvatica*; Milla et al. 2010 for *Quercus ilex, Quercus coccifera, Pistacia lentiscus, Arbutus unedo*; Gormsen et al. 2005 for *Prunus* spp. and *Betula* spp.; Marta et al. 2010 for *Vitis vinifera*). Those studies indicate how to set up biological indicators aiming to plan a user-friendly predictive model based on phenology for application in natural environments. Traits such as closed and hybernant buds, ripening buds, half-opened and opened buds, sprouting phase with the development of the leaves, fully developed leaves phase and so on, will provide descriptive molecular variables with significant relation to QTLs and efficient genome selection and breeding for phenology (Menzel et al. 2006; Alessandrini et al. 2010; Fabbrini et al. 2012). Full-sib progenies from selected

fruit and forest tree clones should be produced and analyzed for novel phenological traits determined by homozygosity for recessive and unexpressed alleles in the heterozygous parental clones. A 3-week wider range of variation compared to the parents has been observed in one hazelnut full-sib progeny for date of bud flush. In the same progeny the "evergrowing" phenotype was expressed in a ratio 3 wild type : 1 evergrowing plants (Catarcione et al. 2009), which provided the basic materials to breed for new clones with a widened "shoulder" for photosynthesis in late fall and winters. The apical and lateral buds of the "evergrowing" phenotype sprout in November–December when the old leaves are still on the branches. This phenological novelty is extremely interesting for deciduous nut crops under mild winters (temperature average of the coldest month no lower than 3 °C). A similar nondormant phenotype in peach named "evergreen" allowed two blooms in one year in warm climates (Rodriguez et al. 1994). So far, few breeders believe in the explosion of useful genetic variance stemming from full-sib progenies of tree species and they just do not try it as a breeding method with the rare exception being when they desire to obtain genome-wide information and test genomic selection (Busov et al. 2011). Sib-mating is thought to cause inbreeding depression and large phenotypic variability in the progeny, making the recognition and recovery of homozygous plants (needed to reveal the recessive, loss-of-function phenotypes) difficult. But, as the "evergrowing" phenotypes show, the homozygosity may involve recessive alleles affecting master gene functions such as the lack of response to chilling, provoking anticipation of the downstream features of flowering, bud flushing, and fruiting. It is expected that full-sib progenies will produce large and interesting new genotypes for recessive alleles affecting many other climate-responsive traits, from plant growth vigor to cold tolerance as observed in hazelnut full-sib progenies and expected in several outcrossed fruit and forest tree crops that underwent clonal selection.

Drought. In response to osmotic stress such as drought, plants respond and adapt by altering thousands of genes, and as a result cellular, physiological, and biochemical processes will be modified. Molecular studies led to the identification of QTLs with large effects on grain yield under drought (Bernier et al. 2007; Venuprasad et al. 2009; Vikram et al. 2011; Ghimire et al. 2012). Dissection of the gene expression associated with mechanisms of dehydration response in an ABA-dependent manner via ABA-responsive element binding factors (*ABF*), *MYC* and *MYB* transcription factors and in an ABA-independent manner via drought-responsive element binding factors (*DREB*), allowed identification of drought-inducible target genes of the *DREB1* transcription factor family (such as the stress-tolerance gene *rd29*), which form the basis of molecular breeding for drought tolerance (Sreenivasulu et al. 2007). miRNAs involved in ABA-mediated stress responses and miRNAs involved in response to drought and salt stress in *Arabidopsis*, *Populus*, *Triticum*, and *Zea* species, have been identified (Khraiwesh et al. 2011). Changes of the expression of siRNAs have also been observed in *Triticum aestivum* under drought stress (Yao et al. 2010).

Given the knowledge on the crucial role of transcription factor and of miRNAs and siRNAs in drought stress responses and DNA methylation, and epigenetic inheritance of stress effects, they will be the sources of a new molecular tool kit to be explored in breeding for tolerance to drought and other osmotic-induced plant stresses.

Alteration of Cell Root Metabolism Under Submergence. Flooding or complete submergence affects more than 16 million hectares of cultivated rice lands of the world in lowland and deep-water rice areas with estimated annual economic loss of more than US$600 million (Septiningsih et al. 2009). Many countries are experiencing, as a result of climate change, frequent severe storms causing excess waterflow in rivers and coastal deltas, and consequent flooding of agricultural areas. Submergence tolerance is controlled by a single major QTL on chromosome 9 termed *Sub1* (Xu and Mackill 1996). Sequencing the *Sub1* region in a submergence-tolerant line derived from the *O. sativa* ssp. *indica* landrace FR13A, revealed the presence of a cluster of three linked genes encoding putative ethylene responsive factors (ERF), *Sub1A*, *Sub1B*, and *Sub1C*. *Sub1A* was subsequently identified as the major determinant of submergence tolerance (Xu et al. 2006). Overexpression of allele *Sub1A-1* in a submergence-intolerant *O. sativa* ssp. *japonica* conferred enhanced tolerance to the plants, downregulation of *Sub1C* and upregulation of alcohol dehydrogenase 1 (Adh1), indicating that *Sub1A-1* is a primary determinant of submergence tolerance. The FR13A *Sub1* locus was introgressed into a widely grown Asian rice cultivar using MAB and Septiningsih et al. (2009) developed submergence-tolerant rice varieties, while also studying the effects of *Sub1* in different genetic backgrounds. The new variety maintains the high yield and other agronomic characters of the recurrent parent and is tolerant to submergence. The MAB strategy successfully culminated three decades of research on submergence tolerance at IRRI that began in the 1970s (HilleRisLambers and Vergara 1982), and is a perfect case study of collaboration between conventional breeders and molecular biologists that resulted in development of six submergence-tolerant varieties. The effective methodologies in this research study can serve as a perfect case study for future researchers as the increasing vulnerability of rice farming to flash floods and other abiotic stresses is currently being provoked by the recent trends in climate change.

3.6 Conclusions

Genomics can be applied to plant breeding for adaptation under climate change when an integrated platform of multiple components such as high-throughput molecular techniques, cost-effective protocols, and precise determination of quantitative trait expression, are coupled with efficient breeding methods based on molecular information. Forest trees are the primary biodiversity component that will take advantage of such a platform and help agroforestry intervention to sink as

much as possible CO_2 into multiple use (bioenergy, biomaterials, functional and pharmaceutical products, ecosystem services, and framework for biodiversity conservation) biomass.

The availability of thousands of ESTs, DNA chips, expression-profiling techniques, full-length cDNAs (to identify intron–exon boundaries, alternative splice sites, and to clearly define gene-coding regions within the genomic sequence, as well as for comprehensive gene-function analyses at the transcriptional and translational levels), is of paramount importance for elucidating the role of gene sequences for stress tolerance in forest and crop species. The availability of ESTs from a diverse set of cDNA libraries provides information on transcript abundance, tissue location, and developmental expression of genes. Mutants, introgression libraries, and advanced transformation techniques, are available for functional genomics studies.

The increasing number of reference genomes for molecular biological studies especially in the forest tree and crop species is extremely useful for acquiring an almost unlimited number of DNA markers and genes for mining and cloning genes to be targeted in selection and breeding programs. Fingerprinted and end-sequenced bacterial artificial chromosome (BAC) libraries can serve for aligning sequenced genomes and for genus genomics to provide compelling evidence for a high level of conservation of gene order and molecular cloning.

The forward genetics approach is useful for identifying functionally important alleles from a known difference at the phenotype level. SNPs are genetic markers identified by such an approach. "Ecotilling" is a forward genetics approach developed for detecting multiple types of SNP in natural populations. SNP can also be discovered by reverse genetic methods such as TILLING based on full sequencing, denaturing high-pressure liquid chromatography (DHPLC), and cleavage with the cel1 enzyme followed by polyacrylamide gel electrophoretic analysis.

T-DNA insertion and transposon tagging libraries are reverse genetic methods primarily used for functional analysis based on loss-of-function analyses. Gain-of-function approaches such as T-DNA activation tagging and gene overexpression are powerful complements to insertional mutagenesis. A library of enhancer trap lines has been developed which will facilitate the detection and isolation of regulatory elements.

Although currently available RNAi and other knock-out methodologies could be used for uncovering the function of newly discovered genes, the preliminary results from these approaches in downregulating gene expression necessitate further support and optimization. The regulatory role of snRNA and the generation of inherited epialleles under stress conditions are research areas that will provide promising hints for discovering new genes for stress tolerance in the near future. However, new tools and technologies developed in genomics are expected to greatly enhance, but not replace, the conventional breeding process. Genomic selection and selection for multiple genes and/or multiple traits are examples of where MAS and traditional breeding pedigree and backcross methods would be advantageous for pyramiding multiple genes that confer resistance to diseases or adaptive quantitative traits. Germplasm evaluation, genome selection, hybrid prediction, and seed and clonal

quality control, with emphasis on long-lived perennial forestry and fruit tree species and widely grown annual crop species (e.g., rice, wheat) will greatly benefit from genomics applied to breeding for climate resilience. In perspective, it is expected that by combining population genetics strategies favoring pollen dispersal from the forests under climate change with ecotonal agroforestry practices based on congeneric plant introductions from stressed environments or genomic selections from progenies under stress conditions, it will be possible to establish and manage artificial hybrid zones where heterozygosity, phenotypic plasticity, hybrid vigor, and high CO_2 sink capacity provide the buffering conditions to withstand climate pressure. Two examples of such a strategy are the ecotonal interaction of coppiced chestnuts and chestnut orchards, and the reproductive interaction of large populations of feral hazelnut plants in managed broadleaf forests with the nearby hazelnut orchards in certain Italian (e.g., Latium) and Transcaucasian (e.g., Georgia) geographical areas. The enlargement of the established ecotonal zones and the promotion of other local ecotonal and resilient interactions by afforestation with conifers, *Populus*, *Prunus*, *Malus*, *Olea* plantations, in several world areas where such species flourish in agricultural and forestry land, will contribute to the global and effective subtraction of GHG, sustainable land management, landscape and biodiversity conservation, and several other services to the human society.

Appendix: Framework of Assessments of Climate Change Indicators Related to Agriculture (Fig. 3.3) (Adapted from: EEA 2000; IPCC 2007c; Tollefson and Gilbert 2012)

Driving Forces

Activities that increase emissions of radiatively active gases provoking the "greenhouse effect" (GHG emissions and effect) on the tropospheric temperature.

Pressure

Radiative forcing of the atmosphere due to the increased concentrations of radiatively active gases and aerosols, which absorb and emit radiation within the thermal infrared range determine the greenhouse effect and consequent earth surface temperature increase and global warming.

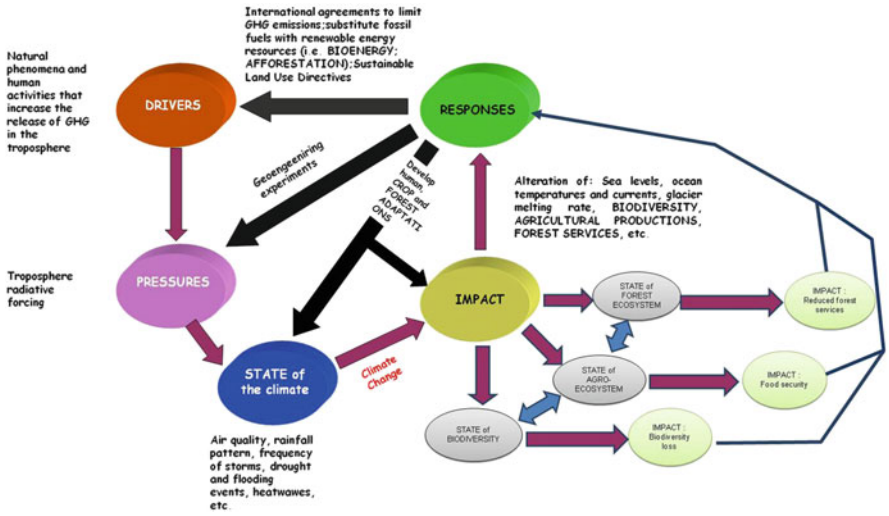

Fig. 3.3 (Figure appendix) DPSIR conceptual framework of the interdependent components of the climate aspects that need support from genetics, genomics, and breeding

State

The main weather and environmental components of the Earth that are affected by global warming are: air quality, rainfall pattern, frequency of storms, drought and flooding events, heatwaves, etc. Globally and locally significant modification of these components compared to a temporal base-line status, contribute to "climate change."

Impact

Sea levels, ocean temperatures and currents, glacier retention, biodiversity, agricultural production, forest services, etc., are affected by climatic changes and the melting of the Greenland ice sheet, the dieback of the Amazon rainforest, and a shift in the West African monsoon may be predicted. Alteration of climatic conditions beyond the tolerance of species, may cause a shift in the timing of life-cycle events (e.g., blooming, migrating), shifting range boundaries (e.g., moving poleward), or the density of individuals within their ranges, changing morphology (e.g., body size). In natural stands, changes in reproductive pattern and allele frequencies for adaptive genes will be observed (Fig. 3.3).

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Chapter 4
Molecular Mapping and Breeding for Genes/QTLS Related to Climate Change

Maria Stefanie Dwiyanti and Toshihiko Yamada

Abstract Through selection by humans, crop plants are adapted to produce optimal yield in the areas where they are cultivated. Climate changes may cause stress to plants, disturb plant growth, and decrease plant yield. Food shortages due to crop failure may cause hunger particularly in poor countries. Therefore, it is important to develop new crop cultivars that can adapt to climate changes. This chapter summarizes quantitative trait loci (QTL) analysis and findings for candidate genes of traits related to tolerance to drought, heat, salinity, macronutrient and micronutrient deficiency, flooding, frost, particularly in major cereal crops. In addition, QTL studies on flowering time of cereal crops are also deliberated. Flowering time is a critical plant phase that determines the transition from the vegetative to the reproductive phase. The optimal time of flowering largely affects the overall yield. Information obtained from QTL analysis has been utilized in the development of stress-tolerant cultivars. Indeed, several stress-tolerant cultivars have been released in stress-prone areas. However, QTLs for most of the traits have not been elucidated. Fortunately, development in sequencing technologies has accelerated elucidation of the genomic regions conferring stress tolerance. This chapter also provides information regarding several recent technologies and approaches in QTL/gene mapping and also in plant breeding.

4.1 Introduction

Through selection by humans, crop plants are adapted to produce optimal yield in the areas where they are cultivated. Climate changes (warmer winters, high temperatures during summer, drought, sea level rise, and increased heavy precipitation), together with its side effects such as high salinity and deficiency of

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macronutrients and micronutrients may cause stress to plants, disturb plant growth, decrease yield, and as a consequence decrease agricultural production (IPCC 2007). Crop failure may cause food shortage and hunger in poor areas. Therefore, it is important to develop new crop cultivars that can adapt to climate changes.

Quantitative trait loci (QTL) analysis has been utilized to determine genomic regions associated with stress-tolerance traits. QTL analysis uses a segregating population derived from two cultivar parents contrasting in stress-tolerance level. Major QTLs for traits such as flowering time, tolerance to high salinity, submergence, or frost/cold were determined quickly, and through fine mapping the candidate genes for QTLs were also identified. However, in more complex traits such as drought and heat stress tolerance, some QTLs identified in one study may not be detected in studies using different mapping populations or stress conditions. To overcome this problem, meta-QTL analysis has been used to identify consensus QTLs across different populations and environments. Along with advances in sequencing technologies and also a decrease in sequencing cost, genomewide association studies (GWAS) became more common in identifying single nucleotide polymorphisms (SNPs) or indels associated with stress tolerance. GWAS enables researchers to identify SNPs and indels without creating mapping populations, but instead utilizing varieties and landraces available in germplasm collections (Mitchell-Olds 2010). This approach shortens the time needed to develop a mapping population. Moreover, GWAS can be applied to minor crop species that have limited availability of genomic information or molecular markers.

The first part of this chapter will describe QTL analysis performed to identify traits involved in tolerance to drought, heat, salinity, submergence, cold and frost, and macronutrient and micronutrient deficiency. For drought, heat, salinity, submergence, and macronutrient and micronutrient deficiency, we will focus mainly on studies conducted on major cereals such as rice, maize, and wheat. For cold and frost, we will focus on studies using temperate grasses such as wheat, barley, perennial ryegrass, and meadow fescue. Flowering QTL studies are also included because flowering is the most critical development stage to determine crop yield. The second part of this chapter will introduce several applications of QTL analysis results through marker-assisted selection (MAS), transgenics, and challenges in developing stress-tolerant cultivars. The third part will deliberate on new technologies that have been adapted to identify QTLs/genes contributing to stress-tolerance traits.

4.2 Molecular Mapping of Genes/QTLs for Tolerance to Stresses

4.2.1 Drought Tolerance

Approximately, more than 40 % of rice growing areas and temperate maize cultivation areas depend on rain as the water source (Campos et al. 2004; Courtois et al. 2009). Crop yield in these areas is vulnerable to drought, and as a consequence, food production may decrease due to prolonged drought. Therefore, the ability to maintain yield level under drought stress is important for cereal crops. A direct approach to identifying drought-tolerant varieties is by evaluating the grain yield under drought stress. QTLs associated with high grain yield under reproductive-stage drought have been identified in rice (Lanceras et al. 2004; Bernier et al. 2007; Kumar et al. 2010; Vikram et al. 2011; Venuprasad et al. 2012), maize (Ribaut et al. 1997; Tuberosa et al. 2002; Swamy et al. 2011), durum wheat (Maccaferri et al. 2008), bread wheat (Li et al. 2007), and many other crops. Another approach is to identify QTLs responsible for traits related to water extraction ability, which generally focuses on root morphology such as deep root length, root thickness, and root dry weight. Some major QTLs for deep root length have been identified in rice (Steele et al. 2006, 2007; Uga et al. 2011) and maize (Trachsel et al. 2009). QTL introgression from the deep-rooting rice varieties "Kinandang Patong" and "Azucena" into the shallow-rooting variety "IR64" has succeeded in improving the root length of rice (Steele et al. 2006, 2007; Uga et al. 2011).

Meta-QTL analysis is utilized to identify consensus QTLs across different mapping populations and different test conditions (Goffinet and Gerber 2000; Veyrieras et al. 2007). This method has been applied in determining consensus QTLs for grain yield (Swamy et al. 2011), maize flowering time (Chardon et al. 2004), soybean nematode resistance (Guo et al. 2006), earliness in bread wheat (Hanocq et al. 2007), and drought tolerance-related QTLs for rice (Courtois et al. 2009; Khowaja et al. 2009) and maize (Hao et al. 2009). Meta-QTL analysis for grain yield under drought stress showed that several QTLs coincide with QTLs for root and leaf morphology, and contain stress-inducible and ABA-response genes, growth and development-related genes, and sugar transport-related genes (Swamy et al. 2011). Several QTLs for grain yield under drought conditions were located near the *Sd1* locus on chromosome 1 of rice (Swamy et al. 2011). *Sd1* is a major locus responsible for semidwarf rice (Monna et al. 2002). It is interesting to note that semidwarf rice has been selected for cultivation because of plant lodging reduction and increase in grain yield (Monna et al. 2002). *Sd1* encodes GA20-oxidase that catalyzes the last step of active GAs biosynthesis (Monna et al. 2002). The relationship between semidwarf characteristics and drought tolerance is also observed in other cereal crops (Swamy et al. 2011). Major QTLs for wheat grain yield under drought were detected near *Rht-b1*, the orthologous locus of *Sd1* on

chromosome 4B (Swamy et al. 2011). Courtois et al. (2009) collected data that consisted of 1,467 QTLs associated with 29 root parameters in rice, including root number, maximum root length, root thickness, and root/shoot ratio. The data was collected from 24 independent papers using more than 10 different mapping populations. The analysis determined 119 consensus QTLs, and found that most QTLs related to root morphology formed a cluster on chromosome 1. Meanwhile, a study on drought tolerance in maize collected QTL data from 22 experiments (Hao et al. 2009). Two hundred and thirty-nine QTLs detected under water stress and 160 QTLs detected under control conditions were compiled. As a result, 39 consensus QTLs under water stress and 36 consensus QTLs under control conditions were identified (Hao et al. 2009). Several genes related to stress response such as genes encoding NCED, a carotenoid cleavage enzyme and CBF1/DREB transcription factors were identified within the meta-QTLs.

Besides water extraction ability, the ability to use water resources is also important. This is called water use efficiency (WUE), which is defined as follows.

From the physiological viewpoint, WUE is defined as moles of carbon gained in photosynthesis in exchange for water used in transpiration. From the farmers' and agronomists' viewpoint, WUE is the yield achieved from the water made available to the crop through precipitation and/or irrigation (Condon et al. 2004). The common method to measure WUE is carbon isotope discrimination (Δ; Farquhar et al. 1989). Δ is the ratio of 12C to 13C (Farquhar et al. 1989). Natural variation in leaf Δ among *Arabidopsis* accessions is related to variation in transpiration efficiency (Masle et al. 2005). QTL analysis of transpiration efficiency in *Arabidopsis* identified the *ERECTA* gene as the transcription efficiency regulator (Masle et al. 2005). The *ERECTA* gene encodes a putative leucine-rich repeat receptor-like kinase (LRR-RLK), which is involved in inflorescence development, stomatal density, epidermal cell expansion, and mesophyll cell proliferation. Lines carrying the *erecta* mutation showed increased stomatal density, and also increased stomatal conductance. High stomatal conductance means higher loss of water to the air. In drought conditions, low stomatal density is important to reduce water loss. Near-isogenic lines (NILs) carrying *ERECTA* from the high transcription efficiency variety showed higher leaf transpiration efficiency and higher plant dry weight/water used ratio (Masle et al. 2005). *ERECTA* is also identified as candidate gene in determining grain yield of rice (Swamy et al. 2011). *ERECTA* is also conserved among angiosperm species; therefore there is a high possibility that it is involved in regulating stomatal density in other species.

Perhaps the stay-green trait is the easiest indicator for evaluation of drought tolerance. The stay-green characteristic has been identified in rice, sorghum, maize, and durum wheat (Xu et al. 2000; Sanchez et al. 2002; Jiang et al. 2004; Zheng et al. 2009; Kumar et al. 2010). Stay-green plants retain their chlorophyll and have delayed leaf senescence under drought conditions, whereas the leaves of normal plants usually senesce. The stay-green characteristic is important particularly during the postflowering stage, because stay-green plants can keep more photosynthetically active leaves to transfer enough nutrition to grains. Fourteen stay-green QTLs were detected in maize (Zheng et al. 2009), and 46 QTLs were detected in a

rice doubled-haploid (DH) line population (Jiang et al. 2004). Three QTLs were identified in a wheat recombinant inbred line (RIL) population derived from a cross between stay-green cultivar "Chirya 3" and nonstay-green cultivar "Sonalika." The QTLs were named *QSg.bhu-1A*, *QSg.bhu-3B*, and *QSg.bhu-7D* (Kumar et al. 2010). However, only *QSg.bhu-1A* was consistently detected over 2 years of experiment. Four stay-green QTLs (*Stg1*, *Stg2*, *Stg3*, and *Stg4*) were identified using three sorghum RIL populations derived from a cross of two inbred lines including "B35," a cultivar resistant to postflowering stress, and "Tx7000," a cultivar sensitive to postflowering stress (Xu et al. 2000). *Stg1* and *Stg2* were located on linkage group A, *Stg3* on linkage group D, and *Stg4* on linkage group J. *Stg1*, *Stg2*, and *Stg3* were consistently identified in different trial locations over 2 years of experiment, whereas *Stg4* was identified in different trial locations over 1 year of experiment (Xu et al. 2000). Further analysis showed that *Stg1*, *Stg2*, and *Stg3* were collocated with three QTLs controlling the chlorophyll content (*Chl1*, *Chl2*, and *Chl3*). Although the candidate genes have not been determined, *Stg1* and *Stg2* contain genes for heat shock proteins, cell membrane ATPase, ABA-responsive genes, and key photosynthetic enzymes (NADP-dependent malate dehydrogenase, chlorophyll a/b binding protein, and Rubisco) (Xu et al. 2000).

4.2.2 Heat Tolerance

By the end of the twenty-first century, global temperature is predicted to increase by about 2–4 °C (IPCC 2007). Higher temperature accelerates plant development, including flowering time. For bioenergy plants, heat stress can reduce biomass production. In cereal crops, heat stress reduces anther dehiscence and pollen fertility rate. It subsequently reduces grain filling, and also the overall yield. Heat stress thresholds differ among plant species and cultivars (Prasad et al. 2006; Wahid et al. 2007). Heat tolerance also varies depending on development stage. Generally, plants are most sensitive during flowering and grain filling rather than in the vegetative stage (Maestri et al. 2002; Prasad et al. 2006; Jagadish et al. 2007).

Several parameters have been used to determine heat tolerance: pollen germination rate, pollen tube length, spikelet sterility, cell membrane thermostability, chlorophyll stability index, canopy temperature depression, grain quality change, and antioxidant level (Matsui et al. 2001; Prasad et al. 2006; Paliwal et al. 2012). Heat tolerance is sometimes related to higher stomatal opening, which is intended to cool plant tissue by releasing more water to air. However, this approach is not beneficial when water is scarce, a condition that usually accompanies heat stress.

Despite the complexity of heat tolerance regulation, several stable QTLs have been identified in wheat (Paliwal et al. 2012). QTLs were identified in a mapping population derived from heat-tolerant hexaploid wheat cultivar "NW1014" and a heat-susceptible "HUW468". QTLs were identified on chromosome 2B, 7B, and

7D for the heat susceptibility index (HSI) for thousand grain weight (HSITGW), HSI for grain fill duration (HSIGFD), and canopy temperature depression (CTD).

The heat shock protein (HSP) has been identified as a candidate gene for heat stress tolerance in rice (Maestri et al. 2002; Ye et al. 2012). Proteomic analysis studying the heat stress effect during anthesis in heat-tolerant rice cultivar "N22" and heat-sensitive rice cultivars "IR64" and "Moroberekan" showed that the heat shock protein content increased in "N22" as a sign of the heat tolerance of "N22" (Jagadish et al. 2010). QTL and molecular analysis also showed HSP as a candidate gene for heat stress tolerance in *Arabidopsis* (Hong and Vierling 2000). HSP101 was mapped to the QTL region for heat stress tolerance in *Arabidopsis* (Hong and Vierling 2000). However, although HSPs are conserved among plant species in terms of high sequence similarity, the specific function in each species may vary (Maestri et al. 2002), therefore further research is needed to elucidate the function of each HSP in heat-stress tolerance.

4.2.3 Salt Tolerance

Approximately 830 million ha worldwide are affected by salinity (Rengasamy 2006), and the extent will increase due to extensive irrigation, drought, and the increasing level of seawater (Pitman and Lauchli 2002). The presence of salt in soil at seed sowing decreases the germination rate (Foolad 1999). A high level of salt in soil during the vegetative stage inhibits plant growth and causes leaf chlorosis, subsequently leading to a decrease in crop yield (Parida and Das 2005; Thomson et al. 2010). High salt inhibits plant growth in two ways (1) concentrations of salt in the soil reduce the ability of plants to absorb water (the osmotic effect of high salinity) (Lauchli and Grattan 2005); (2) when salt exists in plant cells, the cells may be damaged and the metabolic and photosynthetic capacity of the plant declines (Lauchli and Grattan 2005). Studies on *Arabidopsis*, rice, barley, and tomato have used plant condition parameters (root growth, germination rate, or seedling growth) to identify QTLs conferring salt tolerance (Mano and Takeda 1997; Foolad 1997; Foolad and Chen 1999; Foolad et al. 2001; Prasad et al. 2000; Quesada et al. 2002). QTL analysis showed that salt tolerance is controlled by polygenes, and tolerance at the germination stage and the seedling stage is controlled by different mechanisms (Foolad and Chen 1999).

Salt-tolerance during vegetative growth is also associated with the ability of plants to control Na^+ ion concentration in the leaves through exclusion or sequestration of Na^+ (Munns and Tester 2008). Several QTL analyses in rice, wheat, and *Arabidopsis* used the Na^+ concentration or the Na^+:K^+ ratio to identify genes responsible for maintaining low levels of Na^+ in the leaves and enhancing salt tolerance. For example, Gregorio and Senadhira (1993) studied the exclusion of Na^+ ions and increased K^+ in order to maintain a low Na^+:K^+ ratio in the shoots of three salt-tolerant *indica* rice cultivars: "Nona Bokra," "Pokkali," and "SR26."

QTLs for salt tolerance were extensively studied using an RIL population from a cross between the salt-susceptible variety "IR29" and salt-tolerant "Pokkali" (Gregorio 1997). A major QTL called *Salto1* associated with the Na^+:K^+ ratio in shoots and salinity tolerance at the seedling stage was identified in chromosome 1 (Gregorio 1997; Bonnila et al. 2002). QTL analysis using an $F_{2:3}$ population from a cross between the susceptible cultivar "Koshihikari" and salt-tolerant cultivar "Nona Bokra" also identified *Salto1* as a QTL for salt tolerance (Lin et al. 2004). This QTL was designated *Shoot K^+ concentration* (*SKC-1*) because it is associated with shoot K+ ion concentration (Lin et al. 2004). These QTLs are responsible for salt tolerance during plant vegetative growth. Map-based cloning of the *Salto1* region identified the gene *OsHKT1;5* (Ren et al. 2005). *OsHKT1;5* produces a protein that is highly similar to high-affinity K^+ transporters (HKT-type transporters) that play an important role in maintaining Na^+ and K^+ in shoots and leaf blades (Ren et al. 2005). These HKT-type transporters maintain low Na^+ concentration in leaf blades by excluding Na^+ from the xylem sap of leaf sheaths. The proteins also enhance the concentration of K^+ in leaf blades and sheaths.

HKT-type transporters are also involved in salt tolerance of wheat and *Arabidopsis* (Huang et al. 2006; James et al. 2006; Rus et al. 2006; Byrt et al. 2007). Two HKT-type transporter genes (*TmHKT7-A2* and *TmHKT1;5-A*) were mapped to *Nax1* and *Nax2,* QTLs for shoot Na^+ exclusion in durum wheat (genome AABB; Huang et al. 2006; James et al. 2006). A major QTL for salt tolerance *Kna1* was also mapped in bread wheat. The genomic region of *Kna1* corresponds to *Nax2* in durum wheat, suggesting that the two regions are orthologs (Byrt et al. 2007). The ortholog of TmHKT7-A2 TmHKT1;5-A, and OsHKT1;5 in *Arabidopsis* is AtHKT1;1 (Rus et al. 2006; Horie et al. 2009). The *athkt1;1* mutant increased Na^+ concentration of the xylem sap and conversely reduced the Na^+ content of the phloem sap (Sunarpi et al. 2005). The mutant also has increased salt sensitivity, while complementation with *AtHKT1;1* restores the salt tolerance of the plants (Sunarpi et al. 2005), indicating that *AtHKT1;1* is involved in salt tolerance in *Arabidopsis*. TmHKT7-A2 TmHKT1;5-A, OsHKT1;5, and AtHKT1;1 are included in class 1 HKT-type transporters (Horie et al. 2009).

Besides *Salto1*, cellular ion homeostasis in rice is also regulated by several other QTLs. Thomson et al. (2010) identified a QTL for shoot Na^+:K^+ ratio in chromosome 9 in "Pokkali"-derived RILs. QTL analysis using an F_2 population derived from a cross between salt-tolerant *japonica* rice mutant, "M-20" and the sensitive original variety "77-170" (Zhang et al. 1999) identified a major gene for salt tolerance on chromosome 12. The gene encodes a plasma membrane H+-ATPase that produces a proton and electrical gradient. The gradient is used as the force for transport and regulation of Na^+ and Cl^- uptake. Koyama et al. (2001) utilized an RIL population derived from a cross of two *indica* cultivars to perform QTL analysis associated with low Na^+ ion uptake and regulation of the Na^+:K^+ ratio. Two QTLs associated with regulation of the Na^+: K^+ ratio were found on chromosome 1 and 4 (Koyama et al. 2001). The QTL on chromosome 1 coincided with the QTL for Na^+ ion uptake. It is unknown whether the QTL is the same as *Salto1* or not, because of the different location due to different markers flanking the QTL

(Koyama et al. 2001). Besides HKT-type transporters, *Nax1*, *Nax2*, and *Kna1* also contain genes encoding intracellular Na^+/H^+ antiporter (NHX) and Salt Overly Sensitive 1 (SOS1). SOS1 is a putative Na^+/H^+ antiporter (Shi et al. 2002), and the overexpression of *SOS1* improved salt tolerance of *Arabidopsis* (Shi et al. 2002).

Recently, a QTL controlling salt tolerance and ABA sensitivity was identified in *Arabidopsis* using an RIL population derived from a cross between "Landsberg *erecta*" (salt and ABA sensitive) and "Shakdara" (salt and ABA resistant) (Ren et al. 2010). The QTL was designated *Response to ABA and Salt 1* (*RAS1*) (Ren et al. 2010). The *RAS1* gene of the sensitive accession "Landsberg *erecta*" encoded a protein with 230 amino acid residues, whereas *RAS1* of the resistant accession "Shakdara" encoded a protein truncated at its C-terminal with only 209 amino acid residues. Knockdown or loss of *RAS1* led to enhanced salt tolerance and ABA insensitivity, whereas introducing *RAS1* to tolerant cultivars led to salt and ABA hypersensitivity (Ren et al. 2010). Therefore, *RAS1* is the negative regulator of salt tolerance.

4.2.4 Flood and Submergence Tolerance

While drought is the major issue in global climate-change-related crop production, flooding becomes a major problem in monsoon areas. Flooding affects rice, maize, wheat, barley, oats, and sorghum (Xu and Mackill 1996; Setter and Waters 2003; Zaidi et al. 2003; Hattori et al. 2009; Promkhambut et al. 2010). Prolonged flooding, particularly flooding that submerges entire plants causes hypoxia in plants, stops photosynthetic activities, and as a result, kills the plants. Two different forms of flood tolerance have been developed by plants. To face deepwater flooding that lasts for several months and involves water from a few to several meters, elongating the internodes to keep the top leaves above the water's surface helps plants to survive. Another type of flood is the flash flood which arrives suddenly but lasts no longer than a few weeks. Seedlings are the most affected by flash floods. Susceptible young seedlings will use all available energy to grow above the water, and as a result, will die after having consumed all energy reserves when the water finally drains. Submergence-tolerant plants will not grow (stunting) to avoid excessive energy consumption. When a flood ends, the tolerant plants will start growing again using their conserved energy.

Research on flood tolerance has been done extensively in rice. Rice is a staple food in the flood-prone areas of South Asia and Southeast Asia. More than 16 million ha of rice lands of the world in lowland and deep-water rice areas are affected by flooding due to complete submergence (http://www.knowledgebank.irri.org). By positional cloning, Hattori et al. (2009) identified two major QTLs *Snorkel1* (*SK1*) and *SK2* for deepwater flood tolerance on chromosome 12. *SK1* and *SK2* encode ethylene-responsive factor type transcription factor (ERF). The ERF domain is also possessed by DREB transcription factors, which are known to

regulate response to drought and high temperature stress. Under low oxygen conditions, ethylene increases. Ethylene then induces the expression of *SK1* and *SK2*. Overexpression of *SK1* and *SK2* in nondeepwater rice resulted in internode elongation, even in nonflood conditions, suggesting that *SK1* and *SK2* regulate the internode elongation. Although the mechanism of how *SK1* and *SK2* promote internode elongation is still unclear, it is suggested that gibberellic acid (GA) may be involved in this method. The reason is under deepwater conditions, uniconazole (a GA biosynthesis inhibitor) inhibits internode elongation in deepwater-tolerant rice (Suge 1987; Hattori et al. 2009). However, no QTLs related to deepwater tolerance are found to contain GA biosynthesis genes. Further research using transgenics is needed to elucidate the relationship of *SK1* and *SK2* with GA.

Using a cross between an *indica* submergence-tolerant rice "IR40931-26" and a sensitive *japonica* rice "PI543851," one major QTL *Submergence1* (*Sub1*) controlling flash-flood tolerance was located near the centromere of chromosome 9 (Xu and Mackill 1996). "IR40931-26" was derived from the strongly submergence-tolerant cultivar "FR13A." *Sub1* contributed to approximately 70 % of the phenotypic variance in submergence tolerance (Xu and Mackill 1996). Using positional cloning, three genes encoding ethylene response factor (AP2/ERF) were located in the *Sub1* region, and were named *Sub1A*, *Sub1B*, and *Sub1C* (Fukao et al. 2006; Xu et al. 2006). *Sub1B* and *Sub1C* are present in all tolerant and susceptible cultivars studied so far, whereas *Sub1A* exists only in the tolerant cultivars (Xu et al. 2006). Two alleles of *Sub1A* exist in the *indica* rice cultivars: *Sub1A-1* in tolerant cultivars and *Sub1A-2* in sensitive cultivars. The contribution of *Sub1A-1* to submergence tolerance was confirmed by expression analysis in the sensitive cultivar "Swarna" (Xu et al. 2006). Recently, new QTLs for submergence tolerance have been found through QTL analysis using mapping populations derived from two moderately tolerant varieties, "IR72" and "Madabaru" (Septiningsih et al. 2012). Several progenies showed higher survival rates than the "FR13A"-derived tolerant variety "IR40931." Four QTLs were identified on chromosome 1, 2, 9, and 12; the QTL on chromosome 9 was *Sub1*. The *Sub1* allele was inherited from "Madabaru," while other QTLs contain tolerant alleles from IR72. These QTLs may be used to enhance submergence tolerance besides *Sub1*.

When *Sub1A* is overexpressed, *Alcohol dehydrogenase-1* (*Adh1*) is upregulated (Xu et al. 2006). *Adh1* is enhanced in young root under low oxygen conditions, in response to dehydration, low temperatures and to abscisic acid presence, proving the involvement of *Sub1A* in stress response. Since *Sub1A* is an ERF, the *Sub1A* expression is upregulated in response to increased levels of ethylene under flash-flood conditions. However, *Sub1A* does not promote internode elongation as observed in *SK-1* and *SK-2*, but instead enhances the accumulation of mRNA and protein of two suppressors of GA signaling, *SLENDER RICE1* (*SLR1*) and *SLR1-like 1* (*SLRL1*) (Fukao and Bailey-Serres 2008) that are correlated to inhibition of internode elongation under flood conditions.

Even though *Sub1A* has a different function compared to *SK-1* and *SK-2*, the similarity of their ethylene response domain (ERF domain) is high. *Sub1A* and *SK-1*

shared 63.3 % of similarity in the ERF domain, while *Sub1A* and *SK-2* showed 65 % similarity in the ERF domain. Phylogenetic analysis based on the ERF domain, showed that *SK1*, *SK2*, and *Sub1A* are grouped within the same family group (Hattori et al. 2009). It is interesting to analyze the reasons as to why genes with similar structure have opposing functions, and how the plants evolved genes to adapt to specific environments (e.g., flash floods or deepwater).

4.2.5 *Macronutrient and Micronutrient Deficiency Tolerance*

Nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S) are major nutrients needed for normal plant growth. In addition, boron (B), copper (Cu), iron (Fe), chlorine (Cl), manganese (Mn), molybdenum (Mo), and zinc (Zn) are also needed in small quantities (micronutrients). Among these major nutrients, many soils lack N, P, and K because plants need them in larger amounts. The next common deficiency, particularly in lowland rice fields, is zinc (Zn) (Neue and Lantin 1994; Quijano-Guerta et al. 2002).

Although NPK deficiency can be alleviated by fertilizer application, soil conditions such as drought, high concentrations of other elements, pH, and soil temperature can decrease the efficacy of fertilizer application. Drought decreases the amount of nutrients diffused in the soil, and as a consequence, lower amounts of nutrients are available for absorption by plant roots (Pinkerton and Simpson 1986; Hu and Schmidhalter 2005). For example, N application in drought-affected areas did not increase yield as much as application in well-irrigated areas (Smika et al. 1965; reviewed in Hu and Schmidhalter 2005). High concentrations of salt (Na^+) in the soil inhibit the absorption rate of K, because K^+ absorption competes with Na^+. The presence of aluminum (Al) and Fe in the soil decreases the amount of P that can be utilized by plants, because P forms a complex with Al and Fe that cannot be absorbed by plants. This phenomenon is stronger in acid soil with pH less than 6. Moreover, P deficiency often occurs in areas that are highly eroded, or contain high calcium carbonate ($CaCO_3$) because P and Ca form an insoluble complex. Low temperature during the early growing season also inhibits P availability. Since nutrient deficiency often occurs in soil that is affected by drought, salinity, and acidity, development of crop cultivars with high mineral use efficiency will save fertilizer costs and benefit the farmers.

QTL analysis for N use efficiency (NUE) has been performed in the model plant *Arabidopsis* (Loudet et al. 2003) and major crops such as rice, wheat, and maize (Hirel et al. 2001; Obara et al. 2004; Le Gouis et al. 2008). NUE can be measured as shoot dry matter, total N, nitrate, or free-amino acid contents (Hirel et al. 2001; Loudet et al. 2003; Mickelson et al. 2003; Obara et al. 2004; Le Gouis et al. 2008). As in water use efficiency, mineral use efficiency is also regulated by a large number of genes. Some genes show effects only under certain development stages,

or under mineral deficiency. Therefore, QTL analysis under deficiency conditions and QTL analysis under normal conditions may give different results. For example, an NUE study in *Arabidopsis* showed that eight QTLs related to shoot dry matter were detected in nonlimiting N conditions (N+), whereas under N-deficient conditions (N−) only four QTLs were detected (Loudet et al. 2003). Similarly, nine QTLs were related to free amino acid content in N+ conditions but only five QTLs controlled the free amino acid content in N− conditions (Loudet et al. 2003). In temperate maize, the number of QTLs related to grain yield, N content, and N use efficiency were also lower when tested in N− conditions compared to N+ conditions (Hirel et al. 2001, 2007; Gallais and Hirel 2004; Coque and Gallais 2006). Among the QTLs, a QTL for grain yield on chromosome 5 colocalized with a gene encoding cytosolic glutamine synthetase (GS; *gln4* locus) (Hirel et al. 2001). Other grain yield QTLs collocated with GS genes; for example *gln1* and *gln2* on chromosome 1, and *gln3* on chromosome 4 (Gallais and Hirel 2004). The coincidence of NUE QTLs with GS genes was also observed in rice (Obara et al. 2001, 2004; Yamaya et al. 2002) and wheat (Coque et al. 2008). One cytosolic GS gene (*GS1*) was located in a QTL region on the long arm of chromosome 2, which contributed to one-spikelet weight of rice (Obara et al. 2001). Unlike rice and maize, wheat cytosolic *GS* was associated with N content increase in the grain but did not correlate to grain yield components (Habash et al. 2007). There is a high possibility that *GS* is the candidate gene for NUE QTLs in rice, maize, and wheat, because of its central role in plant N metabolism. GS catalyzes the condensation of glutamate and ammonia to form glutamine. Glutamate and glutamine are the N donors in biosynthesis of amino acids, nucleic acids, chlorophyll, plant hormones, and secondary metabolism products. In addition, in higher plants, N is mainly transported as glutamine and asparagine. In addition to the *GS* gene, QTL mapping for grain yield and grain protein yield on a doubled-haploid population from a cross between N stress-tolerant wheat and N stress-sensitive wheat identified 233 QTLs clustered into 82 genome regions (Laperche et al. 2007). Meta-QTL analysis and factorial regression on the result showed that three QTLs were collocated with dwarfing gene (*Rft-B1*), photoperiod sensitivity gene (*Ppd-D1*), and the awns inhibitor gene (*B1*) (Laperche et al. 2007).

Long root hairs, greater root surface area, and deep roots are associated with higher phosphorus uptake efficiency (PUE) under low P conditions. Root morphology is used as a parameter to identify high PUE because P is not mobile in the soils. QTLs for P deficiency-induced root elongation have been identified in *Arabidopsis* (Reymond et al. 2006), wheat (Su et al. 2006), and rice (Shimizu et al. 2004, 2008). Shimizu et al. (2008) found that a QTL for root elongation under phosphorus deficiency (REP) detected on the long arm of chromosome 6 of rice (*qREP-6*) is associated with an increase of tiller number and shoot P content. In contrast, a major QTL responsible for P content in rice tiller was not associated with seedling root growth (Wissuwa et al. 2005). This QTL was mapped to chromosome 12 of rice, and designated *Phosphorus uptake 1* (*Pup1*; Wissuwa et al. 2002). NILs carrying the *Pup1* segment from high PUE parent Kasalath showed higher P uptake

(threefold) compared to Nipponbare (Wissuwa and Ae 2001). The finding suggests that factors other than root morphology are also involved in PUE.

One example of QTL analysis for micronutrient deficiency tolerance is discussed here: tolerance to Zn deficiency. Zn deficiency has been associated with a wide range of soil conditions: high pH soils, peatland, saline soils, and soils with high magnesium and calcium content (Neue and Lantin 1994). Zn deficiency causes leaf bronzing, and plant mortality when severe (Wissuwa et al. 2006), and retarded pollen development in maize (Sharma et al. 1987). Wissuwa et al. (2006) identified two major QTLs for tolerance to Zn deficiency on chromosome 2 and 12 of rice. The QTLs were designated *Zinc-deficiency-induced mortality*-2 (*Zmt-2*) and *Zmt-12*. However, these QTLs did not relate to leaf bronzing. Only one minor QTL was associated with both plant mortality and leaf bronzing (Wissuwa et al. 2006).

4.2.6 Cold and Frost Stress Tolerance

Temperate plants recognize low but nonfreezing temperatures in autumn and winter, develop cold acclimation during winter, and begin growth when temperature rises again as a sign of spring. The length and low temperature level needed as a signal for cold acclimation are variable among and within plant species. If winter temperatures are warmer than usual, plants may not adequately acclimate and may be damaged by frost in spring. A warmer spring may also cause earlier and sparse flowering in wheat and barley, reducing crop yield.

Genomic regions responsible for frost tolerance in wheat were identified using chromosome substitution lines of frost-sensitive "Chinese Spring" and frost-tolerant "Cheyenne" substitution lines (Sutka 1981; Galiba and Sutka 1988). The most effective region in chromosome 5A was designated *FROST RESISTANCE-A1* (*Fr-A1*) (Sutka and Snape 1989; Galiba et al. 1995). Another major QTL, *Fr-A2*, was mapped to chromosome 5, approximately 30 cM apart from *Fr-A1* (Vagujfalvi et al. 2003; Baga et al. 2007). Major frost-tolerance QTLs in barley, *FR-H1* and *FR-H2*, are orthologous to *Fr-A1* and *Fr-A2* of wheat (Francia et al. 2004, 2007). *Fr-A1* cosegregated with vernalization QTL *VRN-1* in most genetic studies (Roberts 1986; Hayes et al. 1993; Sutka and Snape 1989; Limin and Fowler 2002; 2006). Although Galiba et al. (1995) suggested that *Fr-A1* is a gene closely linked to *VRN-1*, Dhillon et al. (2010) suggested that *Fr-A1* is a pleiotropic effect of *VRN-1* because wheat with mutation in *VRN-1* showed higher frost tolerance than wheat with active *VRN-1*. Wheat NILs carrying a winter wheat type of *VRN-1* allele showed higher cold tolerance than lines carrying the *VRN-1* allele for spring growth habit (Limin and Fowler 2006).

The *Fr-A2* locus contains a cluster of *C-repeat binding factor* (*CBF*) genes (Vagujfalvi et al. 2003; Baga et al. 2007). *CBF* genes belong to the *DREB* group, because the genes encode transcription factors that bind to the conserved core sequence CCGAC (c-repeat) in the promoters of genes involved in dehydration and cold response. Eleven *CBF* genes form a cluster within the *Fr-A2* locus, and the

order of orthologs in barley is conserved (Skinner et al. 2005; Miller et al. 2006). Higher *CBF* expression was observed in lines carrying the frost-tolerant *FR-2* allele compared to those carrying the frost-sensitive allele (Xue 2003; Skinner et al. 2005; Vagujfalvi et al. 2005). Among *CBF* genes, *TaCBF14*, *TaCBF15*, and *TaCBF16* transcript levels were more than fourfold higher in the frost-tolerant lines (Vagujfalvi et al. 2005). *CBFs* regulated two cold-regulated genes: *COLD RESPONSIVE PROTEIN 14b* (*COR14b*) and *WHEAT COLD SHOCK 120* (*WCS120*) (Vagujfalvi et al. 2003; Francia et al. 2004; Knox et al. 2008; Galiba et al. 2009). These genes were mapped to the *Fr-A2* locus through expression-QTL analysis. Meanwhile, *HvCBF2* and *HvCBF4* showed the highest expression in frost-tolerant barley, but the genes were not orthologous to *TaCBF14*, *TaCBF15*, and *TaCBF16* (Stockinger et al. 2007; Galiba et al. 2009). The difference in expression level of *CBF* genes may be related to variation in frost tolerance between species (Galiba et al. 2009).

CBF genes also play a role in cold acclimation of perennial ryegrass (*Lolium perenne*) and meadow fescue (*Festuca pratensis*). Four *LpCBF* genes of perennial ryegrass were mapped to linkage group 5 in a position orthologous to the *CBF* clusters in wheat and barley (Tamura and Yamada 2007). One *CBF* gene (*FpCBF6*) was identified in meadow fescue (Alm et al. 2011). This gene was mapped to chromosome 5F and was found to be an ortholog of *Fr-H2* (Alm et al. 2011). Interestingly, although meadow fescue had two QTLs on chromosome 5F, which corresponded to *FR-A1* and *FR-A2*, its vernalization gene *FpVRN1* is located on chromosome 4F, instead of chromosome 5F as in wheat and barley (Alm et al. 2011). This fact supports the hypothesis that different genes on chromosome 5F, not *VRN1*, are involved in cold acclimation.

Cold tolerance is also related to tolerance to drought stress and low ion leakage. Dehydrin is a protein that is involved in cold and drought tolerance by protecting the cell membrane from damage. Dehydrin (*Dhn*) genes are collocated with a cold-tolerance QTL in meadow fescue (Alm et al. 2011). A QTL for electrical conductivity was detected on linkage group 4 in perennial ryegrass (Yamada et al. 2004).

4.2.7 Flowering Time Regulation

Flowering is an important transition step from the vegetative phase to the reproduction phase. Plants use photoperiod and temperature to determine flowering time. Understanding how photoperiod and temperature interact to determine flowering time is important to ensure optimal plant yield. In high latitude areas with four seasons, flowering too late in winter may kill flowers, or not give enough time for grain filling. In drought-prone areas, early flowering rice cultivars are preferred by farmers to escape drought that may occur during the grain-filling period (reviewed in Kamoshita et al. 2008). For biomass crops, late flowering or nonflowering types are chosen to reduce nutrient transfer from stems and leaves to flowers. The next

section summarizes flowering regulation in major crops, including annual crops such as rice and maize and overwintering crops such as winter wheat and barley.

4.2.7.1 Flowering Regulation in Annual Crops

QTL studies on flowering time in rice have been performed mainly using the segregating population derived from a cross between the *japonica* cultivar "Nipponbare" and the *indica* cultivar "Kasalath." "Nipponbare" is sensitive to photoperiod, whereas "Kasalath" is not (Yano et al. 2001). Fourteen QTLs related to flowering time were identified, and six of them have already been fine-mapped. These QTLs were designated *Heading date*1 (*Hd1*)—*Hd14*; based on a definition of flowering in rice because flowering means the start of heading in rice (Yano et al. 1997, 2001; Lin et al. 1998; Yamamoto et al. 1998). Among the 14 QTLs, *Hd1*, *Hd3a*, *Hd3b*, and *Hd6* were related to photoperiod sensitivity (Yano et al. 2001). *Hd1* codes a GATA1-type protein, which is an ortholog of *CONSTANS* (*CO*) in *Arabidopsis* (Putterill et al. 1995). Epistatic interaction was observed between *Hd1* and *Hd3a* (Lin et al. 2000). *Hd3a* showed a high similarity with the *Arabidopsis Flowering time T* (*FT*) gene (Kojima et al. 2002). Under short-day conditions, *Hd1* promotes flowering by upregulating the expression of *Hd3a*. This is in contrast to the *CO-FT* relationship in *Arabidopsis*, because *CO* upregulates *FT* under long-day conditions (Kobayashi et al. 1999; Onouchi et al. 2000; Samach et al. 2000). Meanwhile, the candidate gene for *Hd3b* has not yet been determined, but it is associated with late heading under long-day conditions (Monna et al. 2002). *Hd6* delays flowering under long-day periods and encodes the α-subunit of protein kinase CK2 (CK2α; Takahashi et al. 2001). *Arabidopsis* CK2 interacts with and phosphorylates circadian clock-associated 1 protein (CCA1) (Sugano et al. 1998). Among the *Hd* genes, phenotypic variation in the flowering time of rice was explained mainly by allelic variation of *Hd1* (Takahashi et al. 2009). In addition, *Hd1* and *Hd2* may be involved in flowering response to temperature (Nakagawa et al. 2005).

Besides the *Hd1* pathway, rice flowering time is also controlled by *Early heading date1* (*Ehd1*) (Doi et al. 2004). Under long-day conditions, *Ehd1* promotes flowering (Doi et al. 2004). *Ehd1* is preferentially expressed under short-day conditions even under the absence of *Hd1* (Doi et al. 2004). *Ehd1* encodes β-type response regulator and regulates the expression of *FT*-like genes and several MADS-box genes (*OsMADS14*, *OsMADS15*, and *OsMADS1*) (Doi et al. 2004). *Ehd1* encodes *OsMADS14* and *OsMADS15*, which are orthologs of *Arabidopsis AP1*, which acts downstream of *FT*, *SUPPRESSOR OF OVEREXPRESSION OF CO1* (*SOC1*) and *LEAFY* (*LFY*). Redundancy and antagonistic function of *Hd1* and *Ehd1* may relate to the ability of rice to adapt to a broad range of environments with different temperature and photoperiod requirements for development (Izawa 2007; Takahashi et al. 2009; Ebana et al. 2011).

Another photoperiod pathway gene in rice, *Ghd7,* colocalizes with a QTL for major effects underlying traits related to plant height, heading date, and number of

grains per panicle (Xue et al. 2008). *Ghd7* encodes a CCT-domain protein whose enhanced expression under long-day conditions suppresses *Ehd1* and *Hd3a*, delays heading date and as a consequence increases plant height and panicle size. Five allelic variations of *Ghd7* exist in rice and wild rice (*Ghd7-1*, *Ghd7-2, Ghd7-3*, *Ghd7-0*, and *Ghd7-0a*). Among them, *Ghd7-0* and *Ghd7-0a* were nonfunctional and were found in rice varieties grown in cool areas and with short growing periods (Xue et al. 2008).

Numerous flowering genes in maize have been identified. One of the major genes is *Vegetative to Generative Transition1* (*Vgt1*) (Salvi et al. 2007). *Vgt1* contains a *cis*-regulatory element of the floral repressor gene *ZmRap*2.7, which is located ~70 kb downstream of *Vgt1*. Unlike *Hd1* or *Ehd1*, *Vgt1* does not correlate with photoperiod (Chardon et al. 2004). Nevertheless, allele variation of *Vgt1* is highly correlated with geographical origin (Ducrocq et al. 2008).

4.2.7.2 Flowering Regulation in Plants Requiring Vernalization

Winter perennial plants require some period of low temperature during winter to initiate flowering. The process is called vernalization. Temperature and the length of cold exposure required for vernalization vary among different species and also among varieties within species (Kim et al. 2009). Changes in winter and spring temperature affect the flowering time of plants. For example, high temperature in spring accelerates the heading time of winter wheat (Hu et al. 2005). In addition, if winter temperature is high, winter wheat varieties that require long vernalization will not receive sufficient cold temperatures. As a result, the amount of flower/heading will reduce. The vernalization process has been extensively studied in *Arabidopsis*, winter wheat, and barley (Von Zitzewitz et al. 2005; Distelfeld et al. 2009; Kim et al. 2009). Besides vernalization, flowering of wheat and barley is also regulated by photoperiod (Turner et al. 2005; Hemming et al. 2008; Li and Dubcovsky 2008). Some of the genes involved in the vernalization pathway are also involved in the photoperiod response pathway (for a review see Kim et al. 2009).

The vernalization process in wheat and barley involves the three genes *VRN1*, *VRN2*, and *VRN3*. *VRN1*, *VRN2*, and *VRN3* of wheat were mapped using a segregating population derived from a cross between winter wheat that requires vernalization and spring wheat that does not require vernalization (Danyluk et al. 2003; Trevaskis et al. 2003; Yan et al. 2003, 2004b, 2006). *VRN1* was isolated by map-based cloning of the diploid wheat *Triticum monococcum* and mapped to the long arm of chromosome 5A (Yan et al. 2003). On the basis of gene expression pattern analysis, *VRN1* was also mapped to chromosome 5B and 5D of the hexaploid wheat *T. aestivum* (Danyluk et al. 2003; Trevaskis et al. 2003). The locations of *VRN1* on chromosome 5B and 5D correspond to the location on chromosome 5A (Danyluk et al. 2003; Trevaskis et al. 2003). *VRN2* was also mapped using the diploid wheat *T. monococcum*; it is mapped on the long arm of chromosome 5A

(Yan et al. 2004b). Meanwhile, using the hexaploid wheat *T. aestivum*, *VRN3* was mapped to the short arm of chromosome 7B (Yan et al. 2006).

VRN1 encodes a MADS-box transcription factor that resembles floral meristem identity genes *AP1* and *FRUITFULL* (*FUL*) in *Arabidopsis* (Danyluk et al. 2003; Trevaskis et al. 2003; Yan et al. 2003). *VRN2* encodes a CCT-domain protein of which the homolog in *Arabidopsis* is still unknown (Yan et al. 2004b). *VRN3* is the homolog of the *Arabidopsis FT* gene, which promotes flowering (Yan et al. 2006). In vernalization-requiring wheat cultivars, *VRN2* suppresses *VRN3* expression during summer and autumn. During winter, low temperature induces the expression of *VRN1*. Accumulated *VRN1* promotes inflorescence initiation, and also suppresses *VRN2*. As a result, *VRN3* repression is removed and flowering is induced (Trevaskis et al. 2007). The photoperiod pathway of flowering induction in wheat and barley also involves *VRN3* and *VRN1*. Under long-day conditions, a photoperiod gene *Photoperiod-1* (*Ppd1*) activates *VRN3* (Turner et al. 2005). *VRN3* interacts with bZIP transcription factor FD, and in turn, activates *VRN1* (Li and Dubcovsky 2008). However, as described above, in winter wheat, *VRN2* prevents *Ppd1* activity by repressing *VRN3*.

Mutation in *VRN3* is related to the vernalization requirement of wheat cultivars. Wheat cultivars carrying dominant *VRN3* alleles showed early flowering, and increased *FT* gene expression due to the insertion of a retroelement in the promoter region (Yan et al. 2003). Meanwhile, a dominant *VRN2* allele is responsible for winter growth habit, whereas a dominant *VRN1* allele is responsible for spring growth habit. In the diploid wheat *T. monococcum*, the *VRN2* locus contains two tandemly located zinc finger-CCT-domain genes (*ZCCT1* and *ZCCT2*) (Yan et al. 2004a, b). Both genes are able to confer the vernalization requirement. Recessive cultivars with mutations encompassing all ZCCT genes or in the conserved amino acids of the CCT domains showed spring growth habit (Distelfeld et al. 2009). Allelic variations at the *VRN2* locus of the B genome are associated with variation in heading time of tetraploid wheat, although the effect was not seen after the vernalization process (Distelfeld et al. 2009).

VRN1 genes exist on each genome of hexaploid wheat: *VRN-A1*, *VRN-B1*, and *VRN-D1*. A single dominant *VRN* allele is sufficient to confer spring growth habit, which means the cultivar does not need vernalization to induce flowering (Stelmakh 1987; Allard et al. 2012). Allelic variations were found in *VRN-A1*, *VRN-B1*, and *VRN-D1* (Yan et al. 2004a; Fu et al. 2005). Whereas the allelic variation of *VRN-B1* and *VRN-D1* is determined only by deletion within the first intron, the allelic variations of *VRN-A1* showed an indel within the promoter sequence and also deletion within the first intron of the sequence (Yan et al. 2004a; Fu et al. 2005). Yan et al. (2004a) found at least five allelic variations of *VRN-A1*, with two major types *Vrn-A1a* and *Vrn-A1b*. The *Vrn-A1a* type has duplication in the promoter region, flanked by host direct duplication (HDD). The *Vrn-A1b* type has a 20-bp deletion in the 5′-UTR region and two nucleotide substitutions in the HDD region (Yan et al. 2004a). Interestingly, the *Vrn-A1a* type was dominant in hexaploid spring wheat cultivars released in the United States and Argentina between 1970 and 2004, and also dominant in the spring wheat cultivar from CIMMYT

(Yan et al. 2004a). The results suggest that *Vrn-A1a*-type cultivars spread to Argentina and the United States might be related to the introduction of the semidwarf germplasm from CIMMYT in the 1970s. *Vrn-A1a* is also dominant in the Canadian spring wheat cultivars that show a short growing period (Iqbal et al. 2007). Although the dominant allele of *VRN1* may not be involved in the vernalization process, a different combination of dominant *VRN-A1*, *VRN-B1*, and *VRN-D1* loci affected the precocity (early maturing) of spring wheat cultivars (Stelmakh 1992, 1998). It is also shown that plants with three dominant *Vrn1* alleles need less time to anthesis compared to plants with only one dominant *Vrn1* allele (Allard et al. 2012). Moreover, the time to anthesis is shortened in plants carrying *Vrn-A1* compared to *VRN-B1* or *VRN-D1* (Stelmakh 1992, 1998; Allard et al. 2012). Perhaps allelic variation of *Vrn-A1* is also related to the level of plant precocity.

Vernalization in barley is regulated by *VRN-H1*, *VRN-H2*, and *VRN-H3* (Karsai et al. 2005; Yan et al. 2006; Cockram et al. 2007; Szücs et al. 2007; Wang et al. 2010). The barley *VRN-H1* gene is mapped on the long arm of chromosome 5H, which corresponds to *VRN-A1*, *VRN-B1*, and *VRN-D1* in hexaploid wheat (Galiba et al. 1995; Laurie et al. 1995; Dubcovsky et al. 1998; Barrett et al. 2002; Iwaki et al. 2002; Yan et al. 2003). The *VRN-H2* gene has been mapped in the distal part of chromosome arm 4HL in barley, and corresponds to *VRN2* in wheat (Laurie et al. 1995). *VRN-H3* is mapped to chromosome 7H and an ortholog of *Arabidopsis FT* (Yan et al. 2006). The alleles for spring growth habit (*Vrn-H1*, *vrn-H2*, and *Vrn-H3*) are epistatic to the alleles for winter growth habit (Takahashi and Yasuda 1971). Therefore, only plants possessing a combination of *vrn-H1*, *Vrn-H2*, and *vrn-H3* showed a winter growth habit (Takahashi and Yasuda 1971; Saisho et al. 2011). Facultative spring barley genotypes, which are cold tolerant but unresponsive to vernalization have a dominant *Vrn-H1* allele and recessive *vrn-H2* (Von Zitzewitz et al. 2005). As in wheat, mutation in the first intron of *VRN-H3* caused increased expression and response to vernalization (Yan et al. 2006).

In addition to *VRN* genes, *Ppd* genes are also involved in regulation of winter wheat and barley flowering time. In hexaploid wheat, *Ppd-1* genes exist on chromosome 2A, 2B, and 2D (*Ppd-A1, Ppd-B1*, and *Ppd-D1*, respectively). The major source of photoperiod insensitivity in wheat is the semidominant *Photoperiod-D1a* (*Ppd-D1a*) allele (Worland 1996; Beales et al. 2007; Yang et al. 2009). Mutation in *Ppd-D1a* is likely to be caused by a 2-kbp deletion of a regulatory region that led to misexpression of the pseudo-response regulator (PRR) gene family (Beales et al. 2007). Varieties carrying the *Ppd-D1a* allele show early flowering regardless of photoperiod length (Worland 1996). The allele was probably introduced from the Japanese variety "Akakomugi," which was used to breed semidwarf varieties (Guedira et al. 2010). Mutation in the regulatory region of the *PRR* gene or a closely linked gene of the *Ppd-B1* locus is also thought to confer photoperiod insensitivity (Beales et al. 2007). No mutation in *Ppd-A1* has been reported in hexaploid wheat, but mutation in *Ppd-A1* and alteration in photoperiod sensitivity is reported in tetraploid wheat (Wilhelm et al. 2009).

The ortholog of *Ppd-1* in barley is *Ppd-H1*, which is a major determinant of long-day response (Laurie et al. 1995). Missense recessive mutations in the CCT

domain of barley *Ppd-H1* cause late flowering under long-day photoperiod conditions (Turner et al. 2005). Another photoperiod QTL, *Ppd-H2* contains the barley homolog of *FT* (Faure et al. 2007; Kikuchi et al. 2009).

4.3 Application of QTL Analysis Knowledge in Breeding Stress-Tolerant Crop Cultivars

Despite the complexity of regulation of stress-tolerance traits, several new cultivars have been developed for tolerance to high salinity, submergence, and drought stress (Table 4.1). Rice cultivars with submergence tolerance have been applied in the field. Using marker-assisted backcrossing (MABC), which needs less time than conventional MAS, a tolerant allele of *Sub1A* has been introgressed to rice megavarieties, such as "Swarna," "Samba Mahsuri," "IR64," "Thadokkam 1," "CR1009," and "BR11" (Septiningsih et al. 2009). Among those varieties, "Swarna-Sub1" was released in India, Indonesia, and Bangladesh; "BR11-Sub1" was released in Bangladesh; and "IR64-Sub1" was released in the Philippines and Indonesia during 2009–2010 (Bailey-Serres et al. 2010). A field test in India demonstrated that "Swarna-Sub1" showed a higher yield than "Swarna" under submergence stress (Sarkar et al. 2009).

However, stress-tolerant cultivars still face challenges. In most cases, stress occurs in combination, i.e., drought occurs with heat stress, high salinity with nutrient deficiency, etc. Or, during a season change, drought may occur after flood. Therefore, cultivars developed for certain stress types must be tested against other stresses that may occur in the field. Fukao et al. (2011) tested near isogenic lines with a tolerant allele of *Sub1A* under drought stress which occurs frequently after a flood. They found that *Sub1A,* the QTL for submergence tolerance, also contributes to enhanced drought tolerance in rice. *Sub1A* keeps plants dormant and conserves energy during the submergence period, and promotes tillering after water has receded. In drought conditions, *Sub1A* enhanced recovery from drought at the vegetative stage through reduction of leaf water loss and lipid peroxidation. Through microarray data analysis, *Sub1A* enhanced the expression of genes related to ABA responsiveness, genes associated with acclimation to dehydration, and those that suppress the accumulation of reactive oxygen species (ROS) by increasing ROS-scavenging enzymes (Fukao et al. 2011).

Another approach is to overexpress *DREB1* genes to enhance plant tolerance. *DREB1* is known as a universal stress defense mechanism that can be found across species. It confers tolerance to drought, high salinity, and low temperature (Kasuga et al. 1999; Shinozaki and Yamaguchi-Shinozaki 2000; Ito et al. 2006; Lata and Prasad 2011). Overexpression of the *DREB1A* gene from *Arabidopsis* enhanced drought tolerance in wheat (Pellegrineschi et al. 2004). In another study, overexpression of *DREB1*/*CBF*-type transcription factors enhanced tolerance to drought, high salinity, and low temperature in rice (Ito et al. 2006). However, both

Table 4.1 Examples of breeding crops with increased stress tolerance through MAS breeding

Crops	Tolerance to	Locus/markers/ genes/	Reported in	Note
Durum wheat	High salinity	*Nax*2 (contain *TmHKT1;5-A)*	Munns et al. (2012)	25 % higher yield and reduction in leaf Na^+ in NILs with *TmHKT1;5-A* in saline soils trial
Rice	Submergence	*Sub1A-1*	Siangliw et al. (2003), Neeraja et al. (2007), Septiningsih et al. (2009), Iftekharuddaula et al. (2010)	Introgressed to megarice varieties Swarna, BR11, IR64; Thai jasmine rice KDML105
Rice	High salinity	*Salto1*	Thomson et al. (2010)	High salt-tolerant RIL (FL478) as breeding donor
Wheat	Drought	–	Condon et al. (2004)	Two varieties for high water use efficiency: Drysdale and Rees
Rice	Drought	Chromosome 9 (RM242-RM201)	Steele et al. (2006)	Increase root length of upland variety, Kalinga III

studies showed that while *DREB1*-overexpressing plants grew better than wild type under stressed conditions, they performed worse under control conditions. Constitutively expressed *DREB1* genes keep plants under a "stressed" condition, and uses energy for stress tolerance rather than relocating nutrients to grains. Pellegrineschi et al. (2004) used an rd29A promoter that works specifically under stress conditions to effectively enhance *DREB1* expression under drought, and suppress its expression under normal conditions.

Choosing the right parental pair is important in MAS breeding. Sometimes stress tolerance results from epistatic interaction between two parents; particularly in drought and heat tolerance that are regulated by complex interactions of genes. Although considerable work has been performed to elucidate the genetic basis of both traits, it is still difficult to identify a specific genomic region that is responsible for conferring drought or heat tolerance. Interaction between genes involved in stress tolerance must also be considered while combining several genes (gene pyramiding) to enhance the stress tolerance of a plant.

4.4 Future Approaches to Mapping QTLs/Genes Associated with Stress Tolerance Traits

We can see from the description above that despite enormous effort to identify QTLs related to stress-tolerance traits, candidate genes have been determined only for a few (salt tolerance, submergence tolerance, flowering time, cold and frost tolerance). To accelerate the identification of stress tolerance-related genes, researchers have developed several technologies and also strengthened international collaborations.

4.4.1 Genomewide Association Studies and Genomic Selection

As an alternative to traditional QTL mapping, GWAS has been more commonly used in mapping QTLs/genes related to stress-tolerance traits. The first advantage of GWAS over traditional QTL mapping is that it does not need an a priori hypothesis about candidate gene location. GWAS utilizes SNPs that exist in the genome. By comparing the percentage of certain nucleotide types between stress-resistant and stress-sensitive populations, the relation between a SNP and a phenotype can be determined. This method represents a shortcut compared to the usual method of QTL mapping, which sometimes stumbles on the lack of molecular markers: create a linkage map, QTL mapping, fine-mapping of the QTL region, identification of the candidate gene and lastly, determination of the nucleotide polymorphisms responsible for stress tolerance. The second advantage of GWAS is that it analyzes varieties and without creating mapping population as in QTL mapping, which may take time especially if we want to analyze perennial plants. The third advantage of GWAS is that it can detect evolutionary history or geographical accumulation of natural variation.

Currently, there are two types of GWAS depending on the population type used in the analysis—a population-based approach and a family-based approach (Mitchell-Olds 2010). Population-based GWAS uses populations of unrelated individuals to examine associations between SNPs and phenotypes. Conversely, family-based GWAS uses pedigrees derived from crosses among different founding genotypes. Population-based GWAS uses unrelated individuals, therefore it takes advantage of historical recombination events that have accumulated over thousands of generations. However, a recombinant type may accumulate in a certain population through natural or artificial selection, and create false-positives in the analysis results. Family-based GWAS has a complementary advantage/disadvantage compared to population-based GWAS. It eliminates the false-positive that occurs because of population structure, but recognizes fewer recombination events.

Other advantages and disadvantages for each approach have been reviewed in Mitchell-Olds (2010).

Recently, Atwell et al. (2010) reported GWAS of 200 *Arabidopsis* inbred lines for more than 200,000 SNPs. Genotype data of 200 *Arabidopsis* inbred lines have been stored (http://www.1001genomes.org) as part of a project to discover the whole-genome sequence variation of 1,001 strains (accessions) of *Arabidopsis*. In 2010, whole-genome sequences of 80 accessions were completed (Cao et al. 2011; Schneeberger et al. 2011). Once the genotyping data for these accessions is completed, researchers can use the data to map the trait of interest, therefore reducing the time and effort involved in genotyping. Atwell et al. (2010) have already mapped QTLs related to 107 phenotypes, including flowering, cold tolerance, seed dormancy, salt tolerance, and pathogen resistance traits.

The family-based GWAS approach has been used in maize, which has a high level of outcrossings and large effective population size. Maize geneticists collaborated to develop a maize nested association mapping (NAM) population that consists of 25 groups of inbred lines derived from 25 parents crossed to a fully sequenced B73 genotype (Buckler et al. 2009; McMullen et al. 2009). Each group consists of 200 inbred lines. The NAM population has been used to characterize flowering regulation in maize (Buckler et al. 2009).

Markers identified through GWAS can be used in genomic selection. Genomic selection uses all markers across an entire genome on a population to explain total genetic variance rather than the few markers used in MAS (Meuwissen et al. 2001). The Genomic selection method is centered on a "training population." In the training population, marker genotypic and phenotypic data of each individual are determined. Then, the effect of each marker to phenotype is calculated (= marker effect). On the basis of the marker effect, we can predict the breeding value of an individual by testing its marker genotypes (see for review, Heffner et al. 2009). Genomic selection is useful particularly in breeding programs that want to utilize small-effect QTLs or gene pyramiding.

4.4.2 Marker Transferability Between Species for QTL Mapping in Minor Crops

How about QTL mapping in minor crop species? For minor crops, often called orphan crops, (Armstead et al. 2009), the limited availability of molecular markers and known genome sequence can hinder construction of a linkage map that covers the entire genome, and as a consequence, some QTLs may not be detected. Besides constructing a genome library enriched with SSR sequences, molecular markers from species in the same family can be used in linkage map construction for a minor crop of interest (Wang et al. 2010). A set of 210 SSR markers developed from

wheat, rice, sorghum, and maize were evaluated for their transferability to minor grass species (*Eleusine coracana*, *Paspalum vaginatum*, *Cynodon dactylon*, and *Festuca arundinacea*) (Wang et al. 2005; Saha et al. 2006; Wang et al. 2010). The transfer rate of SSR markers was correlated with the phylogenetic relationship between species. In other study, SSR markers from the temperate grass model plant *Brachypodium distachyon* were tested for transferability to *Miscanthus sinensis*, a potential bioenergy crop (Zhao et al. 2011). Fifty-seven SSR markers chosen to evenly represent location across the *B. distachyon* genome were tested. Out of these *B. distachyon* SSR markers, 86.0 % are transferable to *M. sinensis* (Zhao et al. 2011).

4.4.3 Comparative Genome Analysis

Comparative analysis of linkage maps between different plant species shows that they maintain the same physical localization of genetic loci on the same chromosomal region (synteny) even though they are divergent species. QTL and fine mapping analyses showed that gene sets associated with traits are conserved between species. For example, genes involved in cold acclimation and flowering time are conserved between wheat, barley, and to some extent with perennial ryegrass and meadow fescue. Comparative genome analysis accelerates the discovery of key genes involved in stress tolerance. It is possible to use the sequence information of a gene that has been identified in one species to isolate the same gene in another species, especially in those that do not have sufficient genome information.

4.4.4 International Collaboration for Genetic Information

Currently, information about QTL analysis and mapping populations is stored in public Web sites, therefore researchers can utilize the information for their breeding programs. Availability of molecular marker sequences, linkage maps, and whole-genome sequence data accelerates identification of candidate genes. In 2008, the US Department of Energy Joint Genome Institute released whole-genome sequence information for several major crops and model plants, and has improved the data since then. Currently, whole-genome sequence information is available for 34 species, including *Arabidopsis*, rice, soybean, cotton, maize, sorghum, grape, rapeseed, apple, and poplar (http://www.phytozome.org). The Web site provides not only whole-genome sequence information but also the physical map of genes or putative genes. This information enables researchers to BLAST markers sequence flanking their QTL regions, extract gene information in genomic regions between the flanking markers, and predict the candidate gene (Ducrocq et al. 2009; Jordan

Table 4.2 Databases providing information on genetic and germplasm stock in *Arabidopsis*, and crops belong to the grass family

Database	Species	URL
GrainGenes	Triticeae/*Avena*	http://wheat.px.usda.gov
Gramene	Gramineae	http://www.gramene.org
MaizeGDB	Maize	http://www.maizegdb.org
NBRP	Plant species	http://www.nbrp.jp
Oryzabase	Rice	http://www.nig.ac.jp/labs/PlantGen/english/oryzabase-e/index.html
Panzea	Maize and teosinte	http://www.panzea.org
PlantGDB	Plant species	http://www.plantgdb.org
Phytozome	Plant species	http://www.phytozome.org
Rice Genome Annotation Project	Rice	http://rice.plantbiology.msu.edu/
TAIR	*Arabidopsis*	http://www.arabidopsis.org

et al. 2010). Other web sites also provide genomic information for general plant species or specific to plant species/family (Table 4.2).

Compilation of QTL data in one Web site helps breeders to select molecular markers suitable for their breeding program. Information for the maize NAM population and agronomical traits' QTLs can be obtained from http://www.panzea.org; while for wheat, barley, rice, wild rice, rye, oat, pearl millet, and several other grass species information can be obtained from http://www.gramene.org. The database not only provides information about traits and the related molecular markers, but also the neighboring markers, which can be used in MABC, and segregating populations used for QTL mapping, which is helpful in providing information about parental species.

Germplasms are also an important factor in the development of stress-tolerant cultivars. For example, *Triticum dicoccoides* and *Hordeum spontaneum*, the wild relatives of wheat and barley showed potential as donors for drought and salt tolerance (Nevo and Chen 2010). Wild rice, *Oryza rufipogon* can also serve as a drought-tolerance donor to cultivated rice (Zhang et al. 2006). Meanwhile, genetic variations in flowering time loci *Ppd-H1* and *Ppd-H2* that only exist in wild barley, *H. spontaneum*, can be used as a source for novel allelic variation for photoperiod response in barley (Cockram et al. 2011), which can adapt to warmer climate in high latitudes. Landraces are also potential sources to improve stress tolerance, for example Indian landraces "Pokkali" and "Nona Bokra" that showed salt tolerance. Several programs have been conducted to collect germplasms and characterize the agronomical traits of the stocks, such as NBRP (http://www.nbrp.jp), Oryzabase (a site for rice germplasm that is sponsored by NBRP), Graingenes (list of wheat, barley, and oat genetic stocks, collection owned by several institutions).

Fast progress in bioinformatics technologies and the development of next-generation sequence is accelerating genome sequencing of plant species. Besides genomic analysis, many tools have been developed to identify genes through transcriptome, proteome, and metabolome profilings (see for review, Mochida

and Shinozaki 2010). Knowledge about genetics, transcriptional regulation, metabolic pathways, and also informatics are needed to analyze and interpret the vast amounts of data generated by these analyses. The iPlant Collaborative (http://www.iplantcollaborative.org) provides a network for researchers to learn about various tools for data storage, management and analysis; and sharing knowledge in plant science research analyses.

4.5 Conclusion

QTL analysis has been useful in the identification of genes responsible for traits related to stress tolerance. In several cases, such as high salinity and submergence tolerance, candidate genes for major QTLs have been characterized and introgressed to widely cultivated varieties. With the advance in genome sequencing technologies using next-generation sequencers, QTL/gene mapping and identification are being accelerated and it is becoming possible to perform QTL analysis on minor crops, which often have limited molecular markers. In addition, breeding techniques are also improved, such as MABC and genomic selection. This knowledge will improve our approaches to the development of superior crop cultivars that are able to adapt to present and future climate changes.

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Chapter 5
Genomic Tools and Strategies for Breeding Climate Resilient Cereals

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Abstract Cereal crops are vital for meeting the food, feed and nutritional demands of the world. However, long-term production growth of cereals could be severely affected by the changing climate, which is already exacerbating existing challenges such as drought and heat stresses, insect pests and diseases, and soil degradation, especially in the tropics. While adaptation to climate change would require convergence of appropriate technologies, policies and institutional innovations, the focus of this chapter is on some of the promising genomic tools and strategies that can enhance time- and cost-effectiveness of breeding for climate-resilient cereals. For this, we use maize as a case study, considering the availability of genomic resources, the significance of maize as the number one cereal crop in the world at present in terms of total area and production, the vulnerability of sub-Saharan Africa (where maize is the most important staple food crop) and South Asia (where maize plays a significant role as food and feed) to the changing climates. CIMMYT's experiences and initiatives with regard to designing and implementing modern breeding strategies for developing climate-resilient maize varieties, including high-density genotyping, whole genome resequencing, high-throughput and precise phenotyping, doubled haploids (DH), genomics-assisted breeding (e.g., genome-wide association studies, breeder-ready marker development, rapid-cycle genomic selection, marker-assisted recurrent selection), and crop modeling are particularly highlighted here. The key challenges to the international scientific

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community are (a) to generate high-quality phenotypic data in breeding programs, and integrating the same with modern tools and technologies for accelerated development of climate-resilient germplasm; (b) to better understand the effects of climate change on diversity of cropping systems in different regions; and (c) to effectively monitor the patterns of change both temporally and spatially, coupled with appropriate policies and actions at the farm level.

5.1 Introduction

Tremendous strides have been made over the past several decades by way of development and delivery of a large number of improved cereal varieties adapted to different agroecologies and meeting the diverse demands of the stakeholders, especially through conventional breeding. However, with the world population projected to reach nine billion by 2050, it is imperative to increase cereal production by 37% annually in order to meet the surging food demand (Tester and Langridge 2010). The output of the three major cereal crops, viz. maize, wheat and rice, alone needs to increase by 70% by 2050 in order to feed the world's growing rural and urban populations. At the same time, it is ironic that resource-poor farmers in the developing world are struggling to maintain even existing production levels and make farming profitable in the face of volatile commodity prices and rising energy costs.

Although there is huge potential to increase production of cereals through area expansion in some countries (especially in sub-Saharan Africa), uncontrolled area expansion cannot be a solution for meeting increasing demands, as this could potentially threaten forests, marginal lands, and hill slopes. Moreover, long-term production growth of many crops could be severely affected by global climate change, which exacerbates existing problems such as water and heat stresses, insect pests and diseases, and soil degradation, besides posing several new challenges (Wassman et al. 2009; Shiferaw et al. 2011). Predicted climate change scenarios for Africa include an increase in seasonal temperatures and extreme temperature events, sea level rise in low-lying coastal areas, increased intensity of droughts (IPCC 2007) and a reduction of suitable areas for a range of crops more than any other region (Lane and Jarvis 2007). South and South-East Asia will be particularly hard hit by climate change effects (Lobell et al. 2008; Wassman et al. 2009). It has been predicted if current trends persist until 2050, the yields of major crops in South Asia will decrease significantly by 17% (maize), 12% (wheat) and 10% (rice) because of climate change-induced heat and water stresses (ADB 2009). Lobell et al. (2008) identified wheat production in South Asia alongside rice production in South-East Asia and maize production in Southern Africa to be the most important systems in need of adaptation investments to mitigate the anticipated negative impacts of climate change.

Thus, cereal production may not be able to meet the demands without strong technological and policy interventions that can alleviate the problems posed by

biophysical and socioeconomic constraints, and can boost both production and productivity on the existing land. Modern breeding will also have to rely on effective utilization of time-effective, resource-efficient and cutting-edge technologies, including high-throughput genotyping, precision phenotyping, breeding informatics and decision support tools, for enhancing genetic gains and accelerating development of improved varieties.

We review here some of the promising genomic tools and strategies, supported by important technological advances, in breeding for climate change resilience in food crops, especially cereals. While doing so, we will more often use maize as an example among the cereals due to an array of reasons, including availability of genomic resources, significance of maize as the number one cereal crop in the world at present in terms of total area and production, vulnerability of sub-Saharan Africa (where maize is the most important staple food crop) and South Asia (where maize plays a significant role as food and feed) to the changing climates, and the active involvement of CIMMYT in designing and implementing improved breeding strategies for developing climate-resilient maize varieties.

5.2 Climate Change Adaptation: The Key Breeding Products

Climate change projections suggest more frequent and extreme droughts, and sometimes more than one extreme condition within one crop season (e.g., drought and waterlogging, drought and heat stress), which increase on the short-run the likelihood of crop failures and on the long-run, production declines (Zaidi and Cairns 2011). Since most of the maize production in the developing world is under rainfed conditions, the crop is particularly vulnerable to drought and its yields fluctuate more widely from year to year than is the case for rice and wheat, which are more commonly irrigated.

Breeding strategies for developing climate-ready germplasm must take into account the predicted and significant regional variation as well as increasing temperatures. Temperatures are expected to increase in sub-Saharan Africa by an average of 2.1°C by 2050 (Cairns et al. 2012a). Lobell and Burke (2010) predicted a 2°C increase in temperatures will result in a greater reduction in maize yields than a decrease in precipitation by 20%. Similarly a recent study in Tanzania also indicated that increasing temperatures would result in a greater reduction in maize yields than increased intra-seasonal variability in precipitation (Rowhani et al. 2011). From analysis of over 20,000 historical maize trial yields in Africa, Lobell et al. (2011) reported a yield reduction of 1% and 1.7% for every one degree above 30°C under optimal rainfed and drought conditions, respectively. Maize yields under climate change have been predicted to decrease in nearly 75% of African countries as a result of temperature increases and rainfall changes. This decrease in yield could be an equivalent of 10% of the total maize production in

Africa and in Latin America (Jones and Thornton 2003). Increasing temperatures under climate change has also been highlighted as a major constraint to rice and wheat production. Seasonal temperatures during early grain filling in rice (the most sensitive growth stage to heat stress) are already near threshold temperatures for rice production in Bangladesh, eastern India, southern Myanmar, and northern Thailand (Wassman et al. 2009). In wheat, up to 51% of the production area in the Indo-Gangetic Plains is predicted to experience heat stress by 2050 (Ortiz et al. 2008).

While drought stress and its effects on crop plants has been more intensively analyzed, information on the potential effects of increased temperatures on crop growth and development has been less explored (Paulsen 1994). Heat stress is associated with shortened life cycle (Muchow et al. 1990; Prasad et al. 2006), reduced light interception (Stone 2001), reduced photosynthesis (Crafts-Brander and Salvucci 2002), and increased sterility (Schoper et al. 1987a, b; Jagadish et al. 2012). Sensitivity to supraoptimum temperatures and mechanisms of tolerance depend on the severity, duration and timing of heat stress together with developmental stage of the plant. To apply the knowledge of the mechanisms responsible for yield loss under elevated temperatures within a breeding program, it is essential that the types of heat stress predicted to occur in the target environment are clearly defined (Burke et al. 2009; Wassman et al. 2009). Given that different traits and mechanisms are likely to provide adaptation for different types of heat stress (i.e., varying in duration, intensity, and timing), heat stress environments need to be defined to enable the assessment of the relevance of individual physiological experiments and breeding trials to the target population of environments.

Climate-ready germplasm will have to possess packages of relevant traits, rather than resilience to specific abiotic or biotic stress. A vast amount of research has focused on individual stresses. Separate breeding programs using initial selection under only one stress may result in the loss of valuable alleles or genetic variability for additional stresses. Given that the combination of heat and drought stress is distinctive, crop improvement efforts must focus on the identification of traits and donors associated with combined tolerance to both drought and heat stresses (Barnabás et al. 2008). Research in model species suggests the combination of heat and drought stress is unique and cannot be predicted from the response to individual stresses (Rizhsky et al. 2002, 2004; Mittler 2006). Heyne and Brunson (1940) showed the combined effect of heat and drought stress in maize was greater than the effect of each stress individually. Cairns et al. (2012a) recently showed tolerance to combined drought and heat stress in maize to be genetically distinct from tolerance to the individual stresses, and tolerance to either stress alone did not confer tolerance to the combined effect of drought and heat stress. Given that crops in farmers' fields often experience several stresses at once, these results highlight the need to start focusing on the effects of combined stresses (Voesenik and Pierik 2008).

Development of abiotic stress-tolerant germplasm alone will not be sufficient to further enhance yields under the changing climates. Local adaptation in terms of biotic stress resilience (especially insect pests, diseases, and weeds) will also be

simultaneously required. Rising temperatures and variations in humidity also potentially affect the diversity and responsiveness of pathogens and insect pests, and could lead to new and perhaps unpredictable epidemiologies (Gregory et al. 2009). For example, the gray leaf spot (GLS) disease in maize is now becoming an important disease globally, with high incidences reported in Nepal, China, Bhutan, Colombia, Mexico, Brazil, and several countries in Africa. Incorporating the outputs of global climate models with predicted land use patterns, besides knowledge of the dynamics of insect pests and pathogens, is required to identify emerging insect pests and diseases within each region and incorporate relevant germplasm in breeding pipelines for these regions.

Thus, the ability to quickly develop germplasm combining tolerance to several complex abiotic and biotic stresses will be critical to the resilience of cropping systems in the face of climate change. Conventional breeding methods that rely on extensive phenotypic screening are still important, but not adequate enough for accelerating development of such germplasm with tolerance to multiple stresses. Adoption of innovative and modern breeding methodologies, including mining of novel genetic variation, precision phenotyping and rapid-cycle breeding, is increasingly becoming important. Marker-assisted selection (MAS) is one of the major components of modern breeding, aided by advances in molecular biology, genomics and statistics, and forms the core for genetic enhancement of crop plants.

5.3 Modern Tools, Techniques, and Strategies to Develop Climate-Resilient Crops

To meet the climate change, crops need much better environmental adaptation including innate resilience to cope with more extreme abiotic stresses as well as tolerance to combined stresses. Development of genomic strategies and tools will need to consider responses of different cereals to climate changes, some of which could be crop-specific while others may be shared among different crops. Favorable alleles, genes, and haplotypes need to be identified from diverse germplasm (including landraces and wild relatives, if necessary) and transferred to elite germplasm. Novel strategies to combine molecular markers with accelerated development of elite germplasm (including techniques like doubled haploidy, high-throughput phenotyping, and planting in year-round nurseries) would be needed to fast-track development and delivery of improved germplasm. Here, we highlight some of these important modern tools and strategies.

5.3.1 High-Density Genotyping and Resequencing

In recent years, as the genotyping platforms evolved from gels to chips and sequencing, genotyping throughput has increased from singles to millions of markers per assay (or single to thousands of DNA samples per marker), while the cost per data point has decreased from several US dollars to 1/1,000 cent or less. Single nucleotide polymorphism (SNP) chips have been developed for a number of cereal crops: rice (McCouch et al. 2010; Yamamoto et al. 2010), maize (Yan et al. 2010a), barley (Close et al. 2009), and durum wheat (Trebbi et al. 2011).

In maize, developing chip-based genotyping through Cornell-CIMMYT collaboration brought up three Illumina 1,536-SNP chips (Yan et al. 2009, 2010a), which were soon replaced by the Illumina MaizeSNP50 Beadchip consisting of 56,110 SNPs, with 1 SNP/40 kb, and covering 19,540 genes with 2 SNPs/gene. In rice, SNP discovery with the OryzaSNP project led to identification of approximately 160,000 high-quality SNPs that are informative across 20 diverse rice varieties (McNally et al. 2009). Likewise, resequencing of over 100 varieties through the Rice SNP Consortium (http://www.ricesnp.org) provided an even larger SNP discovery pool from which a one million-SNP chip has been developed (McCouch et al. 2010).

An alternative approach for large-scale genotyping is genotyping-by-sequencing (GBS). A simple and highly multiplexed system for constructing reduced representation libraries was developed for the Illumina next-generation sequencing platform (Elshire et al. 2011). Constructing GBS libraries was based on reducing genome complexity with restriction enzymes, which may reach important regions of the genome that are inaccessible to sequence capture approaches. The procedure has been demonstrated with maize (IBM) and barley (Oregon Wolfe Barley) recombinant inbred line (RIL) populations where roughly 200,000 and 25,000 sequence tags were mapped, respectively (Elshire et al. 2011). With this method, species that lack a complete genome sequence can have a reference map developed around the restriction sites, which can be done in the process of sample genotyping.

Using the GBS system, large-scale high-density genotyping is being employed by the CIMMYT Global Maize Program (CIMMYT-GMP) for improvement of complex traits, and several billion data points have already been generated on the key germplasm. The system is being further optimized to reduce missing data points and improve SNP calls. In the foreseeable future, however, the choice of chip or GBS for genotyping will depend on their cost for genotyping and related data management, analysis and delivery systems.

The genome sequencing of B73 (Schnable et al. 2009) and Palomero, a popcorn landrace in Mexico (Vielle-Calzada et al. 2010) are important landmarks in maize genome research, with significant implications to our understanding of maize genome organization and evolution, as well as to formulate strategies to utilize the genomic information in maize breeding. The Palomero genome is about 22% (140 Mb) smaller than that of B73, and showed a large number of hitherto unreported sequences, implying a large pool of unexplored alleles. Also, more

than 12 genes related to heavy-metal detoxification and environmental stress tolerance were found to be conserved in B73 and Palomero, but absent from teosinte, suggesting that these genes were possibly involved in the domestication process (Vielle-Calzada et al. 2010).

As sequencing becomes increasingly cheap and high throughput, resequencing has become an alternative for genotyping. Large-scale resequencing has been done in rice (Xu et al. 2012a) and maize (Chia et al. 2012) for diversity, evolutionary, and genetic studies. With next-generation DNA sequencing technology (Shendure and Ji 2008), it will be possible to sequence the whole gene bank collection. Maize is the first plant species with a haplotype map (HapMap) constructed. Gore et al. (2009) identified and genotyped several million sequence polymorphisms among 27 diverse maize inbred lines and discovered that the genome was characterized by highly divergent haplotypes. Haplotype-based mapping can be used to replace individual marker-based mapping to improve the mapping power and identify specific alleles in a gene or allele combinations at different loci that contribute to the same target trait (Xu et al. 2012b).

The new genotyping/sequencing technologies and in silico tools now provide immense opportunities for the cereal scientific community to speed up research progress for large-scale diversity analysis, high-density linkage map construction, high-resolution quantitative trait loci (QTL) mapping, linkage disequilibrium (LD) analysis, and genome-wide association studies (GWAS). In addition to powerful next-generation sequencing and genotyping systems, diverse mapping populations are available in crops like maize as international maize genomic resources. For example, the maize "nested association mapping" (NAM) population, comprising 5,000 RILs (200 RILs from each of 25 founders), is an important genetic resource developed in recent years. The NAM population is a novel approach for mapping genes underlying complex traits, in which the statistical power of QTL mapping is combined with the high resolution of association mapping (Yu et al. 2008). Global genetic diversity of maize has been captured in the NAM RILs, which will provide the maize research community with the opportunity to map genes associated with various traits, including resilience to diverse abiotic and biotic stresses.

5.3.2 High-Throughput and (Reasonably) Precision Phenotyping

Advances in phenotyping are also essential for increasing the efficiency of cereal breeding by exploiting information from high-throughput genotyping (Phillips 2009). Phenotyping has evolved from reliance only on direct measurement of the target traits (e.g., grain yield) under stress in breeding for abiotic tolerant crops through analytical breeding (Araus et al. 2008), that implies selecting key secondary trait(s) (i.e., other than the yield or the targeted trait itself), to the remote

inference of whole-plant growth, water status, or even grain yield using remote sensing approaches (Cabrera-Bosquet et al. 2012).

High-throughput phenotyping platforms (HTPP) allow detailed measurements of plant characteristics to be captured to provide reliable estimates of trait phenotypes. These platforms are also useful in modeling (especially taking into account "hidden variables") for predicting genotypic performance in different climate scenarios (under controlled experimental conditions). In recent years, there has been an increasing interest in establishing HTPPs not only by the major private sector institutions, which have been pioneering such endeavors, but also by some of the public research institutions worldwide. This is the case, for example, of "The Australian Plant Phenomics Facility" which includes, up to now, the "High Resolution Plant Phenomics Centre," placed in Canberra, and "The Plant Accelerator" at the University of Adelaide (http://www.plantphenomics.org.au) (Finkel 2009). There are also companies that are actively engaged in developing such facilities (e.g., LemnaTec, http://www.lemnatec.com) at both hardware and software levels for plant phenomics and high-throughput phenotyping (Prasanna et al. 2012a).

There is also an increasing interest to develop relatively low-cost, field phenotyping platforms to overcome the limitation of greenhouse or growth chamber phenotyping (Masuka et al. 2012). Advances are being made in the ability to measure spectral reflectance, plant temperature and architecture at the canopy level throughout the crop life cycle (White et al. 2012). Measurements are made using field platforms with remote sensing cameras in aerial platforms such as balloons, zeppelins or remote-controlled airplanes or "polycopters" (White et al. 2012), or using light curtains and spectral reflectance sensors mounted on a tractor for evaluating crop performance under field conditions (Montes et al. 2011). In addition to the above, improving throughput and precision of field-based phenotyping, using low-cost, easy-to-handle tools, is an area of research that is being intensively pursued, since many of the national agricultural research systems (NARS) and small- and medium-sized seed companies from developing countries cannot afford to establish and maintain expensive high-throughput phenotyping platforms. Intensive efforts are underway through the CIMMYT Global Maize Program to characterize field variability at the key phenotyping sites worldwide, and to improve field-based phenotyping of maize germplasm. This includes approaches like nondestructive estimation of biomass using normalized differential vegetation index (NDVI), monitoring soil moisture using neutron probes/TDR, chlorophyll content using a SPAD meter, canopy behavior using Infrared thermography, etc. (Prasanna et al. 2012a).

Research in developing improved phenotyping technologies and practices for field-based research has tended to focus on the development of new high-throughput tools with little emphasis placed on reducing unwanted environmental effects. Breeding progress relies on genetic variability for the trait of interest, high selection intensity, high broad sense heritability (H) for the trait of interest, and the genetic correlation between yield in the selection environment and the TPE (Falconer and Mackay 1996). Broad sense heritability is related to the proportion of the phenotypic variation that can be attributed to genetic factors. To increase the

efficiency of new high-throughput phenotyping tools greater emphasis should be placed on increasing the genetic "signal."

Recent studies by CIMMYT highlighted the need to reduce plot residual variances under managed drought stress. Combined analysis of more than 700 CIMMYT and national partners' regional maize trials in Southern Africa showed heritability to lower under managed drought stress relative to optimal, low N, and random abiotic stress conditions with plot residual error over threefold higher than genotypic variation (Weber et al. 2012). Similarly, Cairns et al. (2012b) found plot residual variation to be two to three times larger than genotypic variance for grain yield under managed drought stress. These studies highlight the need for measures to reduce the effects of field variability to increase the genetic signal-to-noise ratio to detect real differences between lines. Reducing the size of the residual relative to the genetic component of variance would have a positive impact on heritability levels and expected genetic gains. The most common causes of field variability are soil variability and agronomic management practices (Blum 2011). Soil heterogeneity can represent a significant source of experimental variation and obstruct the detection of the genetic signal (Campos et al. 2011). Measures of soil variability can be incorporated into statistical analysis to reduce experimental error (Hao et al. 2010; Cairns et al. 2011; Prasanna et al. 2012a) (Fig. 5.1). However, the value of these measures still needs be fully established in reducing the error variance relative to the genetic variance (Masuka et al. 2012).

5.3.3 Doubled Haploid Technology

Doubled haploid (DH) technology is now a powerful tool to accelerate the introgression of novel germplasm into elite breeding lines (Forster et al. 2007; Geiger and Gordillo 2009; Prasanna et al. 2012b). A DH plant is formed when haploid cells undergo chromosomal doubling, either spontaneously or by chemical treatment, allowing the production of a homozygous line after a single round of recombination. Published protocols for DH line development are now available for several plant species (Wedzony et al. 2008), including cereals, although the efficiency of producing DH lines varies widely. Doubled haploidy enhances "forward breeding" and provides an opportunity to have an earlier look at the potential of new lines, providing greater knowledge about their environmental adaptability before they are fully tested and further used as parental lines for hybrid development and commercial cultivation. By reducing the time taken to reach homozygosity in conventional breeding technology from approximately seven seasons to two seasons, maternal haploidy-based DH technology in maize offers a great opportunity to increase the efficiency of line development (Chang and Coe 2009; Geiger and Gordillo 2009; Prasanna et al. 2012b). Use of DH technology can potentially enhance the efficiency of recurrent selection- or genomic selection (GS)-based schemes for traits with low heritability, particularly for breeding programs without access to off-season nurseries (Bouchez and Gallais 2000). Furthermore, DH technology

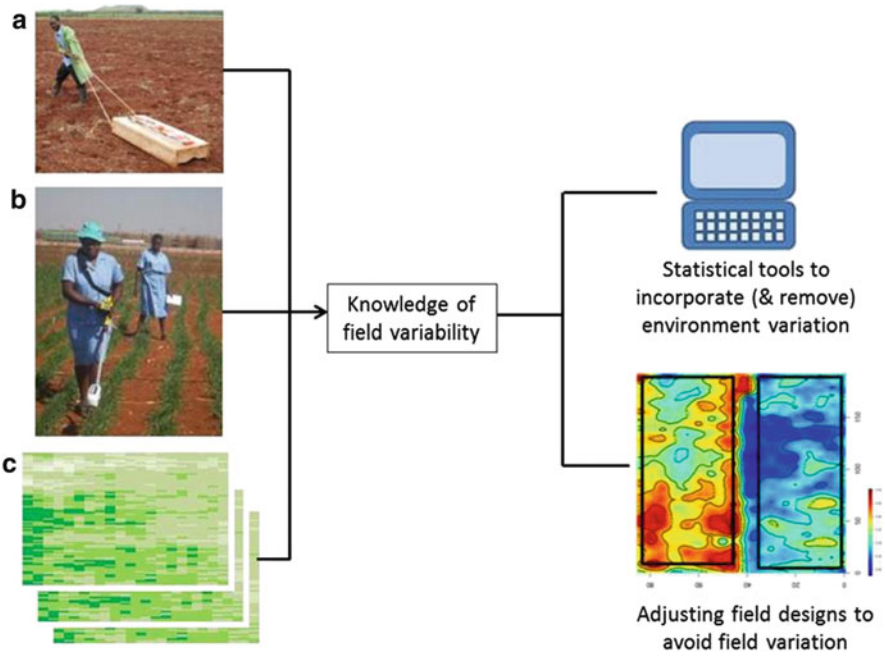

Fig. 5.1 Schematic diagram illustrating the use of (**a**) soil conductivity sensors, (**b**) NDVI, and (**c**) yield mapping, to characterize field variability. This information can be incorporated into statistical designs and/or into field design to avoid placing trials in areas of high spatial variability

enables shifting of resources away from the labor-intensive task of repeated inbreeding to generate inbred lines, allowing more time to be spent on evaluation of the DH populations (Fig. 5.2) for stress resilience and agronomically important traits using a network of phenotyping/breeding sites (Fig. 5.3), and using the selected lines to derive elite hybrids and synthetics.

In maize, the efficiency of spontaneous chromosome doubling was too low for application within breeding programs. Within the last 10–15 years, through in vivo haploid induction using haploid inducers, the DH technology has been adapted by commercial maize breeding programs in Europe (Schmidt 2003), North America (Seitz 2005), and more recently in China (Chen et al. 2009). An estimated 80% of commercial companies now employ DH technology within their temperate maize breeding programs (Phillips 2009). A number of temperate haploid inducer lines with high haploid induction rate (HIR) for commercial use have been derived over the years (Geiger and Gordillo 2009). However, lack of tropically adapted haploid inducer lines previously impeded the application of DH technology in tropical maize breeding programs (Prigge et al. 2012a, b). Since 2007, the CIMMYT Global Maize Program has been intensively engaged with optimization of the DH technology especially for the tropical/subtropical maize growing environments, in partnership with University of Hohenheim, Germany (Prasanna et al. 2012b).

Fig. 5.2 A DH population derived from a CIMMYT hybrid (La Posta Sequia C7-F180-3-1-1-1-BBB/CML449). This DH population is one among several populations being phenotyped at several locations worldwide by the CIMMYT Maize Program for various traits, including drought, heat, and low N tolerance

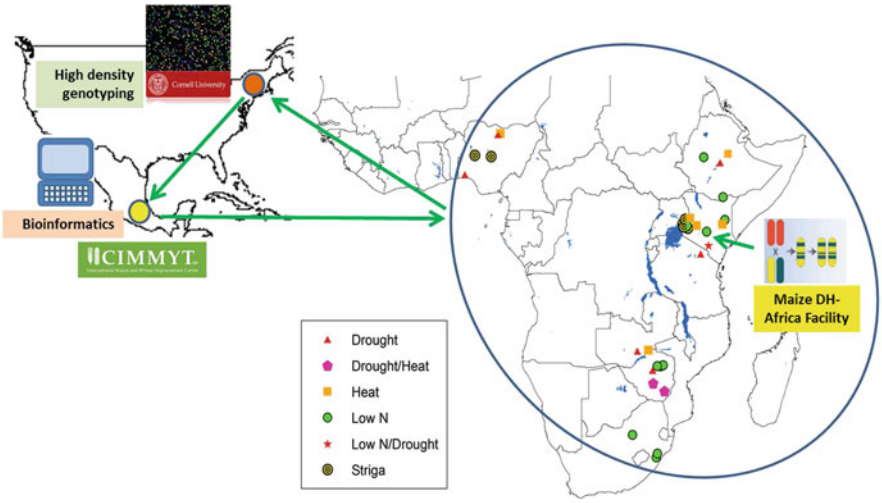

Fig. 5.3 Abiotic stress phenotyping and breeding network for maize established in eastern and southern Africa by CIMMYT, and in west Africa by IITA, with strong linkages for high-density genotyping and bioinformatics support. Similar networks are established by CIMMYT in Latin America and Asia

Tropically adapted inducer lines (TAILS) with a HIR of up to 10% have now been developed through CIMMYT-University of Hohenheim collaboration (Prigge et al. 2011). Experimental evaluation of the first-generation TAILs in two environments (Agua Fria and Tlatizapan in Mexico) over two seasons consistently resulted in average HIR ranging from 9% to 14%. A single-cross hybrid haploid inducer (with high HIR) has been developed using a sub-set of TAILs. The tropicalized haploid inducers are now available for sharing with interested institutions for research or commercial use under specific terms and conditions (http://www.cimmyt.org/en/about-us/media-resources/recent-news/1399-now-available-tropicalized-maize-haploid-inducer-lines). The availability of TAILs is expected to significantly enhance the efficiency of DH line production, increasing

seed set and rates of induction, and reducing the costs of inducer line maintenance and seed production (Prasanna et al. 2012b).

Because DH technology offers a faster way to obtain completely homozygous lines, it can save significant time and resources for implementing genetic studies and/or molecular breeding projects, including:

1. Developing genetic maps (Forster et al. 2007; Chang and Coe 2009), which is one of the widespread applications of DH populations in many crops, including maize.
2. Identification of marker-trait associations using relevant DH populations (with parents of source populations showing significant phenotypic contrast), further leading to potential use of markers in MAS.
3. High-density genotyping of the DH lines for selection of parental lines with complementary genotypes (or haplotypes) in generating hybrids for further testing.
4. Combining seed-chipping technology in MAS of DH lines for simply inherited traits (e.g., provitamin-A enrichment) using reliable markers for favorable genes/alleles with high contribution to phenotypic variation; this could be cheaper, faster, and more effective than phenotyping the DH lines.
5. DH lines could be most useful in implementing genome-wide selection (or GS; Meuwissen et al. 2001; Jannink et al. 2010; Babu et al. 2012) for improving complex polygenic traits with low heritability (e.g., grain yield, abiotic stress tolerance), and when N (population size) is small (Bernardo and Yu 2007; Lorenzana and Bernardo 2009; Mayor and Bernardo 2009).
6. DH and MAS can complement each other for deriving DH lines from biparental crosses when the objective is to obtain lines genetically similar to either parent of the cross (Smith et al. 2008) or to identify recombinants at or flanking specific loci. The most frequent application of this approach would likely be the use of DH line conversion protocols instead of slower conventional backcrosses (Forster et al. 2007).
7. MAS for target traits can be used to eliminate most of the DH lines generated through preplanting selection using molecular markers to avoid handling too many DH lines. Commercial large-scale seed companies typically eliminate over 90% of the DH lines before large-scale field testing.

5.3.4 Marker-Assisted and Genomic Selection-Based Breeding Strategies

5.3.4.1 Marker-Trait Association Analysis

Marker-trait association analysis or trait mapping can be generally defined as identifying genes/alleles/genomic regions that are significantly associated with specific traits. The association can be established in several ways. Two important

approaches are (a) linkage analysis using biparental or multiparental populations; and (b) LD analysis or association mapping using natural populations. There are several articles and reviews on this topic covering a range of crop plants, including cereals (Prasanna et al. 2010; Xu 2010; Xu et al. 2012b). For example, Roy et al. (2011) reviewed the QTL that have been identified in numerous wheat, barley, and rice populations for abiotic stress tolerance (drought, cold, heat, mineral toxicity, salinity, and nutrient deficiencies).

LD or association mapping is now being increasingly used in cereal crops as a mapping strategy, as it offers several advantages compared to linkage mapping, including the time and resources saved from generating segregating or immortal mapping populations, presence of multiple alleles in the population, and higher resolution than linkage mapping. However, there are several factors that could result in false-positives in association detection, of which the most important is the population structure that can be removed through some statistical approaches (Yu et al. 2008). Another constraint is that traits controlled by the genes with rare alleles cannot be mapped effectively and in some cases, novel alleles we are looking for do not exist in the population at all, which can only be mapped using biparental populations with the target allele segregating (Xu et al. 2012b).

To effectively combine the advantages offered by both linkage and LD mapping approaches, a joint linkage-LD mapping strategy has been proposed (Lu et al. 2010). The joint mapping can be done through parallel mapping, which runs linkage and LD mapping using biparental and natural populations separately, or integrated mapping using a single mapping procedure combining the information from both biparental and natural populations. The first joint mapping has been reported in maize using both parallel and integrated mapping approaches (Lu et al. 2010), which involved using three RIL populations and one natural population with 305 inbred lines, genotyped by 2,053 SNP markers. Joint mapping for anthesis-silking interval, an important secondary trait for drought tolerance, identified 18 additional QTL that could not be identified by linkage and LD mapping alone.

Several crop plants, including rice, maize, and sorghum, have been sequenced de novo, and many more, including wheat, are expected to follow; thus, millions of SNPs can be potentially developed for each crop. High-density genetic maps or sequence-based genotyping has significantly improved linkage mapping using biparental populations (e.g., Wang et al. 2011b). High-density SNP data enables GWAS to test all the genes in the genome for their association with target traits. In maize, the NAM population has been used for analysis of leaf architecture (Tian et al. 2011) and quantitative resistance to southern corn leaf blight (Kump et al. 2011). Thus, the next-generation genome sequencing technology, in combination with the GWAS strategy, offer powerful tools for dissecting complex traits.

The first HapMap in maize consists of 3.3 million SNPs discovered using 27 diverse maize inbred lines, with 1 in every 44 bp polymorphic (Gore et al. 2009). Through an international collaboration, over 55 million SNPs were discovered recently, which have been used to develop the second-generation HapMap (Chia et al. 2012). Haplotype-based mapping can now be used to replace individual marker-based mapping to improve mapping power and identify specific alleles

within a gene or allele combinations at different loci that contribute to the same target trait, depending on how a haplotype is constructed (Chia et al. 2012; Xu et al. 2012b). Thus, the new genomic technologies/strategies have the potential to accelerate the detection and cloning of QTL, which enable pyramiding or accumulation of favorable QTL alleles, allowing desired changes in agronomically and nutritionally important traits to be made.

As more and more information on QTLs for a range of important traits in crop plants has become publicly available, approaches for integrated analysis of all such data have received greater attention recently. Two basic strategies of exploiting the currently available information are "meta-analysis" and "in silico QTL analysis," which have been described in detail elsewhere (Xu 2010; Xu et al. 2012b). The first example in maize for meta-analysis is for ADL/NDF-related traits detected in silage maize (Barrière et al. 2008). In another example in maize, QTL meta-analysis has been done for 59 QTLs for traits associated with digestibility and 150 QTL for traits associated with cell wall composition from 11 different mapping experiments; a total of 26 and 42 meta-QTLs were identified for digestibility and cell wall composition traits, respectively (Truntzler et al. 2010).

5.3.4.2 Identifying and Validating Breeder-Ready Markers

The purpose of establishing marker-trait association is to utilize the information in MAS-based breeding programs. However, since the recombination between markers and a specific gene/QTL influencing a target trait is proportional to the power of MAS for major gene-controlled traits, development of genic and functional markers becomes increasingly important. Marker-trait association established for major genes/QTLs using one specific population needs to be validated before it can be used for MAS with target populations. There are some excellent examples for marker validation. For instance, in barley, microsatellite/simple sequence repeat (SSR) markers linked to net-form net blotch resistance QTL (*QRpt6*) and spot-form net blotch resistance QTL (*QRpts4*) were validated in two populations unrelated to the original mapping population. The lines homozygous for the resistant-parent alleles at both marker loci in two other populations had significantly lower infection than lines homozygous for the susceptible-parent alleles at both markers (Grewal et al. 2010).

In maize, a major QTL conditioning resistance to sorghum downy mildew was identified (George et al. 2003) and validated (Nair et al. 2005) in different mapping populations using SSR markers. Another example is the Maize Streak Virus (MSV), a major disease that affects maize productivity in several countries in sub-Saharan Africa. The CIMMYT Global Maize Program has recently fine-mapped and identified SNP markers for a major QTL for MSV resistance, and has validated these markers on a set of DH lines that have been phenotyped for responses to MSV in sub-Saharan Africa (Sudha Nair, personal communication).

Completion of de novo sequencing has facilitated map-based gene cloning with many genes cloned, particularly in rice (Qiu et al. 2011; Miura et al. 2012). Cloned

rice genes include grain number, grain size and weight, heading date, disease resistance, salt tolerance, cold tolerance, submergence tolerance, and yield-related domestication genes (Miura et al. 2012), mainly using the diversity available in the related species. Another gene cloning strategy is based on comparative genomics. With complete reference genome sequences available for many species that are closely related to each other, the comparative genomics approach could become increasingly important for gene cloning and marker development based on sequence homology (Xu et al. 2012b).

Large-scale resequencing of germplasm accessions has resulted in the discovery of many alleles for specific genic loci in cereals, and was followed by functional analysis. These validated alleles can be used to develop functional or breeder-ready markers. Some excellent examples can be found in rice for photoperiod sensitivity, gain size, etc. For example, in rice, the C to A mutation in the second exon of GS3 was reported to be functionally associated with enhanced grain length. Besides the C–A mutation, three novel polymorphic sites, SR17, RGS1, and RGS2, were discovered in the second intron, the last intron, and the final exon of GS3, respectively. The genic marker RGS1 based on the motifs (AT)n was further validated as a functional marker using two sets of backcross RILs (Wang et al. 2011a).

In maize, sequence-tagged, PCR-based markers were developed and demonstrated for use in selecting favorable alleles of *LCYE* (*lycopene epsilon cyclase*), a crucial gene in the carotenoid pathway. Markers for favorable alleles of *LCYE* (Harjes et al. 2008) and for another critical gene in the pathway, *CrtRB1* (*carotene beta-hydroxylase 1*) were developed (Yan et al. 2010b). Allele mining and marker development are also underway for other genes of the carotenoid biosynthetic pathway, including *PSY* (*phytoene synthase*) and *CCD* (*carotenoid cleavage dioxygenase*), offering hope that MAS will soon be possible for several genes which together explain a large proportion of variation for provitamin A in maize (Jianbing Yan, personal communication).

5.3.4.3 Stress Resilient Cereal Varieties Developed Through MAS

An array of breeding products have been released in the last two decades through molecular breeding, including MAS and transgenic approaches, especially by the multinational corporations (reviewed by Xu and Crouch 2008; Xu et al. 2012c), although very limited details are available about the nature, scale, and scope of such operations. MAS-derived varieties and advanced lines combining resistance to biotic and abiotic stresses or improved grain quality have been reported in rice, wheat, maize, barley, and pearl millet (Dwivedi et al. 2007; Prasanna et al. 2010). To date, MAS has been most successful in the selection of resistance to diseases and for improving grain quality. Examples include rice varieties resistant to blast in United States and to bacterial blight in Indonesia and India, and wheat varieties resistant to rust in Canada.

In rice, MAS-derived submergence-tolerant lines have been developed and tested (Xu et al. 2006) for their adaptation to deep-water paddy cultivation in

eastern India and Thailand (Siangliw et al. 2003), and work is in progress to introduce this trait in varieties adapted in Bangladesh, India, Laos, the Philippines, and Vietnam. MAS-derived rice varieties with bacterial blight resistance are already being grown in Indonesia and India. In China, MAS, combined with conventional breeding, has resulted in the development of a group of rice materials and release of varieties with improved disease and insect resistance (Wei et al. 2009; Wang et al. 2009; Li et al. 2010; Xu et al. 2012c).

The first downy-mildew-resistant pearl millet hybrid (Improved HHB-67) released in India was bred by ICRISAT using MAS by improving the male parent with improved resistance to downy mildew (Hash 2005). Breeding products released through MAS have been very much limited from the public sector maize institutions in the developing countries (Prasanna et al. 2010). Major genes/QTLs for resistance to turcicum leaf blight (*Exserohilum turcicum*) and Polysora rust (*Puccinia polysora*) have been pyramided in five elite maize lines in India (Prasanna et al. 2010). In wheat, MAS has already led to the release of two varieties including Lillian (DePauw et al. 2005) possessing the high grain protein content gene (*GPC-B1*) and Goodeve (DePauw et al. 2009) possessing the orange blossom wheat midge resistance gene (*Sm1*).

Although there are only small numbers of reports regarding successful use of MAS in plant breeding, the technology has nevertheless demonstrated its potential as a tool to support conventional genetic enhancement of crops. It is expected that many more successful applications do exist but remain within the confidentiality restriction of commercial breeding companies around the world.

5.3.4.4 Rapid-Cycle Genomic Selection-Based Breeding for Complex Traits

Genomic strategies can contribute significantly to the accelerated development of climate-resilient germplasm, by way of (1) identifying genetic diversity and favorable variation required for climate resilience; (2) identifying traits and genes for tolerance to new and complicated stresses induced by climate change; (3) bringing integrated genomic tools and approaches to manage combining tolerance to abiotic and biotic stresses; and (4) increase genetic gains and breeding efficiency through rapid cycle genome-wide selection or GS in breeding programs.

Traits related to climate change adaptation are complicated and simple MAS strategies may not work well. GS is, therefore, potentially more suitable for improving such complex traits (Meuwissen et al. 2001; Heffner et al. 2010). GS consists of three steps (1) prediction model training and validation, (2) breeding value prediction of single-crosses, and (3) selection based on these predictions. In GS model training, a training population (TP) consisting of germplasm having both phenotypic and genome-wide marker data is used to estimate marker effects. The combination of these marker effect estimates and the marker data of the single crosses are used to calculate genomic estimated breeding values (GEBVs), where a GEBV is the sum of all marker effects included in the model for an individual.

Selection is then imposed on the single crosses using GEBVs as the selection criterion. Thus, GS attempts to capture the total additive genetic variance with genome-wide marker coverage and effect estimates, contrasting with marker-assisted recurrent selection (MARS) strategies that utilize a small number of significant markers for prediction and selection. Markers with effects below the levels of statistical significance are not used in conventional MARS, but used in GS to predict breeding value. This is especially important for quantitative traits conferred by a large number of genes each with a small effect (Rutkoski et al. 2011; Xu et al. 2012b).

Bernardo and Yu (2007) analyzed the prospects of GS for improving quantitative traits in maize and demonstrated that this approach, although more expensive, is superior to MARS for improving complex traits, as GS effectively avoids issues pertaining to the number of QTLs controlling a trait, the distribution of effects of QTL alleles, and epistatic effects due to genetic background. Pilot projects on the implementation of rapid-cycling GS using much higher marker densities are being initiated by CIMMYT on new platforms based on next-generation sequencing technologies. The aim is to make GS-based, open-source, rapid cycle breeding routinely applicable across the CIMMYT and NARS maize breeding programs in sub-Saharan Africa, Latin America, and Asia.

5.3.4.5 Broadening the Genetic Base Through Molecular Prebreeding

For effectively adapting to the changing climate, the genetic base of cultivated crop plants needs to be significantly strengthened. Although the international and national gene banks contain extensive collections, breeders generally confine their programs to a relatively minute portion of elite germplasm available to them. The narrow genetic base of the North American hybrid corn industry has been well-documented (Goodman 2005). Although there are some 250–300 maize races (Goodman and Brown 1988), only one, the Corn Belt Dent, is by far the predominant source of commercial germplasm. Goodman (2005) indicated that the parentage of virtually all commercial US hybrids involves six inbred lines or their close relatives: the Lancaster-type inbreds C103, Mo17, and Oh43, and the Reid-type lines B37, B73, and A632. Similarly, in China, the country with the second largest maize growing area in the world (with ~32 million hectares under maize), the hybrid maize germplasm base has been reported to be quite narrow, with only a few inbred lines having played a central role in hybrid development, such as Mo17, Huangzaosi, 330, E28, Dan340, and 478 (Li 1998; Yu et al. 2007).

A new initiative of CIMMYT, titled "*Seeds of Discovery*" (*SeeD*), funded by the Mexican Government, aims to discover the extent of allelic variation in the genetic resources of maize and wheat, especially in the CIMMYT Gene Bank, through high-density genotyping/resequencing, multilocation phenotyping for prioritized traits, and use of novel bioinformatics tools for discovery, and make available the favorable alleles and haplotypes associated with important traits to the breeders in a usable form (Peter Wenzl, personal communication).

5.3.5 *Breeding Informatics and Decision Support Tools*

The journey from phenotyping and genotyping of individuals from genetic populations to identifying marker-trait associations and finally applying markers in molecular breeding programs depends on a sequential use of a number of decision support tools that facilitate communication and collaboration between molecular biologists, geneticists, bioinformaticians, trait specialists, and breeders towards effective interdisciplinary decision making (Fig. 5.4). Ultimately, molecular breeding programs need to effectively combine MAS with a diverse range of technology-assisted interventions including whole genome scans, advanced biometrical analyses and quantitative genetic modeling that will require increasingly complex facilitating software. Decision support tools that need to support molecular breeding programs, include germplasm evaluation, breeding information management, analysis of genotype-by-environment interaction (GEI), genetic map construction, marker-trait linkage and association analysis, MAS, breeding system design and simulation, etc. (Xu et al. 2012b).

A careful balance of many diverse elements is required to implement a decision support system that provides an optimal combination of time, cost, and genetic gain (Xu et al. 2012b). Such a system would need to include (1) managing and analyzing large amounts of genotype, pedigree, phenotype, and environment data; (2) selecting desirable recombinants through an optimum combination (in time and space) of phenotypic and genotypic information; and (3) developing breeding systems that minimize population sizes, number of generations, and overall costs while maximizing genetic gain for traditional and novel target traits (Xu et al. 2012b).

5.4 Crop Modeling and Forecasting Systems for Complementing Breeding Efforts

Researchers recognize the importance of climatic and genetic causes of genotype-by-environment interaction (G × E), and have been trying statistical models for understanding, overcoming and exploiting G × E, and for deploying germplasm and management practices for specified environments. This has been practiced through multienvironment trials, which include phenotypic measurements of cultivars across environments. However, climate change has greatly increased environmental variability making representation of field variability through experimentation difficult and costly. Moreover, cereal production worldwide is increasingly influenced not only by climate change but also by growing competition for resources, production variability, and dynamic socioeconomic factors, which are rapidly shifting the structure, composition, and distribution of the agricultural production in general and the farming systems that support the livelihoods of smallholder producers and the rural poor in particular. Increasing population

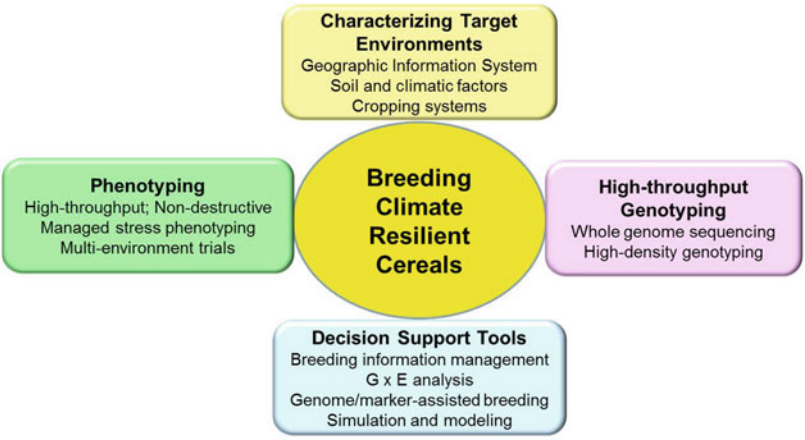

Fig. 5.4 Enhancing the efficiency of breeding climate-resilient cereals requires convergence of high-throughput phenotyping and genotyping, proper characterization of target environments, and appropriate decision support tools

pressure and depletion of natural resources coupled with climate variability and change put agricultural production under risky and uncertain conditions.

Given the above challenges, there is an increasing need to incorporate strategic foresight into decision making in cereal production systems. Joint interdisciplinary ventures to develop new knowledge, tools, and technology are necessary to address complex biophysical and socio-economic problems in a holistic and cost-efficient way. Dynamic crop models are excellent platforms to bring interdisciplinary teams together and create the holistic approach required. They have been successfully applied in plant breeding (e.g., Wang 2011), climate change analysis (White et al. 2012), as well as system analysis, decision support, and policy analysis (Robertson and Carberry 2010). For example, crop models have shown excellent potential for evaluating genetic improvement, and for proposing plant ideotypes for target environments (Boote et al. 2001). They have been used in several aspects of plant breeding including identification of main yield-determining traits, defining optimum selection environments, designing ideotypes that consists of several traits, assisting multilocation testing, and explaining G $\times$ E interactions (Yin et al. 2003). Yin et al. (2003) also proposed an approach that integrates MAS into a model-based ideotype framework to support breeding for high crop yield based on the complementary aspects of crop modeling and QTL mapping. Simulation models can also readily test crop management options and thus several researchers have examined the potential for technological adaptations to climate change (White et al. 2012).

Crop models also have a potential to be used in forecasting, monitoring, and managing crop diseases and pests under climate change. It is expected that rising temperatures and variations in humidity will likely affect the diversity and responsiveness of pests and diseases, and perhaps even create new and unpredictable epidemiologies. This warrants better understanding of pests and pathogen

behavior under different scenarios of climate change using dynamic crop models, which allow for the evaluation of changes in cropping systems and production practices caused by climate change. Moreover, these tools help identify management options for emerging insect pests and diseases at local, regional, and global levels. In summary, besides developing climate-resilient cultivars, greater emphasis also needs to be placed on modeling and forecasting systems.

CIMMYT is currently collaborating with IFPRI (International Food Policy Research Institute) and other CGIAR (Consultative Group on International Agricultural Research) centers to identify promising technologies and to develop strategic foresight and policies for agriculture, particularly in maize and wheat production systems. For example, CGIAR centers, universities, and other partners are working together in the Global Futures for Agriculture Project to estimate the impact of climate change on global food security and to identify promising technologies that could be used to enhance food security under changing climate through a combined use of biophysical and economic models. Besides the activities that are targeted towards calibration and evaluation of crop models for ex-ante and impact analysis in the short and medium term, a long-term strategy is also envisaged to use models in the physiology, breeding, and genetics of maize research to develop varieties that are better adaptable to climate change, drought and heat stresses, besides other biotic, abiotic, and socioeconomic challenges.

5.5 Conclusions and Future Perspective

There is still a huge gap between production and demand of cereals in many regions of the world despite several technological advances in the last few decades. Developing and deploying water- and nutrient-efficient technologies is utmost important to effectively counter the negative effects of increasing human population and associated natural resource degradation, especially in the developing world. While plant breeding has been successful in continuously improving complex traits like drought, future advances in breeding for tolerance to combinations of stresses (e.g., combined drought and heat or abiotic and biotic stresses) will only be possible by combining genetic analyses, high-throughput genotyping and phenotyping (preferably in the field) together with prediction models and molecular breeding (Araus et al. 2008, 2011; Collins et al. 2008; Tardieu and Tuberosa 2010).

An important component of such a strategy should be to strengthen the phenotyping capacity for key abiotic and biotic stresses, especially of the national agricultural research institutions. In addition, measures to facilitate smallholder farmers' access to the improved germplasm will be fundamental in the race to buffer resource-poor farmers from the impacts of climate change, and would have to involve synergistic efforts from both private and public sector institutions.

There is also an immediate need to develop and deploy climate change-resilient maize germplasm vis-a-vis accumulating evidence of the possible adverse effects of extreme climatic events on crop production in the developing world and

identification of climate change vulnerable regions. Whilst some public institutions in the developed countries and the multinational corporations routinely employ MAS approaches to accelerate development of cultivars with resilience to abiotic and biotic stresses, many public institutions in the developing countries are still testing marker applications and taking initial steps towards adopting marker-assisted breeding in a limited number of crops like rice, wheat, and maize (Gupta et al. 2010; Kumar et al. 2010; Li et al. 2010; Prasanna et al. 2010). Various bottlenecks still impede effective adoption of latest approaches of marker-assisted breeding in these countries (Xu and Crouch 2008; Ribaut et al. 2010). Limited human resources and inadequate field infrastructure remain major challenges, although through virtual platforms aided by the information and communication technology revolution breeders now have better access to genomic resources, advanced laboratory services, and robust analytical and data management tools.

The use of higher spatial resolution modeling is essential for the identification of high-priority geographic areas for development and deployment of improved germplasm suited to future climates. Temperature thresholds for current cereal varieties and the interaction of heat stress with other components of climate change (especially drought) must also be considered. The application of biophysical and economic models in crop improvement, decision support and foresight requires implementation of harmonized procedures for data acquisition, incorporating diverse and actual datasets (species and cultivar-specific data, climatic data, soil data, important macro- and micronutrients, pests/pathogens data, crop management practices, genomic data on genes/QTLs/markers for specific traits, and socioeconomic data) in a meaningful way for reliable predictions and practical utility. This cannot be achieved without devoting significant human and financial resources to the generation of quality data on the aspects highlighted above for making crop models of significant value to the scientific community and policy makers.

The key challenges to the international scientific community are (a) to generate high-quality phenotypic data in breeding programs, and integrate the same with modern tools and technologies, including high-density genotyping data, doubled haploidy and decision support tools, for accelerated development of climate-resilient germplasm; (b) to better understand the effects of climate change on diversity of cropping systems in different regions; and (c) to effectively monitor the patterns of change both temporally and spatially (= metapopulation dynamics), coupled with appropriate policies and actions at the farm level.

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Chapter 6
Emerging Concepts and Strategies for Genomics and Breeding

Akshay Talukdar and Pranab Talukdar

Abstract To feed the ever-increasing population on earth, production of food crops must increase at an unprecedented pace with limited inputs and little or no harm to the environment. The target is more challenging in the face of a changing climate scenario. The main focus should be on developing technologies and crop genotypes suitable for the input-poor and low-yielding areas that represent the lion's share of the cultivable areas of the world. Plant breeding is evolving; more so with the advancement of molecular biological sciences. Emerging concepts of structural and functional genomics, transcriptomics, proteomics, and metabolomics approaches contribute towards identification of target genes and eQTL (expression-quantitative trait loci) for effective deployment. The genome editing technologies creating site-directed mutation can facilitate development of nontransgenic designer crop genotypes having wider adaptation. Plant breeding approaches should focus on the natural resources to identify and deploy useful gene(s)/alleles to develop genotypes with enhanced yield potential, better stress tolerance and, quality end products. Improvement in mapping efforts like genomic selection (GS), marker-assisted recurrent selection (MARS), and next-generation mapping, viz. NAM (nested association mapping), MAGIC (multiparent advanced generation intercross) would accelerate breeding progress with improved genotyping and phenotyping facilities. Plant biotechnology and genetic engineering techniques would continue to play a pivotal role in making a crop widely adaptable to the changed climate. Improving crop efficiencies in utilization of solar radiation, inorganic nitrogen, water and other inputs would render crops suitable for

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climate-resilient agriculture. Policies should be in place to make technologies accessible and affordable by all sections of users globally.

6.1 Introduction

Crop yield is a complex trait; it depends not only on genetic factors but to a larger extent on the nongenetic factors as well. Therefore, manipulating the yield potential of crops to meet the ever-increasing demand for food is a big challenge. Given current agricultural growth and crop production, it is quite unlikely that the target of feeding 9.1 billion people on the Earth by 2050 will be met easily. It has been predicted that the current production has to be increased by 70 % to meet the target (FAO 2011). However, the approaches adopted to achieve this goal have to be quite different from those used in the past; it cannot be as harsh to the environment as previous approaches. Therefore, the strategies should be to maximize crop production with less inputs and energies, and with the least or no harm to the environment; this is where science in general and, plant breeding, genetics and genomics in particular have a pivotal role to play.

As crop yield is highly influenced by the environment, the focus should be on beating the environment where the crop is grown. Typically, a crop variety with higher yield potential may not grow well and produce satisfactorily if the required inputs and growing conditions are not met. A larger part of the global crop-producing areas are of the poor yielding or low-input type. Specific approaches and strategies are needed to enhance yield in such environments. Crop varieties designed especially for such situations should therefore be the focus of the climate-resilient crop breeding program in the future.

Reduction of yield loss is indirectly yield gain. More attention is needed in tackling yield-reducing factors such as drought, salinity, heat, flood, frost, etc., whose intensities are predicted to be greater in the changing climate scenario. Plant breeding can play an important role in reducing yield loss by developing varieties suited to local stress conditions and by making them more resilient to biotic (e.g., insects, diseases, and viruses) and abiotic challenges (e.g., droughts and floods). Genes conferring increased drought tolerance may also have a widespread impact on yield (Nelson et al. 2007). Studies estimated that the global yield loss due to biotic stresses averages over 23 % of the estimated attainable yield across major cereals (FAOSTAT 2009). Yield stability is the other factor of importance, which in fact is a difficult breeding target. However, there is evidence for genetic control of stability making it an achievable target (Kraakman et al. 2004). Similarly, transgenic approaches are also likely to improve yield stability (Nelson et al. 2007). A single gene was able to substantially increase yield in many crops including rice and wheat leading to the Green Revolution. In parallel with the increase in yield, sustaining it in the changing climatic scenario is another important issue that needs greater emphasis so as to adopt appropriate strategies for development of suitable technologies.

Plant breeding has been evolving; it has transitioned from a skillful "art" to a technology-dependent "science." Infusion of newer scientific technologies has made plant breeding more precise, productive, and predictable. It has become possible to identify the required plants from segregating progenies even at the seedling stage, representing huge savings in time, energy, and costs. Incorporation of genomics and reverse genetics approaches in plant breeding has shown hope of increasing and sustaining crop production even in changing climatic situations. However, maintaining resistance to rapidly evolving pests and pathogens is going to be an essential mainstay of any breeding program. Interactions between breeders, pathologists, and agronomists must be maintained to ensure that crops and cropping systems change coordinately (Tester and Langridge 2010).

Problems on the agricultural front are emerging with every passing day; challenges are presenting themselves constantly. Newer and more effective strategies and approaches are being used to tackle these problems and challenges. In the field of genetics, genomics, and plant breeding numerous strategies and approaches are being tested regularly. However, only a few are retained over time. In this chapter, emerging strategies and approaches in the field of genomics and plant breeding are discussed in the light of increasing crop production under changing climatic conditions.

6.2 Requirement for New Approaches and Strategies in Genomics and Plant Breeding

Agriculture with intense support from science, technology, and regulatory policies has made substantial progress in terms of producing more food worldwide. Global food grain production has increased from 850 million tons (mt) in 1960 to 2,350 mt in 2007 (Godfray et al. 2010). It has saved millions of people from starvation and untimely death globally, in the developing countries in particular. However, the situation now is not rosy, either; it is intensifying and worsening with the passing of time. Further, the challenges faced today are different in terms of their nature, extent, and attributes. The pressure for enhanced food production is enormous owing to the ever-growing population. About 1.0 billion people were added in a span of 12 years from 1999 to 2011 raising the global population from 6.0 to 7.0 billion (Table 6.1). It is predicted that by 2050, there will be 9.1 billion people on earth. To feed such a huge population, our current productivity has to be increased by about 70 % by this time. However, the global cultivated area of over 1.5 billion ha, which represent some 12 % of the world's land surface, is under tremendous pressure due to rapid urbanization, industrialization, and other nonagricultural uses. As per FAO's estimate, by 2050, 70 % of the global population will be urban. So, more food has to be produced from limited areas. Secondly, irrigation is crucial for crop production. Agriculture is responsible for about 70 % of all fresh water withdrawn for human uses. Water is becoming a scarcer commodity everyday

Table 6.1 Growth of world's population

Year	Population[a]	Time span (years)
1	200 mn	
1650	500 mn	1,649
1804	1.0 bn	154
1927	2.0 bn	123
1960	3.0 bn	33
1975	4.0 bn	15
1987	5.0 bn	12
1999	6.0 bn	12
2011	7.0 bn	12
2027[b]	8.0 bn	16
2050[b]	9.1 bn	23

Source: FAO (2011)
[a]*mn* million, *bn* billion
[b]Projected

leaving less water to produce more food. In large areas in India and China, ground water levels are falling by 1–3 m per year. The quality of produce is becoming a priority among consumers. Energy is becoming costlier leaving less scope not to be efficient. On top of it all, changes in climatic conditions have started showing an impact on crop production through untimely flood, heat waves, and prolonged drought (Ciais et al. 2005; Lobell et al. 2008; Ortiz et al. 2008; Peltonen-Sainio et al. 2010). So, more food has to be produced from a limited area, with less water, minimum energy, and least harm to the environment; a daunting task indeed.

Food security for the growing population is a matter of concern globally. However, the less developed countries are predicted to be particularly vulnerable in terms of food security. The reasons are (1) most of these countries are net importers of cereals (Dixon et al. 2009); (2) many of their national agricultural research services lack sufficient capacity for timely delivery of agricultural technologies (Kosina et al. 2007); and (3) the majority are located in regions that are vulnerable to climate change (Lobell et al. 2008). The net impact of a food crisis would be enormous and would certainly put humanity in threat. It demands preparedness in order to lessen the impact, if one cannot prevent it. The science of genetics, genomics, and plant breeding has to be advanced and utilized fully to meet this challenge.

6.3 Genomic Approach

Genomics can be defined as the generation of information about living things by systematic approaches that can be performed on an industrial scale (Brent 2000). The "information" means the genetic message coded in the DNA sequences, the genome of an organism. Such information is revealed through DNA sequencing approaches, which Weinstein (1998) referred to as "omics." With specialized

objectives and purpose fulfilled, the genomics approaches may be classified as structural, functional, and comparative genomics. Currently, more specialized branches such as proteomics, transcriptomics, and metabolomics have also come into being. All these classes of genomics provide information about genome organization, linkage, protein complement, gene regulation as well as phylogeny and evolutions.

6.3.1 Structural Genomics

One of the specialized branches of genomics is structural genomics, which focuses mainly on the physical aspects of the genome through the construction and comparison of gene maps and sequences, as well as gene discovery, localization, and characterization. With the availability of more and more genome sequences that have been sequenced at a speed never thought of before, it has become imperative to determine structure and assign function to the different genes inscribed in the sequences, a challenge to the scientific community, indeed. However, with advances in the tools of structural biology, the challenge has largely been met through large-scale determination of three-dimensional structural models for all known proteins, protein families or protein domains from which most others can be predicted computationally with a reasonable degree of accuracy. It is believed that the benefit of structural genomics will be much higher than envisaged. The most prominent benefit will be to establish the relationship between one-dimensional sequence information and three-dimensional structure of the protein. Structural genomics may well provide the means of coming to grips with this important intellectual challenge (Burley et al. 1999).

The major goal of structural genomics is to provide a structural template for a large fraction of protein domains. With various approaches, structures of a section of the protein families have been predicted. Approximately 20 % of the known families with three or more members currently have a representative structure. As per estimates, the number of apparent protein families will be considerably larger than previously thought. However, the vast majority of these families will be small, and it will be possible to obtain structural templates for 70–80 % of protein domains with an achievable number of representative structures, by systematically sampling the larger families (Yan and Moult 2005). So, translating the outcome of structural genomics into a practical product that can be applied for human welfare will take some time.

6.3.2 Functional Genomics

The branch of genomics that deals with revealing the function of genes and other parts of the genome is known as functional genomics. It is the emerging field of

molecular biology that is attempting to make use of the vast wealth of data produced by genome sequencing projects to describe genome function in a meaningful way. It primarily uses high-throughput techniques like DNA microarrays, transcriptomics, proteomics, metabolomics, and mutation analysis to describe the function and interactions of genes. The functional genomics approaches are more powerful and robust than the conventional genetic approaches in assessing phenotype largely with respect to the scale and automation involved in the investigation.

Microarray technology, which involves hybridization of unknown samples with a GeneChip containing DNA/RNA probes, is of great use in finding gene/protein function. The use of microarrays for expression profiling was first published in 1995 (Schena et al. 1995) and the first complete eukaryotic genome (*Saccharomyces cerevisiae*) on a microarray was published in 1997 (Lashkari et al. 1997). Plants under biotic and abiotic stresses can be subjected to characterization through microarray analysis. The expression profiles thus generated can provide extensive data for identification of genes involved in the resistance-susceptibility reaction. A first-generation maize GeneChip containing 1,500 ESTs/gene could identify 117 genes that were either induced or repressed 6 h after inoculation with the fungus *Cochliobolus carbonum* (Baldwin et al. 1999). The function of the genes thus identified has to be confirmed on an individual gene basis. A powerful reverse genetics approach like gene-silencing, TILLING, Eco-TILLING, or any other gene knock-out technique should help resolve this issue with better resolution (Marteinssen 1998; Baulcombe 1999).

The variety of methods available for global analysis of protein profiles and cataloging protein–protein interactions on a genome-wide scale are technically complex and demanding. However, newer and improved technology and algorithms for collecting, displaying, and analyzing the vast amounts of quantitative expression data are being developed (Eisen 1998). Such information is yet to be used in plants extensively. However, a lot has been achieved in regard to plant-resistance genes (Michelmore 2000).

6.3.3 Comparative Genomics

Comparative genomics is the analysis and comparison of genetic materials from different taxa including species, subspecies and even genera to study evolution, gene function, and other important traits. The purpose is to gain a better understanding of how species have evolved and to determine the function of genes and noncoding regions of the genome. It also provides insight into the uniqueness of and homology between different taxa. Here, comparison of the sequences are made for gene location, gene structure (with respect to exon number, exon length, intron length sequence similarity), and gene characteristics (e.g., splice sites, codon usage, conserved synteny). It involves the use of computer programs that can line up multiple genomes and look for regions of similarity among them. In recent time, a number of species including crop plants have been fully sequenced and, the

sequences are available in the public domain. Such sequences are valuable genomic tools and have been used for gene identification for specific target traits like drought tolerance, thermotolerance and so on.

In the process of evolution, only a finite number of chromosomal rearrangements have occurred in angiosperm plants. Therefore, large blocks of genetic material would be syntenic between the genomes of related species/genera, and this has been well documented in *Arabidopsis* and *Brassica* species (Gale and DeVos 1998). With more and more crop sequences being available, it would be possible to predict the position of most of the genes, if not all, in each part of the genome. However, for prediction of a gene from a sequence, a number of considerations as well as comparisons are required to be made. For simplicity and efficiency of the job of comparison and identifying the number of gene(s) to the maximum correct possible, a number of computer software programs are used. For example, GenScan, GeneWise, Procrustes, Rosseta, SGP1 (Syntetic Gene Prediction), CEM (Conserved Exon Method), GenomeScan, SGP-2, TwinScan, SLAM, and DoubleScan are commonly used for specific purposes. Similarly, a wide range of genome comparison tools is now available, viz., DIALIGN, ASSIRS (Acclerated Search for SImilarity Regions in Chromosomes), MUMmer (Maximal Unique Match (mer)), and GLASS (GLobal Alignment SyStem). Chain et al. (2003) categorized them as (1) pair-wise local alignment comparison tools; (2) global alignment tools: pair-wise alignment, multisequence alignment and multigenome alignment; (3) substring maximum-exact-match tools; and (4) alignment viewing tools. All of these tools have their own niches, advantages, and limitations; so the user has to determine which one is applicable for a specific purpose.

One of the challenges in comparative genomics is to distinguish "orthologs" from "paralogs," particularly in large diverse resistance gene families (Michelmore 2000). Similarly, the chromosomal positions of resistance gene candidate sequences seem not to be preserved between grass species (Liester et al. 1998). For example, homologs of the *RPM1* gene are missing from susceptible genotypes of *Arabidopsis* (Grant et al. 1998; Stahl et al. 1999). Further, in several species, resistance genes seem to be either telomeric or close to the centromere. It may happen because chromosome rearrangements often involve changes close to the telomere and centromere; therefore chromosomal position may contribute to the lack of synteny of some resistance genes (Michelmore 2000).

The advantage of sequence similarity is that the PCR primer designed based on such sequences should be applicable for all species having similar sequences. Using such a concept, a large number of resistance-gene candidate sequences have been cloned from diverse species (Meyers et al. 1999; Rivkin et al. 1999; Pan et al. 2000). In addition to gene and regulatory gene identification, comparative genomics may provide information regarding allelic variations, which may be helpful in reasoning the specificity at molecular level. Such study has been reported in resistance genes of crop plants (Ellis et al. 1999).

An important application of comparative genomics has been found in Sorghum (*Sorghum bicolor*) whose genome sequence information has become an important reference for genomics, transcriptomics, and other applications of systems biology

in sugarcane (Paterson et al. 2009; Wang et al. 2010). Phylogenetically, sorghum is very closely related to the *Saccharum* genus and current commercial sugarcane hybrids; both belong to the subtribe Saccharinae of the family Poaceae. Comparison of ESTs from sorghum with sugarcane revealed 97 % mean sequence identities as against 93 % and 86 % with maize and rice, respectively (Paterson et al. 2009). Further, comparison of 20 sugarcane BACs to their respective homologous regions in sorghum led to the identification of 209 and 189 genes from sugarcane and sorghum respectively, based on ESTs and gene-calling algorithms (Paterson et al. 2009; Wang et al. 2010). This shows the practical utility of comparative genomics for accumulating information in less studied crops.

6.3.4 Transcriptomics

Transcriptome refers to the complete set of transcripts in a cell, and their quantity, for a particular developmental stage or physiological state. Transcriptomics i.e., the study of the transcriptome aims to comprehensively profile all the information that appears in the RNA pool within a system (e.g., a cell, body fluid, or tissue). Understanding the transcriptome helps in interpreting the functional elements of the genome and revealing the molecular constituents of cells and tissues, and also in understanding development and diseases. The key aims of transcriptomics can be noted as: to catalog all species of transcript, including mRNAs, noncoding RNAs, and small RNAs; to determine the transcriptional structure of genes, in terms of their start sites, 5′ and 3′ ends, splicing patterns and other posttranscriptional modifications; and to quantify the changing expression levels of each transcript during development and under different conditions (Wang et al. 2009). It is important to note that the information carried in the transcriptome is not necessarily a direct recapitulation of information from the genome, nor is it generated only during DNA-dependent RNA synthesis. In fact, the sequence of an RNA molecule can become modified by a number of processes, including differential splicing and RNA editing. Therefore, transcriptome profiling needs validation through various methods including real-time quantitative polymerase chain reaction (Q-PCR) (Tsiridis and Giannoudis 2006).

A number of technologies varying in approach have been developed for estimation and quantification of the transcriptomes. The hybridization-based approaches typically involve incubating fluorescently labeled cDNA with custom-made microarrays or commercial high-density oligo microarrays. Specialized microarrays have also been designed and are available. Genomic tiling microarrays that represent the genome at high density allow the mapping of transcribed regions to a very high resolution, from several base pairs (bp) to ~100 bp. In general, the hybridization-based approaches are high-throughput and relatively inexpensive. Conversely, the sequence-based approaches directly determine the cDNA sequence. The sequencing of cDNA or EST libraries through Sanger chemistry is relatively low throughput, expensive and generally not quantitative. Tag-based

methods including serial analysis of gene expression (SAGE), cap analysis of gene expression (CAGE) and massively parallel signature sequencing (MPSS) are high-throughput and can provide precise, "digital" gene expression levels. In recent time, RNA-Seq (RNA sequencing), a high-throughput DNA sequencing method has been used for both mapping and quantifying transcriptomes. It has successfully been applied to *Scharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Arabidopsis thaliana*, mouse, and human cells (Lister et al. 2008; Nagalakshmi et al. 2008).

For effective identification of candidate gene(s), expression analysis has been combined with genetic or quantitative trait loci (QTL) mapping, an approach called "genetical genomics" (Jansen and Nap 2001). In this approach, total mRNA/cDNA from the tissue/organ of each individual of a mapping population (e.g., recombinant inbred line, RIL) is hybridized with the microarray carrying a high number of cDNA fragments representing the tissue of interest, and quantitative data are recorded against each gene on the filter (de Koning and Haley 2005; Fig. 6.1). The expression data are then subjected to QTL analysis. The QTL so-detected are called expression QTL (eQTL). The genetical genomics unravels genes and gene products that are involved in metabolic and regulatory (e.g., developmental) pathways. For each gene (cDNA) or gene product analyzed in the segregating population, QTL analysis will pinpoint the regions of the genome influencing its expression. Thus genetical genomics can identify candidate gene(s) by combining the QTL information from all genes and gene products that are analyzed. It will indicate what portion of the variation in gene expression maps to the genes themselves (*cis*-acting factors), as opposed to other genomic locations (transacting factors) (Jansen and Nap 2001). The approach of regulatory network construction by combining eQTL and mapping and regulatory candidate gene selection has been used for studying genes associated with flowering behavior in *Arabidopsis* (Keurentjes et al. 2011). With the advent of more sophisticated analytical tools and powerful software for data analysis, it will find more footage in the crop improvement program in the future.

6.3.5 Metabolomics

Each living cell produces an array of metabolic intermediates or end products valuable for its survival or defense, called metabolites. Levels of metabolites can be related to the response of the cells to the genetic or environmental changes. The set of metabolites synthesized by a biological system constitute its "metabolome" (Oliver et al. 1998). The metabolomes share some basic functional groups viz., hydroxyls, alcohols, steroids, alkyls, benzyl rings, etc. The combinations of these functional groups lead to the development of unique compounds varying in their solubility, stability, melting points, and reactivities typical of plant metabolism (Roessner and Beckles 2009). Simultaneous detection and analysis of such compounds has led to the emergence of a new field of "omics" called "metabolomics," that is, the description of the metabolic state of a biological

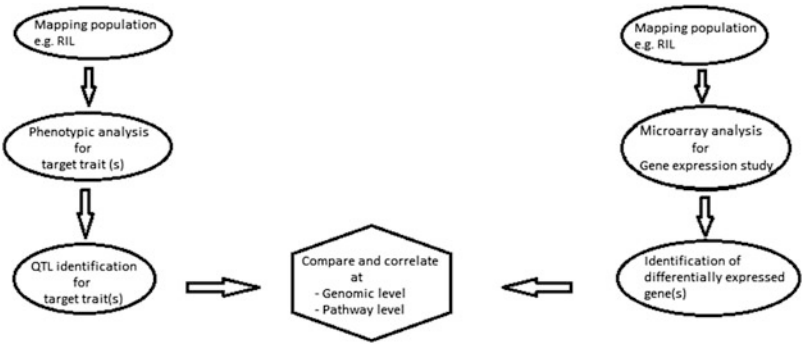

Fig. 6.1 eQTL combines QTL analysis with gene expression

system in response to environmental and genetic perturbations (Oliver et al. 1998; Fiehn et al. 2001; Villas-Bôas et al. 2007). A number of analytical technologies are needed to enable the separation, detection, and quantification of the metabolomes present in a cell. The most common platforms are liquid and gas chromatography both coupled with mass spectrometry (LC-MS and GC-MS) and nuclear magnetic resonance spectroscopy (NMR).

The primary objective of metabolomics is to associate the relative changes in quantitative metabolite levels with functional assignments so as to understand and predict the behavior of a complex system like plants (Oliver et al. 1998). The metabolic profile provides a readout of the metabolic state of an individual that cannot be obtained directly from DNA genotyping, gene expression, or proteomic profiling analyses. The metabolic changes would be the key in identification of the enzymes involved in the biochemical pathways, which in turn would be linked to the underlying gene. Several studies have been reported using metabolomic approaches for gene function analysis and QTL identification (Kazuki 2006), genotype discrimination (Taylor et al. 2002) as well as metabolite characterization so as to identify the regulatory keys and gene function (Saghatelian et al. 2004). With the advancement of analytical tools and techniques and, sophistication of data handling software, metabolomics holds great promise. The pharmacometabolomic approach (Clayton et al. 2006) is useful for providing information on pathways that contribute to determination of the individual pharmacokinetic and pharmacodynamic behaviors of a drug associated with response as well as insight into the mechanisms responsible for individual variation in terms of drug side effects and toxicity (Corona et al. 2012).

Like other important plant traits such as yield and flowering time, metabolite levels in plant tissues (m-trait) are also a quantitative trait, and QTLs responsible for such m-traits (e.g., level of seed vitamin E) have been identified (Giles 2007). By taking advantage of the high-density linkage map of molecular markers, a number of causal genes responsible for each mQTL could be deduced for further investigation of regulatory systems in complicated plant metabolism pathways. The analysis of the relationship between m-traits and other important agronomic and

biological traits such as yield, taste, and biomass in tomato indicated that there are certain correlations among these traits (Grossman and Takahashi 2001; Schauer et al. 2006). The growth rate of *Arabidopsis* seedlings is to some extent predictable from the metabolome signature (Meyer et al. 2007). In the near future, metabolomics could also play a key role in the evaluation of genetically modified crops, and in understanding plant systems and, developing further biotechnology applications.

6.3.6 Proteomics

A central dogma of molecular biology states that the genetic information written in the DNA molecule is passed through mRNA, to ultimately express in terms of protein; the path is not straight forward, though. The presence of posttranslational modification and posttranslational truncation of proteins, and protein–ligand interactions are a few examples that illustrate the complexity of the system. Therefore, the study of proteins has long been important but it gained momentum only during the last decade with the development of technology capable of performing large-scale analyses and identification of proteins (Issaq et al. 2002; Wang and Hanash 2003). This achievement has opened the door for comprehensive studies of proteins related to a genome, called proteomics (Wilkins et al. 1996). Isolation of protein from sample and their identification is more critical. However, introduction of matrix-assisted laser desorption ionization and time-of-flight (MALDI-TOF) mass spectrometry and electro-spray ionization (ESI) tandem mass spectrometry has revolutionized the field.

The technique of proteomics has been used for various purposes including studying the protein–protein interaction and identification of multisubunit complexes. It has already been applied in crops like maize (Chang et al. 2000), chickpea (Bhushan et al. 2007; Pandey et al. 2008), rice, and *Arabidopsis* (Tsugita et al. 1996) to generate useful information for genomics and crop breeding.

6.4 Emerging Concepts of Genomics

6.4.1 Genome Editing

The genome is the store house of genetic information. So, attaining the ability to read and edit genes in any organism has long been a goal in order to have in-depth knowledge and understanding of the genetic control of any cellular activities, and applying such knowledge to improve agricultural productivity, cure human diseases, and so on. A recently developed genomic technology called “genome editing” has promised to realize this goal through an unmatched level of precision

in studying gene function and biological mechanisms potentially in any system ranging from fruit flies, human cell lines, zebra fish to plants and hosts of other organisms. It enables efficient and precise genetic modification via induction of a double-strand break (DSB) in a specific genomic target sequence, followed by the generation of desired modifications during subsequent DNA break repair (Bibikova et al. 2002, 2003). This DSB is induced by a "zinc finger nuclease" (ZFN) (Kim et al. 1996; Bibikova et al. 2001) or by "transcription activator-like effector (TALE) nuclease (TALEN)" (Wood et al. 2011) at desired loci that can be repaired by the error-prone nonhomologous end-joining (NHEJ) (Jackson and Bartek 2009; Lieber 2010; Moynahan and Jasin 2010) method to yield small insertions and deletions (indels) at the break sites. The ZFN and TALEN can effectively be used to introduce into endogenous loci the targeted modifications, namely "gene disruption," "gene correction," and "targeted gene addition."

Gene Disruption. It is the simplest approach of genome editing in which a targeted gene is rendered nonfunctional or knocked out by introduction of error through engineered ZFNs. This process has been used in Drosophila (Beumer et al. 2008), mouse (Geurts et al. 2009), and zebrafish (Doyon et al. 2008) with varying degrees of success. In Drosophila, ZFNs targeting exonic sequences delivered via mRNA injection into the early fly embryo produced up to 10 % of the progeny with mutation for the gene of interest (Beumer et al. 2008). The success has been found to be more pronounced in zebrafish where up to 50 % germline mosaicism at the targeted gene was reported (Doyon et al. 2008; Meng et al. 2008; Foley et al. 2009). Engineered ZFNs were used to knockout the dihydrofolate reductase (*Dhfr*) gene in Chinese hamster ovary (CHO) cells, a mammal cell line. A plasmid encoding the ZFNs was introduced by transient transfection, which resulted in disruption frequencies of up to 15 % of alleles in the cell population (Santiago et al. 2008).

Gene Correction. It involves a homology-directed repair (HDR) mechanism in which a single nucleotide or short heterologous stretches from an episomal donor can be transferred to the chromosome following a ZFN-induced DSB. The endogenous repair machinery uses the investigator-provided donor as a template for repairing the DSB via the synthesis-dependent strand annealing process (Bozas et al. 2009). This technique, also called "allele editing" enables the study of gene function, de novo creation of point mutations at a native locus, and facilitates gene correction. The robustness of this approach was demonstrated in human interleukin-2 receptor-γ (*IL2RG*) (Urnov et al. 2005) and three other genes (Maeder et al. 2008).

Gene correction through ZFNs has also been achieved in plants (tobacco) (Townsend et al. 2009); the ZFN-expressing plasmids were codelivered to tobacco protoplasts along with a linear donor molecule encoding a point mutation that corrects the endogenous gene to an herbicide-resistant form. In 75–96 % of all the herbicide-resistant calli, the correction had occurred.

Gene Addition. The ZFN driven approach has enabled transfer of gene-sized heterologous DNA sequences from an episomal or linear extrachromosomal donor to the target genome. Initially, this work was optimized in flies; it has now

been extended to human beings where transgenes of up to 8 kb in length were added to the genome through ZFNs in the *IL2RG* gene (Moehle et al. 2007). Similarly, ZFNs were used to generate an isogenic panel of mouse ES cells carrying a defined series of alleles for an endogenous gene (Goldberg et al. 2010).

Good news for the plant biologists is that gene addition through ZFNs has been reported in plants including tobacco and maize. In tobacco, ZFNs targeting an endogenous endochitinase gene successfully added an herbicide-resistance marker in nearly 10 % of the cases (Cai et al. 2009). Similarly in maize, ZFNs targeted to the gene encoding an enzyme required for the production of phytate were introduced with a donor carrying an herbicide-resistance marker (Shukla et al. 2009). The ZFN-edited plants were fertile, and showed normal Mendelian fashion of inheritance for the target trait. The success of this approach has promise for use in "trait stacking" in major crop species like rice, maize, wheat, soybean, etc.

The application of ZFN and TALEN techniques and its potential impact in plant breeding is enormous. Testing candidate loci may become a straightforward task, as locus-specific knockouts or allelic replacement allow both functional validation and a direct means of estimating the effects of individual alleles. It would allow editing alleles at loci of known agronomic interest directly in the individual lines, entirely bypassing the process of backcrossing. A likely possibility of this technique in the near future is the targeted replacement of deleterious mutation in elite breeding lines. Thus, genomic editing is likely to be an attractive and potential alternative to current transgenic technologies (Morrell et al. 2012). However, certain issues like specificities, off-target, process optimization, etc., must be resolved carefully before it can be used routinely.

6.4.2 Next-Gen Sequencing

Revealing genetic diversity and putting them to use for crop production is the primary goal of the plant breeding exercises. Modern molecular biological tools including the molecular markers, viz., restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and simple sequence repeats (SSR), etc., have now been used extensively for this purpose. However, each marker system has its own strengths and limitations. Further, speed, efficiency, and accuracy are the other factors limiting the life and utility of these systems. The single nucleotide polymorphism (SNP) is the sequence-based marker system that had overcome many such limitations. However, the cost involved in sequencing the gene or target sequence in order to generate the SNP is very high and, may even be prohibitive in certain cases.

Since the early 1990s, DNA sequence production has almost exclusively been carried out with capillary-based, semiautomated implementations of Sanger biochemistry (Sanger et al. 1977; Hunkapiller et al. 1991). It takes place in a "cycle sequencing" reaction, in which cycles of template denaturation, primer annealing, and primer extension are performed. Each round of primer extension is

stochastically terminated by the incorporation of fluorescently labeled dideoxynucleotides (ddNTPs). In the resulting mixture of end-labeled extension products, the label on the terminating ddNTP of any given fragment corresponds to the nucleotide identity of its terminal position. Sequence is determined by high-resolution electrophoretic separation of the single-stranded, end-labeled extension products in a capillary-based polymer gel. Laser excitation of fluorescent labels as fragments of discreet lengths exit the capillary provides the readout that is represented in a Sanger sequencing "trace." Software translates these traces into DNA sequence, while also generating error probabilities for each base-call (Ewing and Green 1998; Ewing et al. 1998). After three decades of gradual improvement, the Sanger biochemistry can be applied to achieve read-lengths of up to ~1,000 bp, and per-base "raw" accuracies as high as 99.999 % with costs on the order of $0.50 per kilobase (Shendure and Ji 2008).

In order to incorporate speed, accuracy, and automation, several newer and improved technologies have been developed that use cyclic-array sequencing and are generally categorized as next-generation sequencing (NGS) (Shendure and Ji 2008). Basically, the concept of cyclic-array sequencing involves sequencing of a dense array of DNA features by iterative cycles of enzymatic manipulation and imaging-based data collection (Mitra and Church 1999). It has been commercially utilized in a number of products like 454-sequencing, solexa technology, the SOLiD platform, the Polonator, and the HeliScope Single Molecule Sequencer technology.

These platforms have a lot in common and differences in their work flows (Table 6.2). Library preparation is accomplished by random fragmentation of DNA, followed by in vitro ligation of common adaptor sequences. The clonally clustered amplicons to serve as sequencing features can be generated by approaches like in situ polonies (Mitra and Church 1999), emulsion PCR (Dressman et al. 2003) or bridge PCR (Fedurco et al. 2006). The sequencing is done through synthesis, i.e., serial extension of primed templates through polymerase or ligase (Brenner et al. 2000; Mitra et al. 2003; Shendure et al. 2005; Turcatti et al. 2008). Data are acquired by imaging of the full array at each cycle.

Advantages of second-generation or cyclic-array strategies, relative to Sanger sequencing, includes (1) in vitro construction of a sequencing library, followed by in vitro clonal amplification to generate sequencing features, which circumvents several bottlenecks that restrict the parallelism of conventional sequencing (that is, transformation of *E. coli* and colony picking). (2) Array-based sequencing enables a much higher degree of parallelism than conventional capillary-based sequencing. As the effective size of sequencing features can be of the order of 1 μm, hundreds of millions of sequencing reads can potentially be obtained in parallel by rastered imaging of a reasonably sized surface area. (3) Because array features are immobilized to a planar surface, they can be enzymatically manipulated by a single reagent volume. Although microliter-scale reagent volumes are used in practice, these are essentially amortized over the full set of sequencing features on the array, dropping the effective reagent volume per feature to the scale of picoliters or

Table 6.2 Comparison of next-generation sequencing platforms

Platform	Library/template preparation	Sequencing principle or NSG chemistry	Read length (bp)	Strength	Weakness
454	Fragment, Mate-pair, emulsion PCR	Polymerase (Pyrosequencing)	200–300	Longest read length, fast run time	Asynchronous, homopolymers (e.g., CCC, AAA) enhances error rate, high reagent cost
Solexa	Library consists of a mixture of adapter flanked fragments, uses bridge PCR	Polymerase (Reversible terminator)	75–100	Most widely used platform	Multiplex capacity of the sample is low
AB SoLiD	Libraries consist of a mixture of short adapter flanked fragments; uses emulsion PCR	Noncleavable probe, cyclic sequencing by ligation (octamer with two base encoding	50	Error can be corrected by ligaze enzymology, primer reset and "two-base encoding"	Read length is low, run-time is long
Polonator	Mate-pair, uses emulsion PCR	Cyclic sequencing by ligation (nonamer)	26	Relatively cheaper; can be adjusted to other NGS chemistry	Shortest read length
HeliScope	Fragment, Mate-pair, Single molecule	Reversible terminator principle	25–32	No clonal amplification is needed; problem of homopolymer can be tackled.	Low read length, higher error rate

femtoliters. Collectively, these differences translate into dramatically lower costs for DNA sequence production (Shendure and Ji 2008).

The disadvantages of second-generation DNA sequencing includes (1) shorter read-length, i.e., read-lengths are currently much shorter than conventional sequencing, (2) low accuracy, i.e., base-calls generated by the new platforms are less accurate than those of Sanger sequencing. In times to come, these parameters will be taken care of to make it more effective in terms of quantity as well as quality.

The important applications of next-generation sequencing include (1) full-genome resequencing, (2) mapping of structural rearrangements, (3) "RNA-Seq," analogous to expressed sequence tags (EST) or serial analysis of gene expression (SAGE), (4) large-scale analysis of DNA-methylation, by deep sequencing of bisulfite-treated DNA; and (5) "ChIP-Seq," or genome-wide mapping of DNA–protein interactions, by deep sequencing of DNA fragments pulled down by chromatin immunoprecipitation. However, many more new applications are expected in the coming days (Shendure and Ji 2008).

6.4.3 Gene Discovery and Deployment

Discovery and deployment of a useful gene is a major scientific challenge determining the success of developing plant varieties suitable for climate-resilient agriculture. Therefore, several approaches have been put in place for high-throughput gene discovery. The gene silencing technique that employs double-stranded RNA (dsRNA)-mediated interference of functional genes has been effectively and widely used in plants. Similarly, the virus-induced gene silencing (VIGS) technique has also been used to knockout endogenous genes in a transient manner. In VIGS, the vector virus carries a sequence from the plant; the transcript of both the viral and the homologous endogenous gene are degraded by the posttranscriptional gene silencing (PTGS) mechanism (Kumagi et al. 1995; Burton et al. 2000; Ratcliff et al. 2001). However, these techniques are not smooth functioning in plant systems; transformation efficiency is a primary factor of concern. Gene knockout through transposon and T-DNA insertion were also tested in large scale in plant systems for functional annotation of genes (Krysan et al. 1996). Several insertion mutant populations were developed and gene function could be assigned through analysis of mutant phenotype (Winkler et al. 1998). Although it is a powerful technique, the degree of target specificity is a factor to be considered; sometimes it fails to knock the gene of interest. It might not be so effective in species having more than one genome such as soybean, a paleopolyploid. Targeting induced local lesion in genome (TILLING; McCallum et al. 2000) is the other effective and high-throughput reverse genetics approach for identification and study of mutation in plants. It has been successfully utilized in maize and soybean (Till et al. 2004; Slade et al. 2005; Mizoi et al. 2006; Horst et al. 2007). Such approaches would be of

immense help in identifying useful genes for deployment in breeding programs to develop crop varieties suitable for changed climatic conditions.

6.5 Modern Plant Breeding Approach

6.5.1 Molecular Plant Breeding

Plant breeding is as old as agriculture. However, it as a branch of science and field of research received attention and importance only after Mendel's principles of character transmission were rediscovered and realized i.e., after 1900 AD (Allard 1960). With the understanding of how genes control different traits of importance and become transmitted from one generation to another, plant breeders started incorporating gene(s) of choice to the desired genetic backgrounds through different hybridization and selection techniques. However, genes are not independent of the environment (external and internal), and often show their impact through interactions. The understanding of statistics and biometrical genetics helped in unraveling the role of the major and minor genes and the environment, and showed a way to manipulate such traits i.e., the quantitative traits. The locus that governs such quantitative traits is often called the quantitative trait locus (pl. loci) (QTL, in short). Using genetic principles in breeding, breeders succeeded in enhancing the yield of crops as well as crops' capacity to resist disease and insect-pest attacks to a great extent. With deployment of specific alleles of genes, yield of wheat and rice were enhanced to such an extent that it saved millions of poor people from hunger, particularly in Asia and Africa. This success of plant breeding in the 1970s was christened the "green revolution," and the key person behind it, Norman E. Borlaug, was conferred with a Nobel Prize for Peace. Thus, the success of modern plant breeding is unquestionable and well documented (Fehr 1984). It is difficult to believe that such a feat would have been achieved without the clear understanding of the principles of plant breeding and genetics.

With the rise of global population and corresponding increase in eco-environmental problems, the plant breeding techniques demand modernization for enhanced efficiency and environment suitability. Presently, the techniques of plant breeding need to be such that they deliver hugely in the fastest possible time and with the least harm to ecosystems. This led to the introduction and application of molecular biological tools and techniques for modernization of conventional plant breeding, now called "molecular plant breeding" or "molecular breeding," for short. The last three decades have witnessed the development and use of a number of molecular markers, including RFLP, RAPD, AFLP, SSR, and SNP, etc., for various purposes of crop breeding and experimentations. These markers have enabled the breeders to select the desired plant from a large segregating population in an efficient and environment-independent way. Because of its effectiveness, molecular marker-assisted breeding has become equivalent to the conventional

breeding process for many giant agricultural companies including Monsanto, Pioneer, etc (Paul 2009). Markers have made it possible to breed traits from otherwise discarded varieties back into cultivated crops. The conventional backcrossing program has become more potent with the addition of molecular markers, nowadays called the marker-assisted backcrossing (MAB) program. The success of this technique has been recognized by the transfer of many important traits, disease and insect resistance in particular, into crops including rice, maize, soybean, etc. The most prominent success has been the development of submergence-tolerant rice, which was developed by transferring the submergence-tolerance gene (QTL) (*sub-1*) from an Indian landrace to an improved cultivar (e.g., Swarna) through MAB. Thus, molecular breeding has empowered the breeders to tackle even QTL, which the conventional plant breeder found difficult to handle because of its complex genetics and sensitivity to environmental fluctuations. With the availability of a large number of markers, efficient computing power and incorporation of modern concepts like genomic selection (GS), it has become possible to make breeding decisions based on every gene influencing a trait, not just a few. Although molecular breeding has a long way to go to meet the goal of feeding the millions of people on earth, its promise has begun to catch the hype.

6.5.1.1 Allele Mining

Allele mining is an approach to identify naturally occurring allelic variants at loci of agronomic importance, i.e., those genes that affect crop characteristics and performance. In this sense, it includes analysis of noncoding and regulatory regions ("promoter mining") of the candidate genes in addition to analyzing sequence variations in the coding regions of the agronomically important genes (Rangan et al. 1999; Latha et al. 2004). The alleles of genes that are candidates for the target trait can be identified using a variety of approaches including mutant screens (Johal and Briggs 1992; Whitham et al. 1994; Bishop et al. 1996), QTL analysis (Backes et al. 1995; Xiao et al. 1996; Bernacchi et al. 1998), association mapping (Crossa et al. 2007; González-Martínez et al. 2007), and genome-wide surveys for the signature of artificial selection (Vigouroux et al. 2002; Casa et al. 2005; Yamasaki et al. 2005; Chapman et al. 2008). Such novel and important alleles recovered at loci of agronomic importance can be integrated into crop breeding programs using conventional or molecular approaches for combating biotic and abiotic challenges, to enhance yield, improve storage and nutritional qualities. However, success of allele mining operations is dependent on the availability of diverse germplasm collections (Kumar et al. 2010), as wild relatives of the crops are the repository of the useful new alleles not already present in the crop gene pool (Tanksley and McCouch 1997; Gur and Zamir 2004; Johal et al. 2008; Prada 2009).

Two approaches that are generally utilized for identification of sequence polymorphisms for a given gene in the naturally occurring populations includes (1) modified TILLING and (2) sequencing-based allele mining (Kumar et al. 2010). TILLING (McCallum et al. 2000) is a reverse genetics approach that can identify

point mutation in a target gene by heteroduplex analysis (Till et al. 2004). TILLING or its modified version Eco-TILLING is based on the enzymatic cleavage of heteroduplex DNA formed due to mismatch in sequence between reference and test genotype with a single-strand specific nuclease (e.g., Cel-1, S1, mungbean nuclease, etc.) under specific conditions followed by detection through Li-Cor genotyper or capillary electrophoresis (CE) separation. At the site of point mutation, there will be cleavage by the nuclease to produce two cleaved products whose sizes will be equal to the size of the full-length product. The presence, type, and location of point mutation or SNP will be confirmed by sequencing the amplicon from the test genotype that carries the mutation. Conversely, the sequence-based TILLING approach involves amplification of alleles in diverse genotypes through PCR followed by identification of nucleotide variation by DNA sequencing. Both approaches are not cost-effective, as claimed. However, with the advent of newer sequencing technologies, the second- and third-generation sequencing technologies in particular, that are capable of producing at low cost, with more read length and high throughput, sequence-based allele mining is expected to generate data at an economically affordable level.

The most important application of allele mining is the discovery of superior alleles from unutilized natural plant genetic resources. A number of such alleles has already been identified and used in crop improvement. It can also be used to reason the molecular basis of novel trait variations and pinpoint the nucleotide sequence changes associated with superior alleles. It may pave the way for molecular discrimination among related species, development of allele-specific molecular markers, facilitating introgression of novel alleles through MAS or deployment through genetic engineering (Kumar et al. 2010).

6.5.1.2 Quantitative Trait Locus Mapping

The QTL-mapping approach generally begins with the crossing of two parental inbred lines for a number of generations to form preferably a population of recombinant homozygous lines. There are several methods for QTL mapping ranging from the simplest method of single-marker analysis (Sax 1923) to more sophisticated methods such as interval mapping (Lander and Botstein 1989; Haley and Knott 1992), joint mapping (Kearsey and Hyne 1994), multiple regression (Wright and Mowers 1994; Whittaker et al. 1996), and composite interval mapping (Zeng 1994). Analytical complexities are taken care of with various software packages including MAPMAKER/QTL (Lincoln et al. 1993), JoinMap (Stam 1993), QTL Cartographer (Basten et al. 1994), PLABQTL (Utz and Melchinger 1996), QGene (Nelson 1997), and TASSEL (Buckler 2007). This approach has proved to be quite useful for plant breeding and has been successful in identifying loci of large effect and dissecting the genetic basis of fairly simple traits. A detailed discussion of QTL analysis has been given in Chap. 4.

The QTL approach will be important in terms of climate-resilient agricultural activities. Most of the traits particularly important in the event of climate change are

quantitative in nature. Moreover, QTLs for tolerance to traits including drought, heat, frost, flood, etc., have been reported in different genetic backgrounds. Now, accumulating useful QTLs is the major goal for crop improvement in the changing climatic situation. In this regard, various approaches as described in this chapter will be the foci of the future for climate-resilient plant breeding. QTL mapping for a drought-tolerance trait has been done in different crops including maize, wheat, barley, cotton, sorghum, and rice (Quarrie et al. 1994; Teulat et al. 1997; Sari-Gorla et al. 1999; Saranga et al. 2001; Sanchez et al. 2002; Bernier et al. 2008). In pearl millet, a major QTL associated with grain yield in drought stress environments has been identified on linkage group 2 (LG 2), which accounts for up to 32 % of the phenotypic variation of grain yield (Yadav et al. 2011).

The primary disadvantages of the QTL mapping approach is the time involved in creating populations, limited information and inferences that can be made from alleles in two parental lines, the small number of recombination events captured in most mapping populations and a necessary focus on traits that can be readily and accurately phenotyped (Morrell et al. 2012). To overcome such limitations and to map QTL with higher precision, approaches like marker-assisted recurrent selection (MARS) and genome-wide selection (GWS) have been used for such types of study (Ravi et al. 2011).

6.5.1.3 Association Mapping

Association or linkage disequilibrium (LD) mapping approaches assess the correlation between phenotype and genotype in populations of unrelated individual lines. The association-mapping panels (Risch and Merikangas 1996) sample more genetic diversity, can take advantage of many more generations of recombination, and avoid the generation of time-consuming crosses that are necessary for QTL mapping (Myles et al. 2009). Unlike QTLs identified through biparental mapping strategies that can span tens of megabases, the recombination event that is captured in most association panels enables a much greater genetic resolution. With a large panel and with sufficiently dense genome-wide marker coverage, association mapping can potentially map causative loci to individual nucleotide changes. However, much higher resolution can be achieved through genome-wide association studies (GWAS). Huang et al. (2010) used low-coverage resequencing of the genomes of a panel of more than 500 rice landraces and found 80 loci associated with 14 agronomic traits. Several of these associations were previously characterized showing authenticity of the results. Precision of similar kind was also reported in *A. thaliana* (Atwell et al. 2010). These results suggest limits to the precision available in association mapping studies, particularly in inbreeding organisms (Atwell et al. 2010; Hamblin et al. 2011).

6.5.1.4 Marker-Assisted Recurrent Selection

The plant breeder usually uses a conventional recurrent selection process in order to increase the frequencies of desirable QTL alleles at multiple loci. Now, two related approaches have been proposed for similar purposes (1) F_2 enrichment followed by inbreeding (Howes et al. 1998; Wang et al. 2007); and (2) MARS (Edward and Johnson 1994; Hospital et al. 1997; Johnson 2004; Bernardo and Charcosset 2006). In both these approaches, the starting material usually is an F_2 population. The primary goal is to develop an improved recombinant inbred (RI) in the case of self-pollinated crops and with superior testcross performance for cross-pollinated crops. In F_2 enrichment, the F_2 plants with undesirable alleles are culled in order to increase the frequency of desirable alleles that in another way increases the probability of recovering inbred with desirable alleles at all the loci. Usually, this selection cycle is run only once, because a second or third round of enrichment adds little advantage (Wang et al. 2007). However, in MARS multiple cycles of selection are performed based on markers (Edward and Johnson 1994; Johnson 2004; Eathington et al. 2007). The step in MARS involves (1) identifying F_2 plants or F_2-derived progenies that have the desirable allele at most of the target QTLs, (2) recombining selfed progenies from these selected individuals, and (3) repeating the procedure for 2–3 cycles. In the MARS approach, a selection index is created based on weight given to markers according to the relative magnitude of their estimated effects on the trait (Lande and Thompson 1990; Edward and Johnson 1994). The selection index is given by $M_j = \Sigma b_i X_{ij}$, where M_j is the marker score of the jth individual, b_i is the weight given to the ith marker locus and X_{ij} is given score 1 if the jth individual is homozygous for the marker allele with favorable effect, and -1 if the individual is homozygous for the marker allele with un/less favorable effect. The value of the b_i weight can be obtained from multiple regressions of trait values on X_{ij} (Lande and Thompson 1990; Hospital et al. 1997). MARS is now being used in several crops. Success has been reported in sweet corn where MARS increased the frequency of the favorable alleles from 0.50 to $\geq$0.80 at 18 out of 31 markers used in selection (Edward and Johnson 1994), and five marker loci became fixed for the favorable alleles. Similarly, in another F_2 population, the frequency of the favorable marker allele increased to $\geq$0.80 at 11 out of 35 markers used in selection. This shows the power of the approach. It has now been tried in other crops including wheat and success is underway. A possible disadvantage of MARS is the necessity of extra numbers of generations for cyclic selection based on markers (Bernardo 2008).

6.5.1.5 Genome-Wide Selection or Genomic Selection

GWS refers to marker-based selection without significance testing and without identifying a subset of markers associated with the trait (Meuwissen et al. 2001). Here, the effects of all the markers on the quantitative trait (i.e., breeding value) are

fitted as random effects in a linear model. Trait values are then predicted as the sum of an individual's breeding values across all the markers used, and selection is subsequently based on these genome-wide predictions. Therefore, the first step of genomic selection (GS) is the estimation of marker effects and the designing of genomic prediction models from a training population. The second step consists of two or three cycles of early-generation genotypic selection and intercrossing, allowing accumulation of favorable alleles. GWS leads to high correlations between predicted and true breeding value for a quantitative trait (Meuwissen et al. 2001). It has been shown that GS can increase efficiency of breeding for yield potential in elite x elite crosses, but it cannot introduce new genetic variability into the most adapted backgrounds. Trait-based breeding will combine and introgress yield potential traits coming from various genetic resources into the most adapted recipient parent (Reynolds et al. 2011).

6.5.1.6 Next-Generation Mapping of Complex Traits

Mapping complex traits is always difficult and challenging. Various approaches have already been proposed and used with limited success. In recent times, next-generation sequencing (NSG) approaches have also been used for this purpose. Basically, NSG mapping is comprised of three major steps (1) development of the mapping population, (2) extraction of DNA and preparation of libraries, and (3) assembling short reads and genotyping. The NSG approaches have the potential to map QTL more effectively. However, such approaches may also lack sufficient power. Whole-genome resequencing (WGR) (Huang et al. 2009), restriction site-associated DNA (RAD, Baird et al. 2008) and phenotype-based selection and introgression followed by whole-genome resequencing (PSIseq, Earley and Jones 2011) are NSG-based mapping approaches. WGR and RAD use a bulk segregant population and require large mapping populations to detect multiple loci with weak effect (Ehrenreich et al. 2010). In PSIseq, populations with a divergent complex trait are hybridized and then selected for a specific phenotype across multiple generations of backcrosses. The trait of interest is selected for each generation, and offspring are mated to the other parental line expressing the unselected phenotype (introgression and backcrossing). Over multiple generations of selection and backcrossing this hybrid population becomes homozygous for the majority of the unselected parent's genome while loci from the selected parent, which contribute to the selected trait, remain. Using high-throughput sequencing, the breakpoints of introgression are mapped, which eventually map the regions harboring genes influencing the trait. The power and effectiveness of PSIseq have been shown in the Drosophila system. However, it can be used in other systems as well. It does not require existing high-quality reference genomes. Unlike other NSG mapping approaches, it can find multiple loci with small mapping populations (Earley and Jones 2011). With the wider applications of NSGs, these mapping approaches will find a popular place in plant breeding programs of the future.

6.5.2 *Next-Generation Mapping Populations*

A multiparent populations concept, called next-generation populations (NGPs), has evolved and been used recently with the aim to overcome the shortcomings of biparental QTL mapping and association mapping populations. In principle, it combines the controlled crosses of QTL mapping with multiple parents and multiple generations of intermating. The NGPs are often larger than traditional QTL populations, and many lines are crossed in parallel; this increases the rate of effective recombination per generation and maximizes "genetic map expansion," thereby improving genetic resolution compared to traditional biparental mapping (Rockman and Kruglyak 2008). Like association mapping panels, NGPs will more effectively sample rare alleles than typical biparental populations. It also has the power to overcome some of the difficulties of association mapping, including population structure and the unknown frequency of causative mutations. Further, it allows better estimation of allelic effects than is possible under standard association mapping approaches purely because of equal contribution of all the parents involved (Macdonald and Long 2007).

6.5.2.1 Nested Association Mapping Population

The nested association mapping (NAM) population is a form of next-generation population. Here, diverse strains are crossed to a reference parent (Yu et al. 2008). The resultant F_1s are self-fertilized for several generations in order to develop a series of RILs (Fig. 6.2). The members of the RIL families are either sequenced or genotyped and compared with the reference lines. In maize, 25 diverse corn lines were used as the parental lines and each parental line was crossed to the B73 maize inbred to produce an F_1 population. The F_1 plants were then self-fertilized for six generations in order to create a total of 200 homozygous RILs per family, for a total of 5,000 RILs within the NAM population.

NAM combines the advantages and eliminates the disadvantages of two conventional methods for identifying QTLs: linkage analysis and association mapping. In principle, the linkage analysis depends upon the recent genetic recombination while the association mapping takes advantage of historic recombination. Linkage analysis suffers from low-resolution mapping while association mapping needs extensive knowledge of SNPs. The NAM takes advantage of both historic and recent recombination events and, eliminates the disadvantages associated with both of the traditional approaches. It has successfully been utilized in the study of numerous traits in maize (Buckler et al. 2009; Brown et al. 2011; Kump et al. 2010; Tian et al. 2011). The NAM population has tremendous potential to be used in genome-wide association studies and for study of agronomic traits useful for changing climatic conditions.

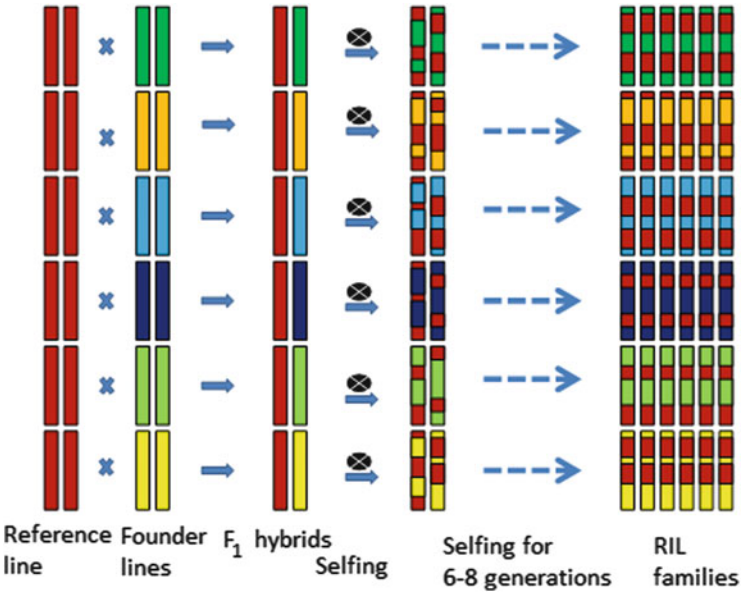

Fig. 6.2 Schematic diagram of NAM population generation

6.5.2.2 Multiparent Advanced Generation Intercross Population

The multiparent advanced generation intercross (MAGIC) is another form of next-generation population, which involves intercrossing of multiple parents, forming a single large population (Cavanagh et al. 2008; Kover et al. 2009) (Fig. 6.3). It is also called recombinant inbred advanced intercross line (RIAIL) populations (Rockman and Kruglyak 2008). Multiparent crosses have a long history in plant breeding (Harlan and Martini 1929; Suneson 1956) and have been a source of considerable insight into evolutional processes in crops (Allard et al. 1972; Clegg et al. 1972). However, adoption of such mapping populations will vary with the crop species depending upon the ease or complexities with which crosses can be made. For example, designs that involve repeated outcrossing (e.g., MAGIC) are difficult to implement in self-pollinated crops such as soybean (Cavanagh et al. 2008).

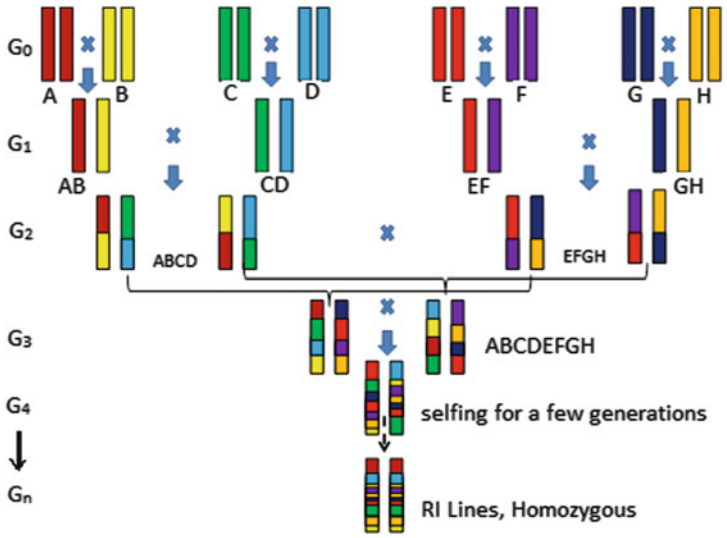

Fig. 6.3 Schematic diagram of MAGIC population generation

6.6 Advanced Approaches for Utilization of Plant Genetic Resources

6.6.1 Introgression of Desirable QTL Through Wide Hybridization

Wild or weedy relatives of crop species are an important reservoir of agronomically superior traits. Such species possess the genes/alleles that helped it survive in changed climatic conditions over the years. Such wild relatives of crops have contributed several important traits for improvement of current cultivars of rice, wheat, maize, barley, and other crops (Hajjar and Hodgkin 2007). In the recent past, the genetic and cytogenetic approaches have contributed significantly towards such transfer of genes from alien species into various crops including rice (Ashikari et al. 2005) and potato (Maxted et al. 2007). An understanding of its genetic and genomic basis would help in planning breeding programs for transfer of the desirable genes from such species to otherwise elite crop cultivars in a more effective and efficient way. The molecular approaches have the technical ability to overcome problems such as linkage drag, shattering, etc., associated with transfer of desirable alleles from wild species. Several molecular marker-supported approaches have been utilized for efficient transfer of desirable traits from wild species into tomato, barley, rice, and so on. Approaches like introgression line (IL, Eshed and Zamir 1995), single segment substitution lines or 3-S lines (Talukdar and Zhang 2007), chromosome segment substitution lines (CSSLs, Wang et al. 2004), and stepped aligned inbred recombinant strains (STAIRS; Koumproglou et al. 2002) have

shown promise in transfer of traits mostly governed by major genes. However, these can also be used for other traits for enrichment of genetic variations and development of hardy crops that can survive and sustain themselves against the vagaries of nature.

6.6.2 Advanced Backcross QTL Analysis

It has been proved time and again that despite the inferior phenotype, the exotic germplasm contain QTLs that can increase the yield and quality of elite breeding lines. However, these QTLs are often linked with undesirable traits making their application difficult. Efforts to overcome such hurdles have motivated the development of a new molecular breeding strategy, referred to as the advanced backcross (AB) QTL method (Tanksley and Nelson 1996), which integrates QTL analysis with variety development, by simultaneously identifying and transferring favorable QTL alleles from unadapted to cultivated germplasm. The AB-QTL strategy has so far been tested in tomato, rice, barley, maize, pepper, etc. In this approach, QTL and marker analyses are performed in advanced generations, like BC_2 or BC_3. During the development of these populations, a negative phenotypic and/or genotypic selection pressure is exerted against the unfavorable genes particularly originating from the donor parent. This helps in the reduction of the percentage of the donor-parent genome simultaneously reducing the alleles that could otherwise interfere with yield and other field performance traits (Tanksley and Nelson 1996). In the case of cross-pollinated crops, field testing can be conducted on BC_2S_1 or BC_3S_1. Conversely, for crops where commercial hybrids are more commonly used, the BC_2 or BC_3 plants are crossed with a tester variety to generate BC_2F_1 or BC_3F_1 families. Once favorable QTL alleles are identified, only a few more marker-assisted generations are required to develop near-isogenic lines (NILs) that can be field tested and used for variety development. Therefore, a cycle of AB-QTL analysis (i.e., QTL discovery, NIL development and testing) represents a direct test of the underlying assumption of QTL breeding: that beneficial alleles identified in segregating populations (i.e., BC_2 or BC_3 in the case of AB-QTL) will continue to exert their positive effects when transferred in the genetic background of elite lines (Grandillo and Tanksley 2003). This process not only results in improved elite varieties, but it also provides a strategy for selectively expand the genetic base of crop species, especially those with a narrow germplasm base. In the effort to breed crops for changing climatic conditions, this approach might prove to be extremely useful as it offers a way to transfer the desirable QTLs from the wild and weedy species to the otherwise elite genotypes, which might lack the gene(s) for adaptability to the harsh environments that are predicted to occur in the years to come. Further, use of high-density molecular marker maps would ensure transfer of the desirable QTLs in a more precise manner making the fear of linkage drag remote.

6.6.3 Association Mapping

Association mapping, also known as linkage disequilibrium (LD) mapping is a powerful tool to resolve complex trait variation down to the sequence level by exploiting historical and evolutionary recombination events at the population level (Risch and Merikangas 1996; Nordborg and Tavare 2002). As compared to traditional linkage mapping analysis, the advantages of association mapping are (1) increased mapping resolution, (2) reduced research time, and (3) greater allele number (Yu and Buckler 2006). Basically, there are two approaches for association mapping (1): a candidate-gene based approach, which relates polymorphisms in selected candidate genes that have purported roles in controlling phenotypic variation for specific traits; and (2) a genome-wide or genome scan approach, which surveys genetic variation in the whole genome to find signals of association for various complex traits (Risch and Merikangas 1996). For the candidate-gene approach, information regarding the location and function of the genes involved in either biochemical or regulatory pathways are required, which are currently available for most of the model plants and a number of other crop species, as well. At the same time, the availability of hundreds of thousands of SNPs at affordable cost through resequencing has prompted researchers to move toward genome-wide association analyses of complex traits. The *Arabidopsis* HapMap provided a powerful catalog of genetic diversity with more than one million SNPs at an average rate of one SNP every 166 bp (Clark et al. 2007). The unique advantage of association mapping is that it harnesses the genetic diversity of natural populations to potentially resolve complex trait variation to single genes or individual nucleotides, and hence its results have wider applicability.

Linkage disequilibrium or gametic phase disequilibrium is the measurement of the degree of nonrandom association between alleles at different loci. The difference between observed haplotype frequency and expected based on allele frequencies is defined as D. It is given as, $D = p\mathrm{AB} - p\mathrm{A}\ p\mathrm{B}$, where pAB is the frequency of gamete AB; pA and pB are the frequency of allele A and B, respectively. In the absence of other forces, recombination through random mating breaks down the LD with $D_t = D_0(1 - r)^{\mathrm{t}}$, where D_t is the remaining LD between two loci after t generations of random mating from the original D_0. Several statistics have been proposed for LD, and these measurements largely differ in how they are affected by marginal allele frequencies and small sample sizes (Hedrick 1987). The estimates of both D' (Lewontin 1964) and r^2 (Hill and Robertson 1968) have been widely used to quantify LD. In terms of identifying SNPs or haplotypes significantly associated with phenotypic trait variation, r^2 is the most relevant LD measurement. Typically, r^2 values of 0.1 or 0.2 are often used to describe the LD decay. Though LD is affected by many factors (Ardlie et al. 2002), LD due to linkage is the net result of all the recombination events that occurred in a population since the origin of an allele by mutation, providing a greater opportunity for recombination to take place between any two closely linked loci than that in linkage analysis (Holte et al. 1997; Karayiorgou et al. 1999). Among other factors,

the reproduction mode of a species partly determines the level of LD in a diverse population (Flint-Garcia et al. 2003). Generally, LD extends to a much longer distance in self-pollinated crops, such as soybean, wheat, than in cross-pollinated species, such as maize. The shorter the LD decay, the greater the mapping resolution, and vice versa.

Association or LD mapping often encounters problems of spurious association generated by population structure and familial relatedness (Yu and Buckler 2006), which needs to be taken care of through statistical treatments. Several statistical approaches viz., structured association, genomic control, mixed model, principal component, etc., can be adopted for explicit or ad hoc adjustment (Yu et al. 2006).

The size of the sample taken for association mapping remains relatively small. In many recent association mapping studies, only about 100 lines were investigated. However, unless the functional locus has a very large effect and tested markers are in high LD with this locus, it will be difficult to identify marker–trait associations with a small population, regardless of whether the candidate-gene or genome-scan approach is used. Simulations with empirical maize data showed that a large sample size is required to obtain high power to detect genetic effects of moderate size (Zhu et al. 2007). Similarly, the number of markers to be used is also an important consideration for candidate-gene association mapping studies. Generally, the number of required markers is much higher for biallelic SNPs than for multiallelic SSRs. As a starting point, the number of SSR markers should be about four times the chromosome number of that species, i.e., at least two markers per chromosome arm. However, chromosome length, diversity of the species and the particular sample, cost and availability of different marker systems will be the ultimate determining factors.

A variety of software packages are available for data analysis in association mapping (Table 6.3). However, TASSEL and STRUCTURE are the most commonly used software for association mapping in plants. TASSEL is also used for calculation and graphical display of linkage disequilibrium statistics and browsing and importation of genotypic and phenotypic data. STRUCTURE software typically is used to estimate the Q matrix (Pritchard et al. 2000). However, to resolve specific issues, specialized software has to be used.

Association mapping or LD mapping was initially developed in human beings. However, it has an equal or even better foothold in animal and plant systems. It has successfully been used in almost all the major crops such as maize, soybean, barley, wheat, tomato, sorghum, and potato, as well as tree species such as aspen and loblolly pine, to name a few. With the availability of the genome sequences of the important crop species and sophisticated analytical tools, the association mapping approach is expected to provide more useful information to breeders allowing them to develop designer crops with the capability to sustain themselves during global climate change. This approach has been advanced further incorporating the benefits of both linkage mapping and association mapping, in an approach called NAM. In this approach, a large number of lines (founder lines) are crossed to a single genotype (reference line). The F_1 thus produced are selfed for 6–8 generations to generate a series of RIL families (Fig. 6.2). This multiparent mapping population is

Table 6.3 List of a few software packages commonly used for components of association analysis

Software	Source	Remarks
ASREML	http://www.vsni.co.uk/products/asreml	Basically designed for animal breeding data analysis. However, it can be used for plant breeding data. It uses a mixed model analysis system
EINGENSTRAT PCA	http://genepath.med.harvard.edu/~reich/Software.htm	It is used for association analysis, PCA
MTDFREML	http://aipl.arsusda.gov/curtvt/mtdfreml.html	It uses mixed model analysis of data primarily of animal breeding, can also be used for plant breeding data
R	http://www.r-project.org/	A generic software convenient for simulation work
SPAGeDi	http://ebe.ulb.ac.be/ebe/SPAGeDi.html	It is fit for genetic and relative kinship analysis
SAS	http://www.sas.com	It is also a generic software package used widely for data analysis
STRUCTURE	http://pritch.bsd.uchicago.edu/structure.html	It is widely used for analysis of population structure
TASSEL	http://www.maizegenetics.net	It is used for association analysis, analysis of LD; has a component of linear and mixed model for data analysis

gaining popularity among breeders for its applicability to creating the wider variability necessary in improving crops species for wider adaptation.

6.6.4 Multiparent Advanced Generation Intercross

Most of the traits of agronomic importance are polygenic, i.e., controlled by polygenes. Such traits are difficult to analyze for their effects and inheritances. Since these traits are governed by polygenes each with small, similar and additive effect, they hence show a continuous variation in the segregating populations. Most importantly, the effects of such genes are not pronounced enough and hence are highly influenced by the environment making estimation of genetic effects more complex. Therefore, complex statistical and biometrical approaches are applied in the analysis of such traits (commonly called quantitative traits). Genetic variations expressed by such traits are an important source for genetic analysis. For genetic studies and, more particularly for molecular mapping and analysis of QTLs, a series of synthetic population, viz., backcross, RILs, and doubled-haploid (DH) lines are used. Usually, such populations (except DH) are developed by crossing two diverse genotypes. The F_1 generation is either selfed (to produce F_2) or backcrossed with one of the parents (usually the recurrent parent) to produce backcross populations. The resultant generations starting from F_2s may be advanced through the single

seed descendent (SSD) approach to produce RIL. These populations, called mapping populations have their own niche and limitations. The most serious limitation is the limited variability i.e., the variability is limited to present in the two parents only. Further, the resolutions with which the QTLs are mapped are statistically poor. QTLs identified by these mapping populations have confidence intervals of 5–20 cM (Wilson et al. 2001; Louder et al. 2002; Ungerer et al. 2002), which corresponds on average to 1.2–4.8 Mb (million base pairs) covering hundreds of candidate genes. To overcome such limitations, a series of populations have been developed by various workers over a period of time. The unique feature of these new generation mapping populations is that they involve more than two genotypes (parents) in their population, and are amenable to replications over time and place that improves the power and resolution of QTL mapping significantly. One such mapping population is MAGIC (Kover et al. 2009) (Fig. 6.3). In this population, a number of accessions (founder lines) are intermated in a complete diallel fashion i.e., each accession is crossed with all other accessions as a maternal and paternal parent. The F_1 thus produced are then intermated through four generations of random mating. From the F_4 families, 2–3 MAGIC lines (MLs) are derived by selfing for four to six generations. Precautions are to be taken to avoid assortative mating through staggered planting or planting the same families multiple times (see Scarcelli et al. (2007) for the detail procedure). The MLs thus derived are subjected to phenotyping in replicated trials and genotyping with molecular markers for detection of SNPs. Through appropriate statistical treatment and uses of analysis software, QTLs are detected with higher resolution. This approach does not necessitate repeated genotyping and allows study of trait correlations, genotype-by-environment interactions, and the genetic basis of phenotypic plasticity. As it is developed through several intercrossings, and can be replicated unlimitedly, associations are located with greater accuracy and, QTLs are mapped with higher resolution. This has successfully been utilized to map QTLs for bolting time, days to germinate, and glabrous and erecta traits in *A. thaliana* (Kover et al. 2009). MAGIC populations are being developed in the UK and CSIRO, Australia to incorporate a large proportion of the genetic diversity within elite wheat varieties from around the world. It also enables the discovery of the best combinations of genes for important traits. CSIRO has developed two MAGIC populations. The first is a four parent population and includes lines adapted to all breeding regions in Australia, with genetic diversity covering approximately 80 % of Australian material. The second population is an eight parent population and includes cultivars from six countries (Australia, Canada, China, Israel, United States, and Mexico) (CIMMYT) (CSIRO 2011). Such MAGIC populations would enable genetic dissection of the QTLs and their deployment for the genetic improvement of crops.

6.7 Specific Issues for Future Breeding Activities

Breeding crop varieties especially suitable for climate-resilient agriculture should invariably consider a number of climatic factors in addition to the efficiency of the crops in utilizing energy, water, nutrients as well as radiation.

Radiation Use Efficiency. Basic research shows that crop productivity and adaptability can be increased many fold through manipulation of photosynthetic mechanisms. The C_4 plants (example, maize) are more efficient than the C_3 plants (example, rice) owing to their high carbon fixation efficiency. The increased concentration of atmospheric CO_2 owing to global air pollution has become beneficial to the C_3 plants. It is estimated that 0.3 % of the observed 1 % rise in global wheat production can be attributed to this increase (Fisher and Edmeades 2010). Reynolds et al. (2011) mentioned that crop productivity increase must achieve a number of broad objectives simultaneously (1) increase of crop biomass through modification of radiation use efficiency (RUE), (2) improvement of targeted adaptation of reproductive processes to major crop agro-ecosystems thereby permitting increases in RUE to be translated to grain weight; and (3) enhancement of the structural characteristics of the crop plants to ensure grain yield potential without sacrificing quality due to lodging. However, a multidisciplinary approach is needed to achieve the targets of crop genetic improvement in terms of its suitability to changing climatic conditions.

Nitrogen Use Efficiency. Usually, high-yielding varieties and hybrid crops are nutrient demanding; they need more chemical fertilizer, timely supply of water in addition to chemical protection from time to time. Over or indiscriminate application of chemical fertilizer, nitrogenous fertilizer in particular, has become a matter of concern in the light of water and environmental pollution. It has emerged as a key target in the changing climatic conditions (Tester and Langridge 2010). Therefore, for current breeding programs, it is important to develop crop varieties or hybrids that (1) need lesser amounts of nitrogenous fertilizer, and (2) are highly efficient in their use of the nitrogenous fertilizer. Enhancing or incorporating a biological nitrogen fixation (BNF) capability to the crop varieties could be an important option. Efforts in this direction are ongoing with encouraging progress reported.

Breeding for Zero Tillage Systems. The zero tillage approach is gaining popularity among crop producers as a conservation measure for the earth. It causes less damage to the soil structure and thus saves energy needed for field preparation to a greater extent. Its adoption and large-scale use is going to change the spectrum of diseases and insect pests attacking the crops. For such situations, newer breeding strategies have to be designed as the conventional approaches would hardly work. Similarly, breeding approaches have to be changed to meet the need of multiple cropping systems and cropping sequences, for which interactions between agronomists and the breeders is very important.

GM Crops. The production and evaluation of genetically modified (GM) crops, which permit generation of variations that may or may not be available in the natural population, will remain an active area of research in the near future. The power and success of this technology has been proved by the significant control of insect pests through deployment of a gene for proteinaceous toxins from the bacterial genome. Likewise, development of β-carotene-rich rice has brought hope for the technology as well as to humanity (Mayer et al. 2008). GM crops with tolerance to abiotic and biotic stresses have been developed in different parts of the world and are in different phases of testing. GM genotypes with tolerance to heat, drought, frost, etc., will be the focus of the future. However, the future focus should be the discovery and characterization not only of genes but also of promoters that provide accurate and stable spatial and temporal control of the expression of the genes (Moller et al. 2009). Similarly, development of marker-free transgenic crops should be on the agenda to avoid political obstructions. The GM technology has the promise to support plant breeding activities for climate-resilient agriculture and will inevitably be deployed for most major crops in the future (Tester and Langridge 2010).

Targeted Genome Editing Technology. Transfer of genes/QTLs from unadapted genotypes to an elite genotypic background is often expensive, time consuming, and also suffers from the problem of linkage drag. However, recently developed technologies viz., zinc finger nucleases (ZFN) (Weinthal et al. 2010) and transcription activator-like effector (TALE) nucleases (Bogdanove and Voytas 2011) have the potential to tackle such problems. These technologies involve the use of sequence-specific designer nucleases that cleave targeted loci, enabling creation of small insertions and deletions (indels), insertion of novel DNA, or even replacement of individual alleles. The usefulness of these techniques has been established even in crops (Shukla et al. 2009; Morbitzer et al. 2010). Perhaps, it would not be impossible to target the replacement of deleterious mutations in elite breeding lines (Morrell et al. 2012). Such technologies should open up a path to develop crops that are suitable for the changing climatic situations.

6.8 Conclusion

To be precise, there is no single solution to the problem of climate change; nor is there any single plant breeding approach to develop crop varieties capable of countering all climatic hazards that are envisaged as a consequence of changing climatic variables. However, the process of breeding climate-resilient crops would include a few obvious considerations (1) generation of variability: the variants with desirable traits can either be searched for in the available germplasm or created through hybridization, mutation, or transgenic approaches; (2) testing, analysis, and identification: the variability generated or collected needs to be tested for its fitness to the changed climatic variables. For this matter, artificial screening facilities or

phenomic facilities would be useful. The improved plant breeding approaches including molecular tools and techniques, and the genomic approaches would be of great use to identify the target plant or progenies; (3) human resource development: imparting training to research personnel on modern tools and techniques needs to be an important component of such efforts; (4) utilization: finally the tested genotypes should be multiplied and distributed to the end users for wide application. For large-scale adoption, demonstrations can help. In India, such an effort has already been initiated (National Initiative for Climate Resilient Agriculture, NICRA) as a preparatory step towards containing the hazards of climate change in agriculture. Such governmental initiatives and policy support mechanisms are necessary to make the technologies available and accessible to even poor users across the world. In this regard a global initiative is required.

It can be said that a coordinated approach of conventional and modern plant breeding processes coupled with crop management and policy planning can find ways to develop crop varieties and agricultural techniques that can increase crop production and sustain it even under severe environmental conditions. While doing so, the necessity should be to identify and exploit effective alleles at multiple loci through appropriate phenotypic, genotypic, and molecular approaches of selection (Christopher et al. 2007; Crossa et al. 2007). Secondly, to accumulate and utilize greater numbers of favorable alleles, approaches like MARS and genomic selection (GS) should be given due consideration. GS has shown savings of 13 years in the release of improved germplasm in oil palm as compared to conventional approaches (Wong and Bernardo 2008). Such approaches with power, precision, and efficiency should be the foci for crop breeding in climate-resilient agriculture. Thus, it is clear that more is required than what can be provided by traditional breeding approaches alone. Policies should be in place worldwide to make the technologies economically affordable by all sections of users globally. Thus, new genomic and breeding technologies must be developed and employed to accelerate the breeding processes so as to meet the goal of feeding billions of people on earth.

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Chapter 7
Genetic Engineering for Tolerance to Climate Change-Related Traits

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Abstract Climate change is expected to introduce new challenges for sustainable crop production worldwide. High temperature, less water availability, and emergence of new pests and pathogens calls for changing strategies and using biotechnological interventions to meet these challenges to sustaining food supply. Engineering biotic and abiotic stress tolerance will require concerted and combined efforts by plant breeders and biotechnologists alike. Several genes have been identified to have potential in mitigating climate change effects. These can be broadly classified as single-action genes and multiple action genes. Single action genes include osmoprotectants, detoxifying, LEA, HSP, ANPs, and ion transporters which have incremental roles in providing abiotic stress tolerance. Multiaction regulatory genes provide an attractive strategy to improve crop plants as these genes activate a cascade of genes which act together to enhance stress tolerance. CBF/DREB, SNAC, MYB, HSF, and AREB are some candidate genes of this category. Signal transduction genes such as osmosensors, AHK1, SNF1-related kinases are potential candidate genes for engineering stress tolerance in the near future. For insect resistance cry genes will remain the ideal choice however, engineering biotic resistance will involve new technologies such as RNAi and micro RNAs for combating insects and pests. Regulatory genes and genes involved in signal transduction will assume great importance in developing cultivars adapted to the threats of climate change. Here we review the target traits and potential genes for engineering stress tolerance in crop plants to meet climate change challenges for food production.

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7.1 Introduction

Climate change threatens sustainable agriculture with its rapid and unpredictable effects, making it particularly difficult for agricultural scientists and farmers to respond to the challenges from biotic and abiotic stresses. Harsh and unpredictable weather patterns also, can affect the most volatile regions of the world and leave them more vulnerable to instability due to greater hunger and poverty. Global warming is causing changes in temperature at a rate unmatched by any temperature change over the last 50 million years making it all the more important how well agricultural production can be maintained. Farmers everywhere throughout history have adopted new crop varieties to cope with changing environment. However, climate change brings these changes at an astonishing pace. Climate change beside its direct effects on weather will increase both abiotic stresses such as drought, heat, and water-logging; and biotic stresses such as pests and pathogens that affect agricultural systems. The biggest and largely unknown concern, however, are the effects that interactions among various stresses will have on crops and cropping systems making the task of feeding the 9 billion world population by 2050 extremely difficult. It calls for concerted efforts in sustaining food production to meet this challenge. Aspects of climate change that may affect future crop production include changes in both mean and extreme temperatures and changes to available water. Improving adaptation requires development of plants and associated production and management systems to cope with or avoid climate effects. Many complex processes and interactions determine crop yield under climate change and associated strategies should be built over adopting new technologies such as biotechnology.

The green revolution was a turning point in the history of world food production, causing an increase in global food grain production from 876 million tons in 1961 to 2,432 million tons in 2010 (FAOSTAT 2011). But the pace of increase in food production is less than the rate of increase in world population. Hence, food production must be increased dramatically to ensure food security for the world's hungry. It is estimated that food production will have to be increased by 70 % to adequately feed a global population expected to reach 9.1 billion in 2050. In the context of global climate change, which is adversely affecting agriculture, it will not be possible for agricultural production to keep pace with the rate of increase in world population under scarce resources and adverse environmental conditions in the future.

7.2 Abiotic and Biotic Challenges for Crop Production Under Climate Change

Apart from challenges of extreme temperature stress, drought and flooding associated with climatic variability, the incidence and severity of biotic stresses such as pests, diseases, and the invasion of alien weed species will also increase due to climate change. The contribution of plant breeding towards resilience in agriculture in the future is difficult to predict as variability is not present in the crop germplasm for use in the breeding programs (Newton et al. 2011). Biotechnological interventions along with changes in agronomic practices may offer potential solutions to this problem.

7.2.1 Drought and Salt Stress

Climate change is associated with increased water stresses in many regions due to changes in rainfall pattern causing increased temperature under low relative humidity thereby reducing water use efficiency. Frequency and severity of drought are expected to increase in the future, particularly under higher emissions scenarios (Meehl et al. 2007). This will limit the productivity of more than 50 % of the arable land in the next 50 years, and competition between urban and agriculture for water will make the problem worse. The use of saline and brackish water could help alleviate the world's water scarcity. There is an urgent need for crop varieties and cropping systems that conserve water consumption and sustain yields during periods of water shortage. However, developing these varieties of crops is difficult due to the interplay of crop responses to drought at the genomic, biochemical, and physiological levels.

7.2.2 Heat Stress

It is a major factor influencing the yields of cereals and legume crops. Higher temperatures can lead to warmer, less severe winters, which shorten the grain-filling period. Crop productivity is expected to increase at higher latitudes for global average temperature increases up to 1 to 3 °C depending on the crop; on the contrary, it is likely to decline for even small global temperature rises at lower latitudes, especially in the dry tropics. Higher temperatures also cause plants to need adequate water to keep cool making rising temperatures and water availability critical for plant response to climate change. But fruits, vegetables, and grains can suffer even under well-watered conditions if temperatures exceed the maximum level for pollen viability and seed set (Hatfield et al. 2008). Temperature increases

will cause the optimum latitude for crops to move northward; decreases in temperature would cause shifts towards the equator.

The amount and timing of precipitation and changes in the length of season, affected by climate change are also critical, and will affect crops differently. Night-time temperatures have been rising faster than day-time temperatures and some crops are particularly sensitive to high night-time temperatures. Warm nights increase the respiration rate and reduce the amount of photosynthetes in the fruit or grain. Common snap beans show substantial yield reduction when night-time temperatures exceed 80 °F (Hatfield et al. 2008).

7.2.3 Elevated Carbon Dioxide Levels

Carbon dioxide is essential for the carbohydrate synthesis that is required for plant metabolism, crop productivity, and yield. According to the Intergovernmental Panel on Climate Change (IPCC), there has been a steep rise in carbon dioxide concentrations over the past two centuries and it may reach 450–1,000 μmol/L of air by the end of this century, which may likely enhance photosynthesis and overall crop productivity. The higher productivity may be offset by pressure from insects and fungal infections, ozone gas, and variable precipitation, even though the extent to which this occurs will depend on the physiological and biochemical responses of each crop. Higher carbon dioxide levels make plants grow larger but they are often less nutritious. Carbon dioxide also makes some plants more water-use efficient and well adapted to water-limited areas with less than normal rainfall amounts (Hatfield et al. 2008).

7.2.4 Ozone Depletion

Ozone is an important greenhouse gas and plant pollutant that steadily increases due to fossil fuel combustion. Crop leaves absorb O_3 gas during photosynthesis that reduces photosynthetic rates causing leaf death thus adversely affecting crop productivity. Presently, global relative yield losses are estimated to range between 7 % and 12 % for wheat, between 6 % and 16 % for soybean, between 3 % and 4 % for rice, and between 3 % and 5 % for maize due to depletion of the ozone layer. For the year 2000 global crop yield loss due to ambient ozone was estimated to be worth US$14–26 billion. About 40 % of this damage is occurring in China and India (Dingenen et al. 2009). Crop yield loss due to the ambient ozone in the USA was estimated to be worth US$3–5 billion annually (Fiscus et al. 2005).

7.2.5 Flooding and Submergence Stress

One of the most pronounced effects of climate change is the increase in heavy downpours. Precipitation has become less frequent but more intense. Field flooding during the growing season causes crop losses due to low oxygen levels in the soil, increased susceptibility to root diseases, and increased soil compaction due to the use of heavy farm equipment on wet soils. The flooding caused severe erosion in some areas and also an increase in runoff and leaching of agricultural chemicals into surface water and groundwater (Wolfe et al. 2007).

7.2.6 Biotic Stress

Biotic stress caused by bacteria, weeds, insects, fungi, and viruses will affect cropping systems. Temperature is ranked as the most important parameter in determining how insects affect crop yields and some insect species such as flea beetles display signs of overwintering due to warmer winter temperatures. The pathogenic bacteria, fungi, and viruses also respond quite well to temperature as well as to humidity and rainfall. Therefore, as the growing season gets longer and winters become more moderate due to climate change, pressures from weeds, microbes, and insect pests are expected to rise due to enhanced capacity for overwintering, greater mobility, and expanded adaptation zones. Many insect pests and diseases of crop plants thrive due to warming, increasing losses in crop production and thereby necessitating greater pesticide use. Warming aids insects and diseases in several ways. Rising temperatures allow both insects and pathogens to expand their ranges northward. In addition, rapidly rising winter temperatures allow more insects to survive over the winter, whereas cold winters once controlled their populations. Some of these insects, in addition to directly damaging crops, also carry diseases that harm crops. Crop diseases in general are likely to increase as earlier springs and warmer winters allow proliferation and higher survival rates of disease pathogens and parasites (Frumhoff et al. 2007; Hatfield et al. 2008). The longer growing season will allow some insects to produce more generations in a single season, greatly increasing their populations. Finally, plants grown in higher carbon dioxide conditions tend to be less nutritious, so insects must eat more to meet their protein requirements, causing greater destructions to crops. Weeds benefit more than cash crops from temperatures and carbon dioxide levels. Controlling weeds currently costs the United States more than $11 billion a year, with the majority spent on herbicides (Kiely et al. 2004). So both herbicide use and costs are likely to increase as temperatures and carbon dioxide levels rise. At the same time, the most widely used herbicide in the United States, glyphosate (RoundUp®), loses its efficacy on weeds grown at carbon dioxide levels that are projected to occur in the coming decades. Higher concentrations of the chemical

and more frequent spraying thus will be needed, increasing economic and environmental costs associated with chemical use (Wolfe et al. 2007).

7.2.7 Combinations of Potential Environmental Stresses

Stress interactions that have a deleterious effect on crop productivity include drought and heat, salinity and heat, ozone and salinity, ozone and heat, nutrient stress and drought, nutrient stress and salinity, UV and heat, UV and drought, and high light intensity combined with heat, drought, or chilling (Heyne and Brunson 1940; Walter 1989; Weinstein et al. 1991; Miller and Timmer 1994; Welfare et al. 2002; Giraud et al. 2008; Hartikainen et al. 2009). Environmental interactions that do not have a deleterious effect on yield and could actually have a beneficial impact on the effects of each other include drought and ozone, ozone and UV, and high CO_2 combined with drought, ozone, or high light (Sullivan and Teramura 1990; Pääkkönen et al. 1998; Ainsworth et al. 2008). Drought and heat stress, two distinct abiotic stress conditions, occur in the field simultaneously, which causes higher detrimental effects on the growth and productivity of several crops than if each of the several stresses was confronted individually (Pnueli et al. 2002; Mittler 2006). Simultaneous drought and heat stress combine high respiration with low photosynthesis, low stomatal conductance, and high leaf temperature (Rizhsky et al. 2002, 2004). Transcriptome profiling also supports that plants subjected to the drought and heat combination require a unique acclimation response involving over 770 transcripts, not altered by drought or heat stress. Similar changes in metabolite and protein accumulation were also found, with several unique metabolites and at least 53 different proteins accumulating specifically during the stress combination. In addition, at least one plant gene, cytosolic *ascorbate peroxidise 1* (*Apx1*), was found to be specifically required for the tolerance of *Arabidopsis* plants to drought and heat stress combination (Koussevitzky et al. 2008).

The extent of damage caused to agriculture by stress combination underscores the need to develop crops with enhanced tolerance to a combination of abiotic stresses. Drawing upon the limited physiological, molecular, and metabolic studies performed with plants simultaneously subjected to two distinct abiotic stresses, it is not sufficient to study each of the individual stresses separately (Keles and Oncel 2002). A particular stress combination should be handled as a new state of abiotic stress in plants that requires a new type of acclimation.

Herbicide tolerance and insect resistance have been the dominant genetically engineered traits since commercial cultivation of transgenic crops in the early 1990s. Genetic engineering for biotic stress tolerance, although successful, will face novel challenges in the future in relation to feeding an ever-increasing world population from diminishing resources (Park et al. 2011). Therefore, it is important to understand the effects of climate change on causal organisms of biotic stresses like insects, bacteria, fungi, viruses, nematodes, and weeds and deliberate on potential strategies to ameliorate the adverse effects caused by climate change.

7.3 Effect of Climate Change on Causal Organisms of Biotic Stress

The effect of climate change on plant systems has to be considered in conjunction with the effects of climate change on insect pests, pathogens, and weeds (Gregory et al. 2009). This is because plant pathogens and pests are among the first organisms to show the effects of climate change due to high populations, ease of propagation and dispersal, and short generation cycle. Climate change can modify the occurrence and severity of diseases, arthropod pests, and weeds. The impact of climate change can be positive, negative, or neutral, since these changes can decrease, increase, or have no impact on plant diseases, pests, or weeds depending on region or period of time considered. Climate change can alter stages and rates of development of the pathogens (Coakley et al. 1999). It can also modify host resistance by changing the physiology of host–pathogen interactions. Similar interactions are possible in the case of insects and weeds. Shifts in the geographical distribution of hosts and pathogens are also possible (Walther et al. 2002). The impact of climate change on these biotic stressors has to be considered at several layers of hierarchy, from genome to ecosystem, along with the corresponding complexity of their relationships (Savary et al. 2011).

7.3.1 Effect of Climate Change on Insects

Insects are cold-blooded organisms. Therefore, change in temperature may act as the most important climatic factor influencing insect behavior, distribution, development, survival, reproduction, geographic range, and population size (Bale et al. 2002). It is estimated that with an increase in temperature by 2 °C, there might be five additional life cycles of insect per season (Yamamura and Kiritani 1998). Because of an increase in winter temperature, the mortality of insects in the winter season is reducing, which is also resulting in the increase of insect populations (Harrington et al. 2001). It has also been speculated that CO_2-temperature and CO_2-precipitation interactions may act as the key components to determine plant damage by pests in the future (Zvereva and Kozlov 2006). Overall, climate change can affect the population dynamics of insect pests of crop plants through increasingly more types and higher number of insects (Woiwod 1997). Moreover, it has also been shown that the effectiveness of some classes of pesticides such as pyrethroids in controlling insects is reduced at higher temperatures (Musser and Shelton 2005).

7.3.2 Effect of Climate Change on Pathogens

As climate change affects evolution rate, many pathogen characteristics like frequency of generations, proportion of sexual reproduction, and the rate of adaptation also change. Change in environment will definitely impact the plant disease triangle involving the hosts, pathogens, and environment. Temperature has an impact on plant diseases through both the host crop plant and the pathogen behavior. It has been shown that wheat and oat become more susceptible to rust disease with increase in temperature (Coakley et al. 1999). Generally, disease-causing fungi will be invasive at moderately high temperatures. Moisture can affect both host plants as well as pathogens. Many climate change models predicted that higher atmospheric moisture with increased temperature could favor pathogen and disease development. Efficiency of fungicides and bactericides may change with a change in climatic factors. Contact fungicides may get washed away due to frequent rainfall events. Systemic fungicides may be affected negatively by physiological changes like slow uptake due to smaller stomata opening or thicker epicuticular waxes. Climate change may diversify the areas of pathogens, new species may be introduced in the region, new hosts may boost pathogen inoculum levels, and new vectors may alter epidemic dynamics (Garrett et al. 2006). Thus, an increase in climate extremes may promote early, invasive, and repetitive plant disease and pest outbreaks by a wide variety of pathogens (Alig et al. 2002; Gan 2004).

7.3.3 Effect of Climate Change on Weeds

Weeds do not have selection pressure like crop plants, because of the huge genetic diversity in weeds compared to crops. Therefore, changes that occur in environmental conditions like light, water, nutrients, or CO_2 may result in more growth and reproductive responses in weeds. Along with agronomic weeds, there are other more noxious or invasive weeds, which propagate in the vegetative mode. Weeds show a strong response to increase in atmospheric CO_2, and there will be competition between C_3 and C_4 weed species under increased CO_2 concentrations in the future (Ziska et al. 2004). The increase in temperature is also expanding the habitat of weeds into new geographical locations. It is speculated that Witch grass, a robust grass weed, could invade the Central Midwest and California with a 3 °C warming trend (Patterson 1995). At present, Witch grass, a root parasite of maize, is limited to the coastal plain of North and South Carolina, but it may occupy the whole Corn Belt with an increase in temperature by 3 °C. Increase in temperature along with drought-like conditions in some regions may result in thicker cuticle development and increased leaf pubescence, which may reduce herbicide penetration. A change in climatic conditions can alter the efficacy of weed biocontrol agents by potentially altering development, morphology, and reproduction of the target pests (Ziska et al. 2003).

Overall, climate change affects weed biology and weed management in the future will be a more serious problem.

7.4 Biotechnology to Breed New Cultivars for Combating Climate Change

Drought and high temperature are considered to be key stress factors where scientists should expect to see the effects of climate change on plants. With unpredicted high precipitation, flooding, and submergence, stress tolerance is also considered an important target trait. With the knowledge and tools, plant breeders and biotechnologists are expected to make the best use of the huge global effort to meet the challenges posed by climate change. Breeding cultivars that combine drought and temperature resistance will require introgression of stress-tolerance genes from landraces and wild crop relatives to commercial cultivars and evaluating them in a matrix of stress environments. Climate change mitigation will involve development of drought- and temperature-tolerant crop cultivars. The present cultivars recommended for use might not be suitable if the climate changes. However, breeding for a new cultivar usually takes 10–12 years, if the target traits are known. In general, the genetic variation within well-established commercial crop cultivars will allow breeding and selection for traits that deliver the necessary crop adaptation to climate change for only short-term gains.

Plants sense, respond, and adapt to abiotic stresses at the molecular, biochemical, physiological, and whole-plant levels. Stress avoidance is believed to be one of the major mechanisms that plants have evolved and breeders have selected for among targeted traits for drought resistance. Another strategy of maintaining cellular metabolism and plant function during water stress is stress tolerance, i.e., mechanisms observed in resurrection plants. Stress escape is the third major strategy, and has been successfully exploited by introducing early flowering alleles in wheat germplasm to enable biomass accumulation and flowering before the period of major drought during grain fill. Adoption of any combination of these drought-resistance strategies requires a balance between reduced water use and maximizing yield potential.

Breeding crops for climate change is a difficult task and it is anticipated that the application of genomic tools will help in identifying the number and type, as well as the nature of dominance and epistatic interactions, of genes underlying a given quantitative genetic trait. The need to exploit functional genomics for breeding in crops has also led to the establishment of various high-throughput technologies for the study of gene expression such as differential display, DNA microarray, SAGE (serial analysis of gene expression), oligoarrays, EST sequencing, and RNA sequencing. The classic molecular biology approach has allowed identification of candidate genes involved in stress tolerance. Proteins involved in signaling (such as protein kinases) or the control of gene expression (such as transcription factors) are

often favored as they control a number of stress-related genes and have wide-ranging effects. The candidate genes are then tested by modifying their expression in transgenic plants.

7.5 Engineering Abiotic Stress Tolerance in Crop Plants

Plants are exposed to different abiotic stresses that affect their growth, development, and productivity. Being mostly multigenically controlled as any other quantitative traits, it is a challenge to understand the molecular basis of abiotic stress tolerance and to manipulate it as compared to biotic stresses. Abiotic stress can result in the increased synthesis of secondary metabolites and signaling molecules, ion fluxes, an oxidative burst and changes in the transcription of an array of genes. The complex plant response to abiotic stress, which involves many genes and biochemical-molecular mechanisms, is schematically represented in Fig. 7.1. With exception to flooding, most abiotic stresses result in water deficit. Therefore, water deficit is the foremost abiotic stress. The gene products produced due to the genes induced during stress conditions are classified into two groups. The first group includes proteins that probably function in stress tolerance, such as chaperones, LEA (Late embryogenesis abundant) proteins, osmotin, antifreeze proteins, mRNA binding proteins, key enzymes for osmolytes biosynthesis, water channel proteins, sugar and proline transporters, detoxification enzymes, and various proteases. The second group contains protein factors involved in further regulation of signal transduction and gene expression that probably functions in stress response: protein kinases, transcription factors and enzymes in phospholipid metabolism. As drought stress is a multigenic or quantitative trait, traditional plant breeding has not been successful in producing abiotic stress-tolerant cultivars.

A high degree of crosstalk exists between these pathways, as often the plant's physiological and cellular responses to different abiotic stresses are similar. In drought and cold stress for example, two types of molecular responses occur simultaneously: those protecting cells from acute dehydration and those protein factors involved in further regulation of gene expression and signal transduction functioning in overall stress response (Shinozaki and Yamaguchi-Shinozaki 2000, 2007). Other examples include crosstalk between cold and osmotic signaling pathways, as well as cold and abscisic acid (Ishitani et al. 1997). Abscisic acid is a crucial signaling molecule in plant stress responses and it regulates stomatal aperture change. Stress-induced proteins with known functions such as water channel proteins, key enzymes for osmolyte (proline, betaine, sugars such as trehalose, and polyamines) biosynthesis, detoxification enzymes, and transport proteins were the initial targets of plant transformation.

Metabolic traits, especially pathways with relatively few enzymes, have been characterized genetically and appear more amenable to manipulations than structural and developmental traits. However, this approach will overlook the fact that abiotic stress tolerance is likely to involve many genes at a time, and that single-gene

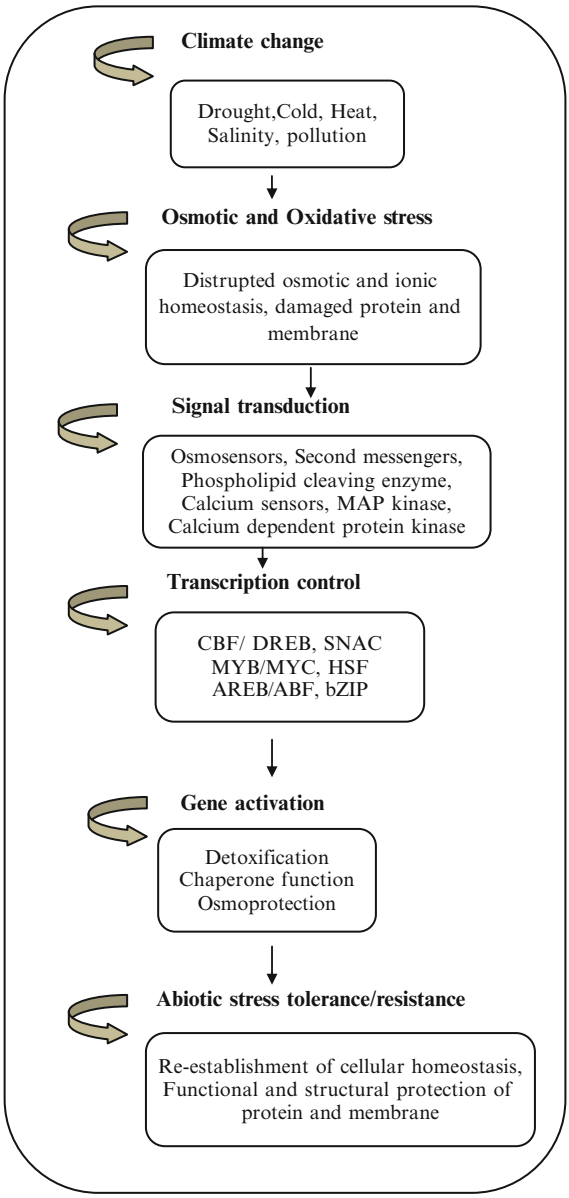

Fig. 7.1 Plant response to abiotic stress to develop tolerance or resistance

tolerance is unlikely to be sustainable (Bhatnagar-Mathur et al. 2008). Therefore, a second "wave" of genetic engineering attempts to transform plants with stress-induced regulatory proteins. Through these proteins, many genes involved in stress response can be simultaneously regulated by a single gene encoding stress-inducible transcription factors (Kasuga et al. 1999), thus offering the possibility of enhancing tolerance towards multiple stresses including drought, salinity, and freezing. It is

interesting to note that this "second wave" has also coincided with a better integration of genetic engineering and plant physiology.

7.5.1 Single-Action Genes

With the advancement of high-throughput DNA technologies, several hundred stress-induced or upregulated genes have been identified specially in *Arabidopsis*, however, the function of only a limited number of gene products are known (Shinozaki and Yamaguchi-Shinozaki 1997). Several stress-associated genes have been evaluated for their contribution to drought or salt tolerance in laboratory studies and some of these genes have been utilized for improving stress tolerance in crop plants through genetic engineering. The plant stress tolerance has recently boosted the identification of stress response genes largely due to the availability of *Arabidopsis* and rice genome sequences. High-throughput transcript and protein analysis has enriched our knowledge in mechanism of stress-activated gene expression. *Arabidopsis* full-length cDNA microarrays have identified novel stress-inducible genes. The high-salinity-inducible genes are also induced by dehydration, emphasizing an extensive overlap of dehydration and salt stress. Global expression profiles also provide an overview of those genes, which are downregulated by dehydration or salt stress. Some of these genes are of direct adaptive value. For example, downregulation of proline dehydrogenase is important for proline accumulation under osmotic stress conditions. In addition, some of the downregulated genes may include negative regulators of stress responses.

7.5.1.1 Osmoprotectants

Severe osmotic stress causes detrimental changes in cellular components. In stress-tolerant transgenic plants, many genes involved in the synthesis of osmoprotectants-organic compounds such as amino acids (e.g., proline), quaternary and other amines (e.g., glycinebetaine and polyamines), and a variety of sugars and sugar alcohols (e.g., mannitol, trehalose, and galactinol) that accumulate during osmotic adjustment have been used so far (Vinocur and Altman 2005). Many crops lack the ability to synthesize the special osmoprotectants that are naturally accumulated by stress-tolerant organisms. Osmoregulation is believed to be the best strategy for abiotic stress tolerance, especially if osmoregulatory genes could be triggered in response to drought, salinity, and high temperature.

Various strategies are being pursued to genetically engineer osmoprotection in plants. The first step involved in obtaining stress-tolerant transgenic plants has been to engineer genes that encode enzymes for the synthesis of selected osmolytes (Bray 1993). Reports involving osmoprotectants including glycine-betaine (Ishitani et al. 1997; Sakamoto et al. 2000; Chen and Murata 2011) and proline (Delauney and Verma 1993; Yamada et al. 2005; Szabados and Savouré 2010) have been

published. Sugar alcohols such as mannitol, trehalose, myo-inositol, and sorbitol have been targeted for the engineering of compatible-solute overproduction, thereby protecting the membrane and protein complexes during stress (Tarczynski et al. 1993; Zhao et al. 2000; Garg et al. 2002; Cortina and Culiánez-Maciá 2005; Patra et al. 2010). Similarly, transgenics engineered for the overexpression of polyamines have also been developed (Roy and Wu 2001, 2002; Waie and Rajam 2003; Capell et al. 2004; Quinet et al. 2010; Alcazar et al. 2010).

There are also some reports showing a negative effect of osmotic stress on yield potential (Fukai and Cooper 1995). Genetic manipulations of compatible solutes do not always lead to a significant accumulation of the compound (except in some cases of proline overproduction) (Chen and Murata 2002; Hong-Bing 2011), thereby, suggesting that the compatible solutes may not always confer stress tolerance. Besides, the results of simulation modeling also suggest that changes in a given metabolic process may end up with little benefit for actual yield under stress (Sinclair et al. 2004). Oversynthesis of osmolytes, if it happens at primary metabolic cost, may not be favorable and should be stress-inducible and/or tissue-specific promoter driven (Garg et al. 2002). Turner et al. (2007) have also shown that osmotic adjustment provided no beneficial effect on yield under drought stress in chickpea.

7.5.1.2 Detoxifying Genes

In most of the aerobic organisms, there is a need to effectively eliminate reactive oxygen species (ROS), which is generated as a result of environmental stresses. ROS are a product of altered chloroplast and mitochondrial metabolism during stress. These species cause oxidative damage to different cellular components including membrane lipids, proteins, and nucleic acids. In order to control the level of ROS and oxidative injury, plants have an antioxidant defense system to scavenge the ROS. These antioxidant systems include various enzymes and nonenzymatic metabolites that may also play a significant role in ROS signaling in plants and could provide enhanced plant resistance to salt stress (Vranova et al. 2002). Ascorbic acid and reduced glutathione employ various enzymes such as superoxide dismutases (SOD), catalases (CAT), ascorbate peroxidases (APX), glutathione S-transferases (GST), and glutathione peroxidases (GPX) to scavenge ROS. The detoxification strategy involves use of these genes for engineering abiotic stress tolerance. Transgenic tobacco overexpressing SOD in the chloroplast, mitochondria, and cytosol have been generated (Bowler et al. 1991; Van Camp et al. 1996) and these have been shown to enhance tolerance to oxidative stress induced by methyl viologen (MV) in leaf disc assays. Overexpression of chloroplast Cu/Zn SOD showed a dramatic improvement in the photosynthetic performance under chilling stress conditions in transgenic tobacco (Sen Gupta et al. 1993) and potato plants (Perl et al. 1993). Overexpression of MnSOD in chloroplasts of alfalfa (*Medicago sativa*) transgenic plants showed lower membrane injury (McKersie et al. 1996), while the tobacco transgenic plants overproducing the

alfalfa *aldose reductase gene* (*MsALR*) showed lower concentrations of reactive aldehydes and increased tolerance against oxidative agents and drought stress (Oberschall et al. 2000).

7.5.1.3 Lipid Biosynthesis Multifunctional Genes

Multifunctional genes are those genes that improve photosynthesis under abiotic stress conditions through changes in the lipid biochemistry of the membranes (Grover and Minhas 2000). Tobacco plants overexpressing the chloroplast *glycerol-3-phosphateacyltransferase* (*GPAT*) gene (involved in phosphatidyl glycerol fatty acid desaturation) from squash (*Cucurbita maxima*) and *A. thaliana* (Murata et al. 1992) showed an increase in the number of unsaturated fatty acids and a corresponding decrease in the chilling sensitivity. Additionally, transgenic tobacco plants with silenced expression of chloroplast w3-fatty acid desaturase (*Fad7*, which synthesizes trienoic fatty acids) were able to acclimate to high temperature as compared to the wild type (Murakami et al. 2000).

7.5.1.4 Late Embryogenesis Abundant Proteins

LEA and LEA-type genes are found universally in plants and LEA proteins are produced in response to dehydration stress and function in stabilization, protection of cytosolic structures, ion sequestration, protein renaturation, transport of nuclear targeted proteins, prevention of membrane leakage, and membrane and protein stabilization. They accumulate in seeds during the late stages of embryogenesis and are associated with the acquisition of desiccation tolerance under drought, heat, cold, salt, and ABA stress (Sivamani et al. 2000; Bartels and Sunkar 2005). LEA proteins are divided into groups based on conserved sequence motifs. Five of these groups have been characterized at the molecular and structural level. Amongst the several groups of LEA proteins, those belonging to group 3 are predicted to play a role in sequestering ions that are concentrated during cellular dehydration. These proteins have 11-mer amino acid motifs with the consensus sequence TAQAAKEKAGE repeated as many as 13 times (Dure 1993). The group 1 LEA proteins are predicted to have enhanced water-binding capacity, while the group 5 LEA proteins are thought to sequester ions during water loss. Constitutive overexpression of *HVA1*, a group 3 LEA protein from barley conferred tolerance to soil water deficit and salt stress in transgenic rice plants (Xu et al. 1996). Constitutive or stress-induced expression of the *HVA1* gene resulted in the improvement of growth characteristics and stress tolerance in terms of cell integrity in wheat and rice under salt- and water-stress conditions (Sivamani et al. 2000; Rohila et al. 2002). Transgenic rice (TNG67) plants expressing a wheat LEA group 2 protein (*PMA80*) gene or the wheat LEA group 1 protein (*PMA1959*) gene resulted in increased tolerance to dehydration and salt stresses due to low water use efficiency (Cheng et al. 2002).

7.5.1.5 Transporter Genes

Plants have to re-establish homeostasis under stressful environments, restoring both ionic and osmotic homeostasis. This has been a major approach to improve salt tolerance in plants through genetic engineering, where the target is to achieve Na^+ excretion out of the root, or their storage in the vacuole. A number of abiotic stress-tolerant transgenic plants have been produced by increasing the cellular levels of vacuolar antiporter proteins. Transgenic melon (Bordás et al. 1997) and tomato (Gisbert et al. 2000) plants expressing the *HAL1* gene showed salt tolerance as a result of retaining more K^+ ions than the control plants under salinity stress. A vacuolar chloride channel, the *AtCLCd* gene, which is involved in cation detoxification, and the *AtNHXI* gene, which is homologous to the *NhxI* gene of yeast, has been cloned and overexpressed in *Arabidopsis* to confer salt tolerance by compartmentalizing Na^+ ions in the vacuoles. Transgenic *Arabidopsis* and tomato plants that overexpress *AtNHX1* accumulated abundant quantities of the transporter in the tonoplast and exhibited substantially enhanced salt tolerance (Apse et al. 1999; Zhang and Blumwald 2001). The Salt Overly Sensitive 1 (SOS1) locus in *A. thaliana*, similar to the plasma membrane Na^+/H^+ antiporter from bacteria and fungi, when overexpressed provided a greater proton motive needed for elevated Na^+/H^+ antiporter activities (Shi et al. 2003). In contrast, the sequestration of excess Na^+ into the vacuole is mediated by the vacuolar membrane-localized Na^+/H^+ antiporter, AtNHX1 (Shi et al. 2000). Studies of *Arabidopsis* plants carrying mutations in *AtHKT1;1* revealed that Na^+-selective transport via AtHKT1;1 has an essential role in Na^+ exclusion from leaves and K^+ homeostasis in leaves during salinity stress (Davenport et al. 2007).

7.5.1.6 Heat-Shock Protein Genes

Temperature stress (high temperature) is considered to be one of the major stresses on crop plants under climate change resulting in changes in the level of enzymes, cellular membrane structure, photosynthesis activity, and protein metabolism. The induction of genes encoding heat-shock proteins (Hsps) is one of the most prominent responses observed at the molecular level of organisms exposed to high temperature (Vierling 1991).

Genetic engineering for increased thermotolerance by enhancing heat-shock protein synthesis in plants has been achieved in a number of plant species, showing positive correlations between the levels of heat-shock proteins and stress tolerance (Sun et al. 2001; Wang et al. 2005a). In plants, HSFs are encoded by a large gene family with different expression patterns and functions. Overexpression of *HsFA2* led to the constitutive accumulation of galactinol and raffinose and improved the tolerance of *Arabidopsis* plants to different environmental stresses (Nishizawa et al. 2006, 2008; Ogawa et al. 2007). Similarly, overexpression of *HSF3*/*HsfA1b* enhanced thermotolerance (Prandl et al. 1998) and raffinose levels under normal

and heat stress conditions (Panikulangara et al. 2004). Genetic manipulation of Rubisco activase to improve its stability at high temperatures is also a potentially important target (Kurek et al. 2007). Superoxide dismutase and catalase have been shown to be associated with good thermotolerance.

7.5.1.7 Anaerobic Polypeptides

The most common situation arising from climate change is yield losses due to lack of water but there are considerable areas that are waterlogged. Waterlogging is not region or country specific, but it can be a problem for wheat growers worldwide, as it is sensitive to heavy wet soils. For cotton, if rainfall occurs during or after irrigation, waterlogging could be serious and cause up to 40 % yield losses. Similarly, rice, the second most-produced food crop is well adapted to flooding of roots and transports O_2 from the aerial parts to roots very efficiently, but problems arise when the whole plant is submerged. This is particularly true in tropical regions such as Eastern India, which are prone to monsoon flooding and it is ranked as the third greatest constraint to rice production. Canola and barley are also sensitive to waterlogging and suffer severe yield losses. Waterlogging and submergence tolerance lead to reduced gas exchange between plant and atmosphere causing hypoxic or anoxic conditions in the root zone. O_2, being vital for the central energy-providing pathway, controls the metabolic activity and energy production of plants. The oxidative phosphorylation pathway, which generates ATP, is adversely affected by flooding or waterlogging conditions. Other oxygen-dependent biochemical pathways are those that involve cytochrome oxidases and desaturases. Some microorganisms use alternate electron-acceptor enzymes rather than enzymes that use O_2 as the terminal acceptor. Plants also have limited adaptations for adjustment to low availability of ATP. Plants have three main fermentation pathways, i.e., ethanol, lactic acid, and plant-specific production of alanine from glutamate and pyruvate, which involves alanine amino transferase. These pathways do not operate under normal O_2 conditions but proceed via de novo induction by low O_2. Submergence-tolerant plants show that alcohol fermentation pathway, H^+ ATPase proton pump, and regulating cytosolic pH are important factors for survival under submergence conditions.

A number of anaerobic polypeptides (ANPs) have been identified, which have a role in submergence and flooding tolerance. These include enzymes (PDC, ADH, LDH, and AlaAT, etc.) involved in the fermentation pathways (Waters et al. 1991; Germain et al. 1997; Porterfield et al. 1997; Muench et al. 1998; Kato-Noguchi 2000; Rahman et al. 2001). These enzymes allow fermentation of carbohydrates to maintain ATP production in the absence of O_2 producing two molecules of ATP per mole of glucose instead of 36 moles produced by oxidative metabolism. This process starts with the activation of a signal transduction pathway, causing metabolic adaptation (induction of glycolytic and fermentation pathway genes) which causes an increase in ethylene production. A cell wall-loosening enzyme XETC (xyloglucan endotransglycosylases) is induced which facilitates loosening of the

cell wall and creation of aerenchyma, which is probably triggered by ethylene. The low-oxygen-induced genes contain an ARE (anaerobic response element) in their promoters. Maize *Adh1*, *Adh2* and *Arabidopsis ADH1*, *LDH*, *PDC* contain these elements and disruption of this motif abolishes gene expression or anaerobic induction of these genes. Studies on the identification/isolation/cloning of genes that are associated with improved flooding stress tolerance have also focused on enzymes of the glycolytic and alcohol fermentation pathways indicating that the respiratory pathway is affected in a major way in response to anaerobic stress. Research on genetically altering the levels of *pdc* and *adh* in tobacco and rice has been extensively carried out to elucidate their role in submergence tolerance. Transgenic rice overexpressing the *pyruvate decarboxylase 1* (*pdc1*) gene has also been developed, which showed a positive correlation of higher PDC activities with survival after submergence (Quimlo et al. 2000).

7.5.2 Multiaction Regulatory Genes

An attractive target category for manipulation and gene regulation is the small group of transcription factors that have been identified to bind to promoter regulatory elements in genes that are regulated by abiotic stresses (Shinozaki and Yamaguchi-Shinozaki 1997). Transcription factors that are a part of the regulon to help prevent the effects of abiotic stress have been constitutively overexpressed to promote a greater amount of tolerance bringing initial stress response. Many of the studies in commercial crops were based upon *Arabidopsis* because not only are structural proteins conserved, but entire stress-tolerance regulons too, making it a valuable model for biotechnological research. The transcription factors activate cascades of genes that act together in enhancing tolerance towards multiple stresses. Dozens of transcription factors are involved in the plant response to drought stress (Vinocur and Altman 2005). Most of these fall into several large transcription factor families, such as AP2/ERF, bZIP, NAC, MYB, MYC, Cys2His2 zinc-finger, SA-inducible DOF protein, and WRKY. Individual members of the same family often respond differently to various stress stimuli. Conversely, some stress-responsive genes may share the same transcription factors, as indicated by the significant overlap of the gene expression profiles that are induced in response to different stresses (Chen and Murata 2002). Transcriptional activation of stress-induced genes has been possible in transgenic plants overexpressing one or more transcription factors that recognize promoter regulatory elements of these genes.

Many genes that respond to multiple stresses like dehydration and low temperature at the transcriptional level are also induced by ABA, which protects the cell from dehydration (Skriver and Mundy 1990). In order to restore the cellular function and make plants more tolerant to stress, transferring a single gene encoding a single specific stress protein may not be sufficient to reach the required tolerance levels (Bohnert et al. 1995). To overcome such constraints, enhancing tolerance towards multiple stresses by a gene encoding a stress-inducible

transcription factor that regulates a number of other genes is a promising approach (Chinnusamy et al. 2005). Therefore, a second category of genes of recent preference for crop genetic engineering are those that switch on transcription factors regulating the expression of several genes related to abiotic stresses.

7.5.2.1 CBF/DREB

CBF (CCAAT-binding factor) is mainly involved in cold stress response and is conserved throughout the plant kingdom including plants that do not cold-acclimate (tomato and rice). The core motif of this *cis*-acting element is CCGAC and the TF that bind to it are CRT-binding factors or DRE-binding proteins. *DREB2* genes are constitutively expressed under normal and stress conditions but their target genes (*rd29a*, *rd29b*, *rd17*, and *LEA14*) are only induced upon dehydration. Two DREB1-like transcription factors, BNCBF-5 and -17 were engineered in *Brassica napus* that resulted in dwarfing and delayed flowering, however, the engineered plants were able to accelerate maximal freezing tolerance during cold acclimation (Savitch et al. 2005). Furthermore, increased rate of CO_2 assimilation and capacity to perform electron transport, and induced the accumulation of chlorophylls, photoprotective pigments were observed more in transformed plants than the wild-type. An *Arabidopsis* CBF1 inserted along with three copies of an ABA-responsive complex (ABRC1) promoter from the barley *HAV22* gene to improve salt, drought, and chilling tolerance in tomatoes resulted in significant tolerance for abiotic stress without a reduction in fruit number, seed number, and fresh weight per plant (Lee et al. 2003). Another positive feature of using the ABRC1-CBF1 was that gene expression was not constitutive. Transforming lettuce with the *Arabidopsis* ABA-responsive binding factor 3 (*ABF3*) gene resulted in drought and stress tolerance in the crop.

Sumoylation and ubiquitination has been found to be involved in transcription factor regulation under abiotic stresses. A recent study showed that a SUMO E3 ligase, SIZ1 and sumoylated ICE1 enhanced its activation of DREB1A/CBF3 (Miura et al. 2007) while DREB2 was shown to be regulated by DREB-INTERACTING PROTEIN 1 and 2 (DRIP1 and DRIP2), which are RING finger E3 ligases, through ubiquitination (Qin et al. 2008). ICE1 was demonstrated to be under the control of HIGH EXPRESSION OF *OSMOTICALLYRESPONSIVE GENE 1* (*HOS1*), another RING finger protein (Dong et al. 2006).

The ERF family comprises APETALA2 (AP2)-type transcription factors that recognize DRE/CRT and function in cold stress responses. ERF proteins share a conserved 58–59 amino-acid domain (the ERF domain) that can bind to two similar *cis*-elements: the GCC box, which is found in several PR (PATHOGENESIS-RELATED) gene promoters where it confers ethylene responsiveness, and the C-repeat (CRT)/dehydration-responsive element (DRE) motif, which is involved in the expression of dehydration- and low-temperature-responsive genes. ERF proteins from one plant species have been shown to function in other plant species, enhancing their potential utility in increasing the stress tolerance of plants

(Jaglo et al. 2001; Gu et al. 2002). However, constitutive overexpression of ERF genes generally causes deleterious effects and this problem can be overcome using stress-inducible promoters. This approach has been used successfully for the DRE binding factor DREB1A: *Arabidopsis* plants that overexpressed DREB1A no longer showed deleterious effects but showed enhanced protection against freezing, drought, and high salinity when the stress-inducible promoter was used (Kasuga et al. 1999).

7.5.2.2 SNAC Transcription Factor

The stress-responsive NAC1 (SNAC1) gene encodes a NAM, ATAF, and CUC (NAC) TF with trans-activation activity and is induced by drought, predominantly in guard cells. Plants overexpressing *SNAC1* do not show the common dwarf phenotype of those overexpressing *CBF*/*DREB1*, revealing a different stress response mechanism (Ito et al. 2006). The strong induction of *SNAC1* gene expression by drought in guard cells suggests an effect in stomatal closure. *SNAC1* also induces the expression of genes encoding proteins related to osmotic adjustment (sorbitol transporter and exoglucanase) and stability of cell membranes, which can be related to stress response. Transformed rice with SNAC1 under the control of a CaMV 35S promoter became more stress tolerant to drought and salt at vegetative stage (Hu et al. 2006). The expression levels in the transgenic plants were located mainly in the leaves where there was curling, which helped in closing stomata and inhibiting water loss. Recently, overexpression of *SNAC2*/*OsNAC6* in rice improved drought and salt tolerance of transgenic plants because of the enhanced expression of a large number of genes encoding proteins with predicted stress-tolerance functions such as detoxification, redox homeostasis, and proteolytic degradation as well. Expression of *SNAC2*/*OsNAC6* is induced by drought, salinity, cold, wounding, and ABA treatment, but decreased by methylation and deacetylation (Nakashima et al. 2007; Hu et al. 2008).

7.5.2.3 MYB Transcription Factor

MYB proteins are widely distributed in plants and have been implicated in the ABA-response, enhancing the sensitivity to ABA and drought tolerance. The promoter region of RD22 (responsive to dehydration) contains MYC (CANNTG) and MYB (C/TAACNA/G) *cis*-element recognition sites (Abe et al. 2003). The MYB protein can also interact with other transcription factors. The MYB transcription factor represents a family of proteins which include a conserved domain, the MYB DNA-binding domain. In contrast to animals, plants contain an MYB protein subfamily which is characterized by the R2R3-type MYB domain (Stracke et al. 2001).

ABA induces expression of *AtMYB2* in *Arabidopsis*, a MYB gene that is also induced in response to dehydration or salt stress. The *AtMyb2* gene is induced by

dehydration and salt stress but not by cold and heat stress and thus *AtMyb2* is responsive to dehydration at the transcriptional level. The putative protein (AtMyb2) encoded by *AtMyb2* has 274 amino acids, a molecular mass of 32 kDa and a putative DNA binding domain that shows considerable homology to plant MYB-related proteins, such as maize C1. These results suggested that an MYB-related transcription factor AtMyb2 is involved in the regulation of genes that are responsive to water stress in *Arabidopsis* (Abe et al. 2003). A drought-inducible MYB gene, designated *BcMYB1*, from drought-tolerant *Boea crassifolia*, was strongly induced by drought stress and also responded to PEG, high salinity and low temperature to some extent. BcMYB1 might be involved in the regulation of gene expression in response to dehydration stress through an ABA-independent pathway, whereas it seems not to be a regulatory component in wound signaling. *BcMYB1* shares high similarity with *AtMYB102* from *Arabidopsis* and both genes respond to water stress. However, their expression patterns were quite different. *AtMYB102* could be induced by exogenous ABA, while *BcMYB1* was insensitive to ABA treatment. In addition, *AtMYB102* expression depends on and integrates signals from both wounding and water stress while *BcMYB1* could hardly be induced by wound signaling. *AtMYB74* and 102 are upregulated by drought stress (Chen et al. 2005).

7.5.2.4 Heat Stress Transcription Factors

Heat is another abiotic stress that can affect the way a plant develops, even as a seedling. High temperature changes the membrane properties of important organelles in rice (Pareek et al. 1998). The response is mediated by the heat shock transcription factor (HSF), which is present in a monomeric, nonDNA binding form in unstressed cells and is activated by stress to a trimeric form, which can bind to promoters of heat shock genes. Heat stress transcription factors are expressed to induce the production of chaperones used to protect proteins from forming negative physiological interactions, such as conformational change in protein structure, so HSFs can be used to increase longevity under heat stress. Modifying tobacco with DS10:HaHSFA9 from sunflower gave its seeds the ability to overexpress the production of HSPs, thereby increasing tolerance of heat stress. Seven days after the 50-°C heat treatment only a handful of the controls germinated whereas 24 % of the DS10:HaHSFA9 transformed plants germinated (Prieto-Dapena et al. 2006).

7.5.2.5 ABA-Responsive Transcription Factor

As in other organisms, transcription factors in plant systems are regulated by phosphorylation. The ABA-responsive transcription factors ABI5 and AREB/ABF of *Arabidopsis* and TRAB1 of rice are regulated by the phosphorylation of multiple Ser/Thr residues. SNF1-related kinase 2 (SnRK2)-type protein kinases

(Kobayashi et al. 2005; Furihata et al. 2006) and Ca^{2+}-dependent protein kinase (CDPK) are good candidates for regulators of AREB/ABFs (Kaplan et al. 2006; Zhu et al. 2007). SnRK2s are activated by osmotic stress or ABA (Boudsocq et al. 2004), while CDPKs are activated by increased intracellular Ca^{2+} levels induced by various stimuli (Harper et al. 2004; Lanteri et al. 2006; González et al. 2012). The regulatory genes/factors reported so far not only play a significant role in drought and salinity stresses, but also in submergence tolerance. More recently, an ethylene response-factor-like gene *Sub1A*, one of the clusters of three genes at the Sub1 locus, has been identified in rice and the overexpression of *Sub1A-1* in a submergence-intolerant variety conferred enhanced submergence tolerance (Xu et al. 2006). Presumably, such transcription factors function as a hub component that integrates multiple signal inputs under abiotic stress conditions.

7.5.2.6 bZIP Transcription Factors

The bZIPs are a large family of transcription factors and one class of bZIP proteins that is linked to stress responses comprises the TGA/octopine synthase (ocs)-element-binding factor (OBF) proteins. These bind to the activation sequence-1 (as-1)/ocs element, which regulates the expression of some stress-responsive genes such as the PR-1 and Glutathione S-Transferase 6 (GST6) genes (Lebel et al. 1998; Chen and Singh 1999). Other bZIP proteins have been implicated in stress signaling, including UV lights and salt/drought stress signaling (Jakoby et al. 2002). For example, the ABRE binding factor (ABF)/ABA-responsive-element-binding (AREB) proteins respond at the transcriptional and posttranscriptional level to drought and salt stress. Logemann and Hahlbrock (2002) demonstrated the complexity of bZIP transcriptional regulation by showing that pathogen responses override UV protection through an inversely related ACGT-containing element (ACE)/ACE promoter motif. Interestingly, two identical ACE motifs constitute both a UV-responsive element and a negatively acting elicitor responsive element, enabling plants to readily shut off a less urgently needed UV-protection program when under attack by a pathogen.

7.5.2.7 Other TFs

A novel transcription factor that inhibits the affects of cold, salt, and drought stress in rice is OsCOIN (*Oryza sativa* cold-inducible) and it leads to an increase of cold, salt and drought stress tolerance. It was determined that proline concentration in the cell helped determine OsCOIN's ability to function (Liu et al. 2007).

7.5.2.8 Signal Transduction Genes

Reports on sensors for abiotic stresses are limited. A number of signaling components are associated with the plant response to high temperature, freezing, drought, and anaerobic stresses (Grover et al. 2001). AHK1/ATHK1, a membrane-spanning histidine kinase in *Arabidopsis*, could act as an osmosensor in yeast cells and presumably in plant cells (Tran et al. 2007; Wohlbach et al. 2008) although the downstream signaling mechanisms are largely unknown. Components of the same signal transduction pathway may also be shared by various stress factors such as drought, salt, and cold (Shinozaki and Yamaguchi-Shinozaki 1999). Although there are multiple pathways of signal-transduction systems operating at the cellular level for gene regulation, ABA is a known component acting in one of the signal transduction pathways, while others act independently of ABA. Abiotic stress signaling in plants involves receptor-coupled phospho-relay, phosphoionositol-induced Ca^{2+} changes, the mitogen-activated protein kinase (MAPK) cascade, and transcriptional activation of stress-responsive genes (Xiong and Zhu 2001).

MAP kinase cascades function as major cellular signaling components in eukaryotes. Genome information allowed identification of 20 MAP kinases, 10 MAP kinase kinases, and 60 MAP kinase kinase kinases in *Arabidopsis* (Ichimura et al. 2002). These MAP kinase components appear to function in several different signaling processes, so they might not constitute simple cascades but rather networks, making it difficult to identify the function of each component. Studies have supported that MAP kinase cascades are involved in salinity and cold stress signaling (Teige et al. 2004), however, the direct upstream factors of MAP kinase cascades have not been identified.

Sucrose nonfermentation 1 (SNF1)-related kinases (SnRK1, SnRK2, and SnRK3) are also reported to function in various stress-tolerance mechanisms and ABA responses. Yeast SNF1 and *Arabidopsis* KIN10/SnRK1.1 and KIN11/SnRK1.1 are presumed to act in the regulation of metabolic pathways (Baena-González et al. 2007). In addition to SRK2J/SnRK2.9, nine *Arabidopsis* SnRK2s are activated under osmotic stress conditions (Boudsocq et al. 2004). SRK2D/SnRK2.2, SRK2I/SnRK2.3, and SRK2E/OST1/SnRK2.6 are strongly activated by ABA. Recent studies showed that these three ABA-activated SnRK2s are essential for ABA signaling (Fujii and Zhu 2009; Nakashima et al. 2009; Umezawa et al. 2009).

Ca^{2+} is one of the most important second messengers in response to extracellular stimuli in plants (Ludwig et al. 2004). Increased levels of intracellular Ca^{2+} are presumably induced under stress conditions by signal molecules such as inositol trisphosphate (IP3), diacylglycerol, inositol hexaphosphate (IP6), cADP-ribose, or reactive oxygen species. A Ca^{2+}-permeable stretch-activated channel of *Arabidopsis* also contributes to Ca^{2+} release upon stimulation (Nakagawa et al. 2007). *Arabidopsis* has 25 SnRK3-type kinases, according to genome information, of which the best characterized is SALT OVERLY SENSITIVE 2 (SOS2)/CIPK24/SnRK3.11, which was identified as an essential factor in the salinity stress response.

In conjunction with SOS3/ScaBP8/CBL10 Ca^{2+}-binding protein, SOS2 activates the plasma membrane Na^+/H^+ antiporter (SOS1) required for salinity tolerance (Mahajan et al. 2008; Luan 2009). *Arabidopsis* has more than 30 Ca^{2+}-dependent kinase (CDPK) genes (Hrabak et al. 2003) known to function under abiotic stress and ABA responses.

7.6 Engineering Biotic Stress Tolerance in Crop Plants

According to various estimates genetically engineered crops saved 15.6 million tons of CO_2 through reduced herbicide and pesticide use in 2007. Therefore, shifts from harmful chemical pesticides and herbicides to biological approaches are an unavoidable requirement for sustainable agriculture. Enhancing resilience to the effects of climate change, particularly to biotic and abiotic stresses, is the most important challenge facing us for sustainable agricultural productivity. Traditional breeding approaches are not enough to keep pace with the speed of adverse changes occurring due to climate change. Utilizing new molecular techniques and designing climate-resilient genetically modified crops is one of the effective options. Transgenic crops were grown on 160 million hectares globally in 2011 and all commercially released crops are for herbicide, insect, or virus resistance, or stacking of these traits (Fig. 7.2). Distribution of economic benefits at the farm level from 1996 to 2009, after commercialization of biotech crops showed that the highest share is by herbicide-tolerant crops with US$30.2 billion followed by insect-resistant crops with US$34.2 billion (ISAAA 2011). A number of genetically engineered crop plants with biotic stress tolerance are either already present in the market or ready for commercial cultivation (Table 7.1).

7.6.1 Genetic Engineering for Insect Resistance

First reports on insect-resistant transgenic plants were published in 1987 (Vaeck et al. 1987) in which transgenic tobacco was generated using insecticidal crystal protein genes of *Bacillus thuringiensis* (Bt). Subsequently, several genes conferring insect resistance have been transferred to crop plants and the first transgenic plant ever commercialized was insect-resistant cotton in 1996. Insect-resistant transgenic crops occupy the second position in terms of acreage among all commercially cultivated transgenic plants. Bt is a gram-positive, ubiquitous soil bacterium that synthesizes crystalline inclusions (Cry proteins) during sporulation or during vegetative growth (Vip proteins). The crystalline structure of the inclusion is made up of protoxins of around 130 kDa, called δ-endotoxins. These Bt crystal proteins are part of a large family having around 410 toxins (Crickmore 2006). Protoxins, when ingested by insects, are solubilized in the alkaline conditions of the insect midgut. The protoxins are then processed by gut proteases to a 65–70-kDa truncated

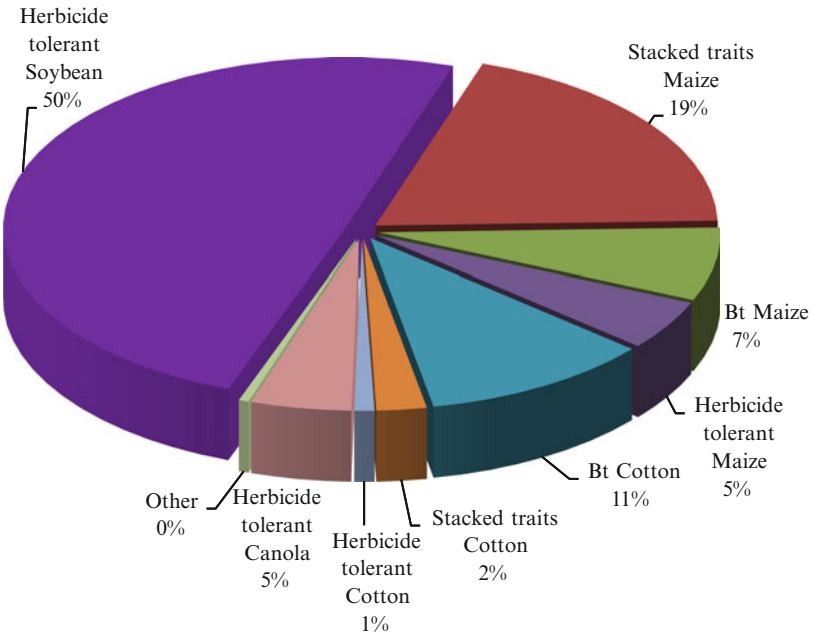

Fig. 7.2 Crop-wise distribution of commercially grown biotic trait related transgenics in 2010. Source of information: ISAAA (2011) (http://www.isaaa.org/)

N-terminal form of active toxins. There are three domains in Bt toxins. Domain II and III, in combination or independently, bind to the receptors on the surface of the cells of the epithelium of insect midgut. This interaction leads to oligomerization of the protein and perforation of the cell membrane. The open channels result in ionic leakage, which destroys the cell, leading to breakdown of the gut, bacterial proliferation, and finally insect death (Bravo et al. 2007).

Members of the Bt toxin family have selectivity for their primary targets in insect families like Lepidoptera (moths and caterpillars), Diptera (flies and mosquitoes), and Coleoptera (beetles). Their current nomenclature is based on amino acid identity (Crickmore 2006). Since these Bt toxins have high specificity, crop plants in which these genes are incorporated do not present a significant risk to human health and the environment in the way chemical insecticides do (Mendelsohn et al. 2003). Many strategies to improve expression levels of Bt toxins have been adopted subsequently as >95 % mortality of insects under heterozygous conditions requires >0.2 % Bt expression of total soluble protein in the tissue (Gatehouse 2008). Plastid genome transformation is one of the strategies by which increased expression of Bt can be achieved. Chloroplast transformation of Bt in tobacco has shown accumulation of 3–5 % of total leaf protein (McBride et al. 1995). But it is difficult to stably transform plastid genomes in other crop plants. The *Cry1Ab* gene has been successfully introduced in soybean (*Glycine max*) plastids and shown to confer resistance to a broad range of pests (Dufourmantel et al. 2005). Another advantage of this

Table 7.1 Genetically engineered crop plants related to biotic stresses ready for commercial cultivation

Crop	Scientific name	Biotic trait/traits
Cotton	*Gossypium hirsutum L.*	Herbicide tolerance, Insect resistance, Insect resistance + Herbicide tolerance
Maize	*Zea mays L.*	Herbicide tolerance, Insect resistance, Insect resistance + Herbicide tolerance, Virus resistance + Insect resistance + Herbicide tolerance, Herbicide tolerance + Herbicide tolerance
Soybean	*Glycine max L.*	Herbicide tolerance, Insect resistance, Modified Oil content + Herbicide tolerance
Alfalfa	*Medicago sativa*	Herbicide tolerance
Argentine Canola	*Brassica napus*	Herbicide tolerance, Herbicide tolerance + Fertility restored
Carnation	*Dianthus caryophyllus*	Herbicide tolerance + Modified flower color
Potato	*Solanum tuberosum L.*	Insect resistance, Insect resistance + Virus resistance
Tomato	*Lycopersicon esculentum*	Insect resistance
Poplar	*Populus nigra*	Insect resistance
Rice	*Oryza sativa L.*	Herbicide tolerance
Sugerbeet	*Beta vulgaris*	Herbicide tolerance
Flax, Linssed	*Linum usitatissumum L.*	Herbicide tolerance
Polish canola	*Brassica rapa*	Herbicide tolerance
Wheat	*Triticum aestivum*	Herbicide tolerance
Chicory	*Cichorium intybus*	Herbicide tolerance + Fertility restored
Tobacco	*Nicotiana tabacum L.*	Herbicide tolerance
Creeping Bentgrass	*Agrostis stolonifera*	Herbicide tolerance
Plum	*Prunus domestica*	Virus resistance
Squash	*Cucurbita pepo*	Virus resistance
Sweet pepper	*Capsicum annuum*	Virus resistance
Papaya	*Carica papaya*	Virus resistance

Source of information: ISAAA (2011) (http://www.isaaa.org/)

technique is that it cannot disperse pollen from transgenic to nontransgenic plants as plastids are maternally inherited. Expressing multiple Cry toxins in one plant can help to control more than one insect pest. Transgenic cotton plants expressing Cry1Ac and Cry2Ab were more toxic to the primary target pest, bollworm (*Helicoverpa zea*), and also two secondary pests, *Spodoptera frugiperda* and *Spodoptera exigua* (Stewart et al. 2001; Chitkowski et al. 2003). Thus, gene stacking is a useful approach to control more insects along with incorporation of other traits. In maize, six insect-resistance genes, viz. Cry34Ab1, Cry35Ab1 and modified Cry3Bb1 for rootworm; Cry1F, Cry1A.105 and Cry2Ab2 for Lepidoptera;

and resistant genes for two herbicides, namely glyphosate and glufosinate-ammonium, have been stacked for insect resistance and weed management (Gatehouse 2008). Identification of novel Bt toxins and improvement of toxicity range of existing genes through protein engineering are other potential strategies (Fang et al. 2007). Site-directed mutagenesis of the *Cry* genes, domain swaps, and fusion proteins are approaches to improving the toxicity range of Bt proteins. It was shown that a hybrid Cry protein with domain I and III from Cry1Ba and domain II from Cry1Ia conferred resistance to potato tuber moth (*Phthorimaea operculella*, a lepidopteran pest) and Colorado potato beetle (*Leptinotarsa decemlineata*, a coleopteran pest) in potato (Naimov et al. 2003). Site-directed mutations in Cry1Ab increased its toxicity towards larvae of gypsy moth (*Lymantria dispar*) up to 40-fold (Rajamohan et al. 1996). The galactose-binding lectin domain (B-chain) from the ribosome-inactivating protein, ricin, was added at the C-terminal of domain III of Cry1Ac and expressed in maize and rice (Mehlo et al. 2005). The fusion protein was able to bind to the Gal-residue of a potential insect receptor along with an *N*-acetyl galactosamine residue by domain III of the toxin. The toxin provided resistance to larvae of stem borer (*Chilo suppressalis*) and leaf armyworm (*Spodoptera littoralis*) along with resistance to a hemipteran pest, the leafhopper *Cicadulina mbila* (Ye et al. 2009). Although, Bt toxins are not effective against hemipterans, the fusion protein provided resistance against leafhoppers because of the presence of the lectin domain.

Since Bt toxins do not target most of the insect pests, there is a need to develop strategies to manage sap-sucking and storage grain pests. Several genes of plant and animal origin have been identified and employed for this purpose. These are mainly proteinase inhibitors (PI), polyphenol oxidase, α-amylase inhibitors (AI), lectins, chitinase, and tryptophan decarboxylase. Many transgenic plants were developed using different serine and cysteine proteinase inhibitors from various plant sources including cowpea, potato, tomato, sweet potato, rice, and bean, but insects were found to be adapting to the ingestion of transgenic plants overexpressing PIs, thus overcoming the deleterious effect of PI (Jongsman and Bolter 1997; Jouanin et al. 1998). Use of PI genes to enhance insect resistance in transgenic crops in an efficient manner requires reassessment of their mechanisms of action, particularly in affecting processes other than digestion (Gatehouse 2011). The case with α-amylase inhibitors is similar. A gene for an α-amylase inhibitor from bean (*Phaseolus vulgaris*) was expressed in garden pea (*Pisum sativum*) under a seed-specific promoter and showed that expression of AI in seeds was up to 3 %. The transgenic lines were resistant to stored product pests like larvae of the bruchid beetle and the field pest, pea weevil *Bruchus pisorum* (Morton et al. 2000). Despite this success, commercialization of this transgenic crop expressing the α-amylase inhibitor gene has not taken place because of systemic immunological responses in mice fed with transgenic peas due to altered posttranslational processing in pea (Prescott et al. 2005). Another class of potential insecticidal toxin is plant lectins. These proteins target hemipteran plant pests, which are not affected by Bt toxins. Overexpression of Mannose-specific snow-drop lectin (GNA) under constitutive or phloem-specific promoters in rice conferred partial resistance towards rice brown plant hopper

(*Nilaparvata lugens*) and other hemipteran pests (Rao et al. 1998; Foissac et al. 2000). Expression of Mannose-specific lectin from garlic (*Allium sativum*) leaves (ASAL) in rice provided partial resistance to hemipterans, and *Rice tungro virus* because of control of its insect vector (Saha et al. 2006). In a similar way, snowdrop lectin from *Galanthus nivalis* was expressed in maize to control corn leaf aphids (Wang et al. 2005a), and fusion protein of ASAL from *A. sativum* and ACA from *A. cepa* into *Brassica juncea* to control mustard aphids (Hossain et al. 2006). Insecticidal activity of the *choM* gene encoding cholesterol oxidase from Actinomyces A19249 towards cotton boll weevil larva was confirmed by expressing it in tobacco (Corbin et al. 2001). Another promising molecule is avidin, which when expressed in transgenic maize conferred resistance to the larvae of three different coleopteran storage pests (Kramer et al. 2000).

Recently, some novel approaches have been exploited such as engineering secondary metabolism for plant defense and volatile communication compounds through overexpression of genes or through RNA interference. When two genes encoding Cyt P450 oxidase and a UDP-glycosyltransferase from sorghum were introduced in *Arabidopsis*, hydrogen cyanide was produced on tissue damage, which resulted in enhanced resistance to attack by the flea beetle *Phyllotetra nemorum* (Kristensen et al. 2005). In another experiment, maize terpene synthase gene *TPS10* was transformed in *Arabidopsis* and resulting plants emitted the sesquiterpene volatile, which attracted parasitoid wasps that attack maize pests (Schnee et al. 2006). Expressing siRNA of corn rootworm V-type ATPase in transgenic maize plants showed suppression of respective mRNA in the insect and significant reduction in feeding damage by insects (Baum et al. 2007). In the future, different modifications in Bt toxins along with identification of new insecticidal molecules and new strategies to control all types of insect pests will help manage insects via transgenic technology.

7.6.2 Genetic Engineering for Disease Resistance

The losses caused due to pathogens are equivalent to those caused by insect pests. Despite the large number of commercialized transgenic crops there are not that many disease-resistant crop species (Punja 2006). However, the future of transgenics for disease resistance is bright as it accounts for 10 % of the total genetically engineered traits approved for field trials in the United States of America (Collinge et al. 2010). Potato is the dominant crop with approximately one-third of all the applications of disease-resistant transgenic plants filed for field trails, followed by tomato, maize, soybean, and wheat. The main limitation is that the plant pathogens are diverse in nature from cellular bacteria and fungi to viruses (Collinge et al. 2008). Most importantly, to date no common toxin has been identified like Bt, which can be applied against all pathogens. There are many taxonomic classes of pathogens with several strains. All of these pathogens are physiologically very diverse from each other. By understanding the molecular basis

of plant–pathogen interactions, several strategies have emerged for disease resistance via genetic manipulations. Many transgenics have been developed against specific pathogens or for broad-spectrum resistance. The strategies involved are manipulation of resistance by R-genes, expression of pathogenesis-related (PR) proteins, antifungal peptides, detoxification of pathogen virulence factors, increasing structural barriers, and manipulation of phytoalexin biosynthesis (Wally and Punja 2010). The major strategies employed in current transgenic crops under approval for field trials are deploying antimicrobial proteins against bacteria and fungi and expression of the coat protein (CP) gene against viruses (Collinge et al. 2010).

7.6.2.1 Bacterial Diseases

Bacterial diseases are the most devastating in cereals, vegetables, and fruit crops with great economic significance. Many molecular approaches, such as the use of the R-gene, antibacterial proteins or peptides, lytic peptides from insects, lysozymes, are employed to develop transgenic crops with resistance to bacterial diseases (Mourgues et al. 1998; Collinge et al. 2008). Other approaches are inhibition of bacterial virulence factors or improvement of natural defense mechanisms of plants by overexpressing defensin genes or enhancing production of reactive oxygen species or modifying signaling pathways (Wally and Punja 2010). Antimicrobial proteins, peptides, and lysozymes are naturally present in all insects, plants, and animals. One of the lytic peptides, cecropin was introduced into transgenic tobacco resulting in increased resistance to *P. syringe* pv. *tabaci* (Huang et al. 1997). Similarly, the *cecropin B* gene was used to reduce development of lesions caused by *X. oryzae* pv. *oryzae* in transgenic rice (Coca et al. 2004). The gene encoding antimicrobial peptide, D4E1, was artificially synthesized and transformed into poplar, which provided resistance to *Agrobacterium tumefaciens* and *Xanthomonas populi* (Mentag et al. 2003; Montesinos 2007). The R-gene approach was exploited in rice using the *Rxo1* gene from maize to confer resistance to *Xanthomonas oryzae* pv. *oryzicola* (Zhao et al. 2005). An enzyme, *AiiA*, from the bacterial strain, *Bacillus* sp. 240B1, responsible for degradation of quorum-sensing signals, was expressed in potato to disrupt the communication system, known as quorum-sensing, used by phytopathogenic bacteria to regulate the expression of genes according to population density. The transgenic potato acquired resistance to a soft rot causing pathogen, *Erwinia carotovora*, by making the bacteria incapable of infecting the host (Dong et al. 2000). However, partial resistance or breakdown of resistance is the main limitation in many of these approaches.

Currently, there are 649 field test applications for bacterial-disease-related transgenic crops in USA awaiting approval. Potato tops the list with 23 % of the applications followed by tomato (16 %), grape (13 %), rice (8 %), and soybean (5 %). The most common genes used for transgenic bacterial-resistant crops in USA field trials are Attacin and Cecropin from giant silk moth, Hordothionin from barley, Indolicidin and Lysozyme from cow, Magainin from African clawed frog,

and Protein kinase, R-gene and transcription factor from rice, tomato and pepper (Collinge et al. 2010). Horticultural crops are predominant in bacterial-resistant transgenics like apple, papaya, pear, citrus, grape, potato, and tomato along with rice, sugarcane, and tobacco.

7.6.2.2 Fungal Diseases

Fungal diseases are the most important factor that contributes to economic losses in almost all the crop species (Wani 2010). It has been reported that fungi cause more than 70 % of the diseases of all economically important crops. Molecular approaches used to develop transgenics for fungal resistance are similar to those used against bacteria. In addition to the use of pathogenesis-related (PR) proteins, antifungal proteins, phytoalexins, plant ribosomal-inactivating proteins, and RNA silencing are employed to impart fungal resistance. Many studies showed resistance to fungal pathogens by using a gene encoding chitinases and glucanases, which degrade the chitin and α-1,3 glucan, the major constituents of the fungal cell wall. Overexpression of *RC7 chitinase PR-3* and *Chi11* (*chitinase*) *Tlp* (*PR-4*) in rice in independent studies conferred resistance to *Rhizoctonia solani* (Datta et al. 2001; Kalpana et al. 2006). Similarly, transgenic peanut acquired resistance to late leaf spot caused by *Phaeosariopsis parsonata* by expressing tobacco chitinase (Rohini and Rao 2001) and transgenic radish showed resistance to *Rhizoctonia solani* by expressing potato chitinase, *Chi 18* (Yang et al. 2009). Another important gene used for resistance against fungi is *gf-2.8* from wheat (*Triticum aestivum*) encoding oxalate oxidase. Transgenics harboring this gene showed resistance against *Sclerotinia sclerotiorum* in soybean (Cober et al. 2003), against *Septoria musiva* in poplar (Liang et al. 2001), and against *Blumeria graminis f. sp. tritici* in wheat (Altpeter et al. 2005). Defensins (PR-12), a class of low molecular weight cysteine-rich peptides, are thought to modify ion uptake within the microorganism's cell membrane. A dahlia defensin gene, *DM-AMP1*, was used in transgenic rice to confer resistance against *Magnaporthe oryzae* and *Rhizoctonia solani* (Jha and Chattoo 2010). Similarly, introduction of mustard defensin in transgenic tobacco provided resistance to leaf pathogen *Fusarium verticillioides* and *Phytophthora parasitica* pv. *nicotianae* and in transgenic peanut to *Phaeosariopsis parsonata* and *Cercospora arachidicola* (Anuradha et al. 2008). A harpin (hypersensitive response and pathogenicity) encoding gene *hrf1* from *X. oryzae* pv. *oryzeae* was introduced in rice, and the resulting transgenic plants showed resistance to all major strains of *M. grisea* (Shao et al. 2008).

From 1987 to 2009, 853 applications were filed for the approval of field trials in USA for fungal-resistant transgenic plants. Among all the crops, maize tops the list with a 24 % share of the applications followed by potato (14 %), soybean (12 %), wheat (11 %), and tomato (5 %). Other important crops for which permission for field trials was sought were tobacco, rice, cotton, barley, sunflower, peanut, and grape. Important genes used for fungal-resistant transgenics are polygalacturonase inhibitor, chitinase, protein kinase, oxalate oxidase, thionin, stilbene synthase, PR

protein genes (*PR-1*, *PR-2* and *PR-5*), and R-genes (*Rpg1* from barley, *Pi9* from rice, *RB2* from *Solanum bulbocastanum* and *Rps1-k* from soybean) (Collinge et al. 2010). Some antimicrobial metabolite-related enzymes such as lignin biosynthesis protein from pea, coenzymeA reductase and divinyl ether synthase from tomato are also exploited for genetic engineering against fungi. Pathogen-specific resistance approaches are more useful to impart resistance to fungal diseases.

7.6.2.3 Viral Diseases

Viruses are widespread in nature and responsible for many crop diseases, especially in vegetables and fruits. The pathogen-derived resistance (PDR) phenomenon was mostly exploited to develop virus-resistant transgenic plants by utilizing part of the virus genome, like expression of coat protein genes, viral replicase genes, or other viral sequences. Using this technique, the resistance was observed at two levels, protein-mediated resistance and RNA-mediated resistance. The first evidence of pathogen-derived resistance was provided by Abel and his group (Abel et al. 1986) by expressing *Tobacco Mosaic Virus* (*TMV*) coat protein in transgenic tobacco, which conferred resistance to the *TMV* virus. Further, a similar approach was successfully used in rice for *Rice Yellow Mottle Virus* (Pinto et al. 1999) and in wheat for *Wheat Streak Mosaic Virus* (Sivamani et al. 2002). This approach of coat-protein-mediated resistance was proved to be successful in controlling *Papaya Ring Spot Virus* (*PRSV*) in papaya (Fermin et al. 2004). Genes other than coat proteins were also used for virus resistance. Use of viral RNA-dependent RNA polymerase was shown to confer resistance for *TMV* (Golemboski et al. 1990). Potato plants transformed with both a sense and antisense transcript of the *Potato virus Y* helper-component proteinase (HC-Pro) gene provided complete resistance against the virus (Waterhouse et al. 1998). Further, many transgenic plants were developed exploiting antisense RNA in transgenics by RNA silencing. Other approaches including use of replicase-mediated resistance; RNA satellites, ribosome-inactivating protein (RIP), ribonucleases, hammerhead ribozyme, and plantibodies were also successfully utilized for the development of virus-resistant transgenics (Dasgupta et al. 2003; Wani and Sanghera 2010). Currently, transgene-mediated RNA silencing and generation of small interfering RNAs (RNAi) are the principal mechanisms used to develop virus-resistant transgenic plants (Mansoor et al. 2006; Sudarshana et al. 2007; Prins et al. 2008; Sanghera et al. 2011).

At present the maximum applications for field test release in USA for disease-resistant transgenic crops are for viral resistance, i.e., 983 compared to 853 for fungal resistance and 167 for bacterial resistance (Collinge et al. 2010). Among these, the proportion of transgenics for viral resistance is maximum in potato (33 %) followed by melon (11 %), tomato (9 %), tobacco (8 %), and squash (7 %). Other important crops are sugarcane, sugar beet, papaya, wheat, maize, soybean, peanut, pea, and grape. Many virus-resistant transgenic crops are already available in the market.

7.6.3 Genetic Engineering for Nematode Resistance

Worldwide losses due to nematodes in crop plants are estimated at US$118 billion. Natural resistance genes for nematodes were the only way to control these parasitic organisms effectively as they cannot be controlled easily by nematicides or other control measures (Atkinson et al. 2012). Where natural resistance is not available the development of nematode-resistant transgenic plants is the only strategy to manage this problem. Strategies such as protease inhibitors (serine protease inhibitors, Cysteine protease inhibitors, Cystatins), R-genes, lectins, nematicidal peptides, endotoxins from Bt, and the RNAi technique will be useful (Atkinson et al. 2003). In the early period of transgenic development for nematode resistance, protein and peptide based transgenic defenses were utilized. When a peptide oryzacystatin, *Oc-1ΔD86* that interferes with nematode replication, was expressed in *Arabidopsis*, the size of female beet cyst nematodes (*Heterodera schachtii*) was greatly reduced (Urwin et al. 1995). When expressed in banana and challenged with burrowing nematodes (*Radopholus similes*), eight transgenic lines showed substantial control (Atkinson et al. 2004). Overexpression of cystatins in plants to interfere with intestinal digestion of dietary protein intake of nematodes from plants, was also found to be useful. A field trial of transgenic potatoes expressing cystatin showed considerable resistance when challenged with potato cyst nematode *Globodera* (Fuller et al. 2008). In another study a cystatin from taro, a root crop, when expressed in tomato provided resistance to *Meloidogyne* (Chan et al. 2010). In another study, two distinct synthetic peptides, which interfere with cyst nematode chemoreception by binding to either acetylcholine esterase or nicotinic acetylcholine receptors, which target the nematode cholinergic nervous system, were used for transgenic development in *Arabidopsis* and potato. In *Arabidopsis* it reduced the number of *Heterodera schachtii* (beet cyst nematode) females whereas in potato plants resistance was observed to *Globodera pallida*, a potato cyst nematode (Liu et al. 2005; Lilley et al. 2011).

Bt toxins, which control insects efficiently, are also shown to have nematicidal properties. Out of many classes of Bt toxins, *Cry5B*, *Cry6A*, *Cry12A*, and *Cry21A* can control various plant parasitic nematodes effectively (Wei et al. 2003). Transgenic tomato plants expressing *Cry6A* were shown to reduce egg production in *Meloidogyne incognita* (Li et al. 2007).

At present, the most widely used strategy to control nematodes is RNAi. Expression of dsRNA complimentary to *16D10*, a *Meloidogyne* parasitism gene in *Arabidopsis*, demonstrated significant reduction in the number of galls and their size along with a reduction in egg formation by nematodes (Huang et al. 2006). Complete resistance to *Meloidogyne incognita* infection by expressing dsRNA for splicing factor or integrase in transgenic tobacco plants was also reported (Yadav et al. 2006). This approach will provide greater numbers of transgenic nematode-resistant crops with the availability of complete genome information of nematodes in the future.

7.6.4 Genetic Engineering for Weed Management

Changes in temperature and carbon dioxide are likely to impart significant direct stimulation of weed growth. Weeds benefit more than cash crops from temperatures and carbon dioxide levels. Weed management is one of the most serious problems in all the cropping systems. Development of herbicide-resistant transgenic crops provided a potential tool for managing weed infestation in a more effective and economic way for zero-tillage agriculture (Duke 2006). In fact, herbicide resistance is the major transgenic trait in the world with 61 % of the area under transgenic crop cultivation (ISAAA 2011). The first genetically modified herbicide-resistant crop was soybean and the herbicide was glyphosate (Padgette et al. 1996). Subsequently, many transgenic glyphosate-resistant crops such as corn, cotton, alfalfa, canola, sugar beet, and bent grass (*Agrostis stolonifera*) were developed (Dill et al. 2008). Glyphosate is a herbicide, which acts by blocking the shikimate pathway through inhibition of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). Blocking of the EPSPS enzyme does not allow the synthesis of aromatic amino acids and causes mis-regulation of the shikimate pathway, which affects the normal growth of the plant (Dill 2005). Transgenic plants resistant to glyphosate were developed using the CP4 EPSPS gene from *Agrobacterium tumefaciens* strain CP4. Another glyphosate-resistant mutated EPSPS gene Zm-2MEPSPS was identified from maize. A third gene used in glyphosate-resistant transgenics was glyphosate oxidoreductase (GOX) from *Ochrobactrum antropi* strain LBAA that degrades glyphosate to glyoxylate, a safe and ubiquitous natural product and aminomethyl-phosphonate (AMPA), a nontoxic compound.

Transgenic crops developed against another herbicide are glufosinate (phosphinothricin) resistant. Glufosinate is a competitive inhibitor of glutamine synthetase, an enzyme essential for the assimilation of nitrogen to glutamine. Two homologous genes, phosphinothricin acetyl transferase (PAT) from *Streptomyces viridochromogenes* and basta *N*-acetyltransferase (BAR) from *Streptomyces hygroscopicus* inactivate the glufosinate (Herouet et al. 2005). Many crop plants have been transformed with these genes, out of which glufosinate-resistant canola is very successful in Canada. Currently "Dual Stack" crop cultivars are commercially available in cotton, soybean and corn, which offer resistance to both glyphosate and glufosinate and provide farmers with a choice between these two broad spectrum herbicides for weed management. There are many health- and environment-related benefits of using glyphosate and glufosinate in herbicide-resistant crops as water, air, and soil contamination by these herbicides along with its effect on nontarget organisms are negligible as compared to other herbicides (Cerdeira and Duke 2006).

At present there are only five genes used to confer herbicide resistance, CP4 EPSPS, GA21, and GOX for glyphosate resistance and *pat* and *bar* genes for glufosinate resistance. Because of overuse of these two herbicides for weed management, a few weeds resistant to these herbicides evolved in many countries. This initiated the need to search for new mechanisms of herbicide resistance so that it

can be incorporated along with the existing ones. A gene encoding dicamba monooxygenase (DMO), which neutralizes dicamba, was cloned from a soil bacterium, *Stenotrophomonas maltophilla* and used to develop dicamba-resistant soybean (Herman et al. 2005). Genes were identified from the *aad* gene family encoding aryloxylkanoate dioxygenate for providing resistance to certain auxin herbicides (Schleinitz et al. 2004; Muller et al. 2006). In the future, no single herbicide resistance trait will be sustainable over the longer term, if a single herbicide type is used continually. Multiple herbicide-resistant transgenic crops with varying modes of resistance that allow the use of herbicide mixtures can provide a sustainable solution to the problem of weed management.

7.7 Conclusions and Future Perspectives

Agricultural productivity worldwide is subject to increasing environmental constraints, both biotic and abiotic due to their high magnitude of impact and wide distribution. It seems unlikely that global food production will satisfy future demand under climate change, and the biotic and abiotic stresses, will be the primary factor for yield losses worldwide. Genetic engineering, i.e., use of transgenes to improve the tolerance of crops to abiotic stresses, remains an attractive option.

Targeting multiple gene regulation will be a better option than targeting single genes. Genetically modified (GM) crops being cultivated today were developed to be herbicide tolerant and resistant to pests. Development of GM crops with traits valuable for poor farmers, especially within the context of climate change—such as resistance to drought, extreme temperatures, soil acidity and salinity—is not yet a reality.

Further, approaches combining the molecular, physiological and metabolic aspects of abiotic/biotic stress tolerance are mandatory for filling gaps in knowledge for whole plant phenotypic response under stress. The degree of recovery from stress, which also has its molecular basis, is as relevant as the response to stress. Application of forward and reverse genetic analysis coupled with genomics and proteomics tools will certainly accelerate our understanding in this area leading to improvements in abiotic and biotic stress tolerance. The molecular identities of stress signaling still remain a mystery and the challenge in the near future will be identification of signaling elements to understand signaling specificities and crosstalks. The most prominent missing elements in biotic and abiotic stress signaling are the sensors or receptors. Overexpression of early-response transcription activators and components upstream of transcription to turn on many downstream effector genes will surpass first-generation genetic engineering, which involved manipulation of stress-related genes. Our understanding of plant stress tolerance can be greatly refined by thorough characterization of individual genes and assessing their contribution to stress tolerance. Mainly master switches such as

transcription factor or upstream signaling molecules are promising candidate genes for biotechnological approaches.

To develop abiotic stress-tolerant varieties and make them available to farmers, teams of scientists from different disciplines are needed at the cellular, plant, and field scales to work together to find ways to manipulate these complex, multilevel processes and improve crop response. It is no secret that breeding for abiotic stress tolerance has always been a challenge, making this task less attractive to breeders. The challenges of abiotic stress breeding tend to be common across crops. Genetic engineering for abiotic stress resistance is showing promise in some crops using transcription factor genes leading to molecular breeding. The transcription factor (TF) genes have potential for the coordinated regulation of stress-induced genes relevant to stress tolerance. There is a growing interest in the use of regulatory genes for developing stress-tolerant genotypes. Regulation of the stress-inducible genes can help in understanding signaling pathways leading to stress tolerance. As multiple stress responses are necessary for plants to endure severe stress conditions, the engineering of a single gene is not sufficient but it is possible for a single TF to control the expression of multiple target genes. At present, there are no transgenic crop varieties being marketed on the basis of improved drought or heat tolerance; Monsanto has experimented with overexpression of transcription factors and RNA chaperones. Nevertheless, Monsanto claims that the yield increases are significant and, of course, these varieties would represent the start of a process of long-term improvement.

Advances in genome sequencing, development of high-throughput omic technologies, quantitative genetics, and bioinformatics offer us a good opportunity to meet the challenges of climate change. Climate change will remain a central focus for existing and future research programs in highly targeted crop improvement with networks and collaborative links developed at national and international levels on climate change. As climate change has far-reaching impacts on food security and impacts are already becoming evident, rapid action must be taken now to develop crops and cropping systems to offer good options for farmers to outweigh its impacts.

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Chapter 8
Participatory Breeding for Climate Change-Related Traits

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Abstract After a review of the effects of climate changes on food security and agricultural production, the chapter relates modern plant breeding, as opposed to farmers' breeding practiced for millennia, with the decrease of agrobiodiversity. It underlines the contradiction between the unanimous recognition of the importance of biodiversity and the tendency towards uniformity of modern plant breeding, which, combined with the increased consolidation of the seed industry, is causing a dramatic decrease of cultivated biodiversity. This is exactly the opposite of what is required to adapt crops to climate changes. Although a suite of traits play an important role in the adaptation of crops to climate changes, it is also important to recognize that climate changes are a moving target and therefore the emphasis should not be so much on which trait to breed for but rather to adopt breeding strategies that allow a highly dynamic and efficient system of variety deployment in farmers' fields. Participatory plant breeding, whose technical aspects are described in detail, has the capability of increasing agricultural production at farm level by exploiting specific adaptation, thus increasing at the same time agrobiodiversity. Participatory plant breeding, integrated with evolutionary plant breeding, should become the model of plant breeding used by the plant breeding programs of the CGIAR centers.

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8.1 Introduction

Today, nobody questions whether climate changes are occurring or not and the discussion has shifted from whether they are happening to what to do about them.

The most recent evidence from the Fourth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC 2007) indicates that the warming of the climate system is unequivocal, as it is now evident from observations of increases in global average air and ocean temperatures, widespread melting of snow and ice, and rising global average sea level.

The report states the following evidence:

- Eleven of the last 12 years (1995–2006) rank among the 12 warmest years in the instrumental record of global surface temperature (since 1850).
- The temperature increase is widespread over the globe, and is greater at higher northern latitudes. Land regions have warmed faster than the oceans.
- The rising sea level is consistent with warming. The global average sea level has risen since 1961 at an average rate of 1.8 mm per year and since 1993 at 3.1 mm per year, with contributions from thermal expansion, melting glaciers and ice caps and the polar ice sheets.
- Observed decreases in snow and ice extent are also consistent with warming. Satellite data since 1978 show that the annual average Arctic sea ice extent has shrunk by 2.7 % per decade, with larger decreases in summer of 7.4 % per decade. Mountain glaciers and snow cover on average have declined in both the hemispheres (IPCC 2007).
- It is very probable that over the past 50 years, cold days, cold nights, and frosts have become less frequent over most land areas, and hot days and hot nights have become more frequent. Heat waves have become more frequent over most land areas, the frequency of heavy precipitation events has increased over most areas, and since 1975 the incidence of extreme high sea levels has increased worldwide. There is also observational evidence of an increase in intense tropical cyclone activity in the North Atlantic since around 1970, with limited evidence of increases elsewhere.
- There is no clear trend in the annual numbers of tropical cyclones, but there is evidence of increased intensity (IPCC 2007).
- Changes in snow, ice, and frozen ground have resulted in more, and larger, glacial lakes, increased ground instability in mountain and other permafrost regions, and led to changes in some Arctic and Antarctic ecosystems (Walker 2007).

Projections to the year 2100 indicate that CO_2 emissions are expected to increase by 400 % and CO_2 atmospheric concentration is expected to increase by 100 % (Fig. 8.1, modified from Cline 2007).

Some studies have predicted increasingly severe future impacts with potentially high extinction rates in natural ecosystems around the world (Williams et al. 2003; Thomas et al. 2004).

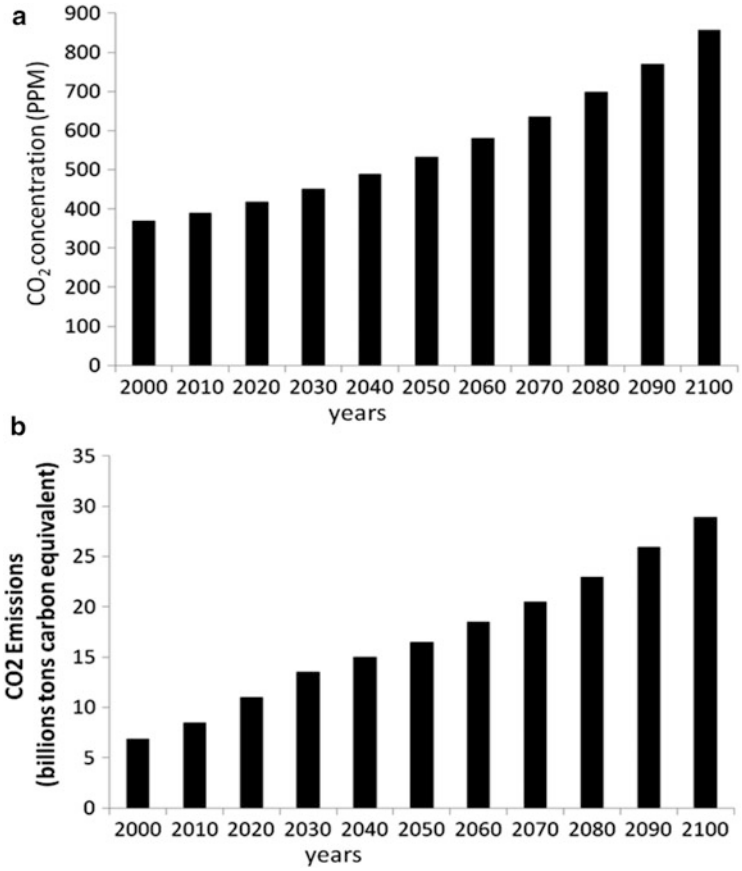

Fig. 8.1 Projected atmospheric CO_2 concentration in parts per million CO_2 (**a**) and projected emission in billion tons carbon equivalent (**b**) (modified from Cline 2007)

More recent evidence (Durack et al. 2012) suggests that dry regions will become drier and wet regions wetter in response to warming. This has been labeled as the "rich get richer" mechanism.

In addition, the IPCC (2007) argues that the impacts of climate change will be distributed differently among regions, generations, age classes, income groups, occupations, and genders (IPCC 2007). Climate change is expected to affect men and women differently because of their different access to assets, opportunities, and decision-making spaces. Gender inequalities, in many developing countries limit rural women's options of migrating to look for off-farm employment (Aguilar 2009) therefore making their livelihoods dependent on climate-sensitive sectors such as agriculture (Skinner 2011). The feminization of agricultural labor is an increasing phenomenon worldwide (World Bank FAO; IFAD 2009). Yet, the majority of the rural women have limited access to productive resources (e.g., land, water, and seed) to farm (Skinner 2011). The International Assessment of

Agricultural Knowledge, Science, and Technology for Development (IAASTD) argues that the feminization of agriculture can represent a further marginalization of small-scale farms because rural women have mostly limited education and access to resources and opportunities (IAASTD 2009). Poor men, conversely, face greater difficulties in fulfilling their socially assigned roles as breadwinners when agricultural revenues are insecure because they lack the financial capital to diversify their livelihoods (Skinner 2011).

There has always been a considerable interest in understanding whether science can attribute any particular drought or hurricane to climate changes (Schiermeier 2011). Thanks to the advances in statistical tools, climate models and computer power, a link has been found between extreme weather and climate change in at least two instances—the catastrophic flooding in the UK in 2000 (Pall et al. 2011) and the late-twentieth-century increase in intense rainfall across the Northern Hemisphere (Min et al. 2011).

8.2 Climate Changes, Food, and Agriculture

Using the results from formal economic models, it has been estimated that, in the absence of effective counteraction, the overall costs and risks of climate change will be equivalent to a 5 % decrease in global gross domestic product (GDP) each year (Stern 2005). If a wider range of risks and impacts is taken into account, the estimates of damage could rise to a 20 % decrease in GDP or more, with a disproportionate burden and increased risk of famine on the poorest countries (Altieri and Koohafkan 2003).

The majority of the world's rural poor (about 370 million of the poorest people on the planet) live in areas that are resource-poor, highly heterogeneous, and risk-prone. The worst poverty is often located in arid or semiarid zones, and in mountains and hills that are ecologically vulnerable (Conway 1997). In many countries more people, particularly those at lower income levels, are now forced to live in marginal areas (i.e., floodplains, exposed hillsides, arid or semiarid lands), putting them at risk from the negative impacts of climate variability and change.

Climate changes are predicted to have adverse impacts on food production, food quality (Atkinson et al. 2008), and food security. One of the most recent predictions (Tubiello and Fischer 2007) is that the number of undernourished people will have increased by 150 % in the Middle East and North Africa, and by 300 % in Sub-Saharan Africa by the year 2080, compared to 1990 (Table 8.1). Enhancing gender equality is recommended as a key strategy to support women's ability to fulfill their roles in food systems and food cultures vis-à-vis their disadvantaged access to resources and opportunities (Jiggins 2011).

Agriculture is extremely vulnerable to climate change. Higher temperatures eventually reduce crop yields without discouraging weed, disease, and pest challenges. Changes in precipitation patterns increase the likelihood of short-term crop failures and long-term declines in production. Although there will be gains in

Table 8.1 Expected number of undernourished in millions, incorporating the effect of climate (from Tubiello and Fischer 2007)

	1990	2020	2050	2080	2080/1990
Developing countries	885	772	579	554	0.6
Asia, Developing	659	390	123	73	0.1
Sub-Saharan Africa	138	273	359	410	3.0
Latin America	54	53	40	23	0.4
Middle East and North Africa	33	55	56	48	1.5

some crops in some regions of the world, the overall impacts of climate change on agriculture are expected to be negative, threatening global food security (Nelson et al. 2009).

Food insecurity will probably increase under climate change, unless early warning systems and development programs are used more effectively (Brown and Funk 2008). Currently, millions of hungry people subsist on what they produce. If climate change reduces production while populations increase, there is likely to be more hunger. Lobell et al. (2008) showed that increasing temperatures and declining precipitation over semiarid regions are likely to reduce yields for maize, wheat, rice, and other primary crops in the next two decades. These changes could have a substantial negative impact on global food security.

In addition, the impacts of climate change include reductions in calories consumption and increases in child malnutrition. Thus, aggressive agricultural productivity investments are needed to raise calories consumption enough to offset the negative impacts of climate change on the health and well-being of children (Nelson et al. 2009).

Foley (2011) proposed five solutions to food and environmental challenges, namely (a) stop the expansion of agriculture, particularly into tropical forests and savannas; (b) close the world's yield gaps between farm's current yield and its higher potential yield; (c) use resources much more efficiently to obtain far more crop output per unit of water, fertilizer, and energy; (d) shift diets away from meat: we can dramatically increase global food availability and environmental sustainability by using more of our crops to feed people directly and less to fatten livestock; and (e) reduce food waste: roughly 30 % of the food produced on the planet is discarded, lost, spoiled, or consumed by pests. For the second of these solutions Foley suggests that the largest and most immediate gain, especially in regions where hunger is most acute are to be expected by improving the yields of the world's least productive farms—a major shift in the research priorities of both national and international agricultural research.

Evidence indicates that if women small-scale farmers had the same access to productive resources—and seed of improved varieties in particular—as men, they could increase yields on their farms by 20–30 %, thereby reducing the number of hungry people in the world by 12–17 % (FAO 2011). Conversely, many have argued that access to food is more related to social marginalization and good governance than to production intensification (Sen 1981; De Schutter 2011;

Tscharntke et al. 2012). Empowering the most marginalized farmers and women farmers in particular, is seen as a means to both improve gender equality and to progress towards hunger and poverty eradication (FAO, IFAD, and WFP 2012).

8.3 How Do People Respond to Climate Changes?

Although the debate about climate changes is relatively recent, people have been adapting to climate changes for thousands of years, for example in Africa. In general, people seem to have adapted best when working as a community rather than as individuals. The four main strategies of adaptation have been (1) changes in agricultural practices, (2) formation of social networks, (3) embarking on commercial projects, such as investing in livestock, and (4) seeking work in distant areas. The first three of these strategies rely on people working together to improve their community (Giles 2007).

In coping continuously with extreme weather events and climate variability, farmers living in harsh environments in Africa, Asia, and Latin America have developed and/or inherited complex farming systems that have the potential to bring solutions to many of the uncertainties facing humanity in an era of climate change (Altieri and Koohafkan 2003). These systems have been managed in ingenious ways, allowing small farming families to meet their subsistence needs in the midst of environmental variability without depending much on modern agricultural technologies (Denevan 1995). The systems can still be found throughout the world, covering some 5 million ha. Such systems are of global importance to agriculture and food production, and are based on the cultivation of a diversity of crops and varieties in time and space that have allowed traditional farmers to avert risks and maximize harvest security in uncertain and marginal environments, under low levels of technology and with limited environmental impact (Altieri and Koohafkan 2003). One of the salient features of traditional farming systems is their high degree of biodiversity, in particular the plant diversity in the form of poly-cultures and/or agroforestry patterns. One example of this traditional farming system is a mixture of barley and wheat known as hanfets, which is practiced since millennia in the Central Highlands of Eritrea and in the northern part of Ethiopia (Woldeamlak and Struik 2000; Woldeamlak 2001; Woldeamlak et al. 2008). Farmers quote yield, yield stability, better resistance to lodging of barley, better resistance to rust of wheat, and better quality of the bread obtained from the mixture as the main reasons for growing this mixture. There are also examples in the same region of more complex mixtures involving bread wheat, durum wheat, six-row barley, and two-row barley. Another famous example is the nine seeds (*Navdanya*) mixture common in some regions of India; the mixture includes barley, little millet, pigeon pea, green gram, chickpea, rice, sesame, black gram, and horse gram.

An additional strategy used in areas with an erratic start of the rainy season, is to have a suite of crops to choose from depending on the timing of the start of the rains. This is, for example, the case in Eritrea where crops such as sorghum, pearl

millet, finger millet, teff, and barley are available to farmers (Ceccarelli et al. 2007). In the case of an early start of the rainy season, farmers plant teff and/or barley, while in the case of a late start they plant sorghum and pearl millet.

A careful observation of these systems shows that farmers tend to dilute the risk associated with practicing agriculture in difficult conditions using various combinations of three levels of biodiversity: different crops, different cultivars of the same crop, and/or heterogeneous cultivars to retain adaptability and to maximize adaptation over time (stability or dependability), rather than adaptation over space. Diversity and heterogeneity serve to disperse or buffer the risk of total crop failure due to unpredictable environmental variation. As we will see later, this is in sharp contrast with the trend of modern plant breeding towards uniformity over space and uniform cultivars.

These strategies of minimizing risk by planting several species and varieties of crops makes the system more resilient to weather events, climate variability and change, and is more resistant to the adverse effects of pests and diseases (Newton et al. 2011), while at the same time stabilizing yields over the long term, promoting diet diversity and maximizing returns even with low levels of technology and limited resources (Altieri and Koohafkan 2003). As we will see later, these strategies are an important lesson to breeding for adaptation to climate changes.

The term "autonomous adaptation" has been used to define responses that will be implemented by individual farmers, rural communities, and/or farmers' organizations, depending on perceived or real climate change in the coming decades, and without intervention and/or coordination by regional and national governments and international agreements. To this end, pressure to cultivate marginal land, or to adopt unsustainable cultivation practices as yields drop, may increase land degradation and endanger the biodiversity of both wild and domestic species, possibly jeopardizing future ability to respond to increasing climate risk later in the century.

One of the options for autonomous adaptation includes the adoption of varieties/species with, for example, increased resistance to heat shock and drought (Bates et al. 2008).

8.4 How Do Crops Cope with Climate Changes?

Adapting crops to climate changes has become an urgent challenge, which requires some knowledge on how crops respond to those changes. In fact plants have responded to increasing CO_2 concentration from preindustrial to modern times by decreasing stomatal density—reversing the change which occurred about 350 million years ago and that led to the appearance of leaves (Beerling et al. 2001; Beerling 2007; Ceccarelli et al. 2010)—as shown by the analysis of specimens collected from herbaria over the past 200 years (Woodward 1987). In *Arabidopsis thaliana*, the ability to respond to increasing CO_2 concentration with a decrease in the number of stomata is under genetic control (Gray et al. 2000); with the dominant

allele (*HIC* = high carbon dioxide) preventing changes in the number of stomata. In the presence of the recessive *hic* allele, there is an increase of up to 42 % in stomatal density in response to a doubling of CO_2. Stomatal density varies widely within species: for example in barley stomatal density varies from 39 to 98 stomata/mm^2 (Miskin and Rasmusson 1970) suggesting that the crop has the capacity to adapt.

We know now fairly well how plants respond to an increase in CO_2 concentration, which has both direct and indirect effects on crops. Direct effects (also known as CO_2-fertilization effects) are those affecting crops by the presence of CO_2 in ambient air, which is currently sub-optimal for C_3 type plants like wheat, rice, and barley. In fact, in C_3 plants, mesophyll cells containing ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCO) are in direct contact with the intercellular air space that is connected to the atmosphere via stomatal pores in the epidermis. Hence, in C_3 crops, rising CO_2 increases net photosynthetic CO_2 uptake because RuBisCO is not CO_2-saturated in today's atmosphere and because CO_2 inhibits the competing oxygenation reaction, leading to photorespiration. CO_2-fertilization effects can include an increase in photosynthetic rate, reduction of transpiration rate through decreased stomatal conductance, higher water use efficiency (WUE), and lower probability of water stress occurrence. Consequently, crop growth and biomass production may increase by up to 30 % for C_3 plants at doubled ambient CO_2. However, other experiments show biomass increases of only 10–20 % under doubled CO_2 conditions. In theory, at 25 °C, an increase in CO_2 from the current 380 to the 550 ppm (air dry mole fraction), projected for the year 2050, would increase photosynthesis by 38 % in C_3 plants. In contrast, in C_4 plants (e.g., maize and sorghum) RuBisCO is localized in the bundle sheath cells in which CO_2 concentration is three to six times higher than atmospheric CO_2. This concentration is sufficient to saturate RuBisCO and in theory would prevent any increase in CO_2 uptake with rising CO_2. However, even in C_4 plants, an increase in WUE via a reduction in stomatal conductance caused by an increase in CO_2 may still increase yield (Long et al. 2006).

However, the estimates of the CO_2-fertilization effects have been derived from enclosure studies conducted in the 1980s (Kimball 1983; Cure and Acock 1986; Allen et al. 1987), and currently they appear to be overestimated (Long et al. 2006).

In fact free-air concentration enrichment (FACE) experiments, representing the best simulation of elevated CO_2 concentrations in the future, give much lower ca. half) estimates of increased yields due to CO_2-fertilization (Table 8.2).

Indirect effects (also known as weather effects) are the effects of solar radiation, precipitation, and air temperature. Keeping management the same, cereal yields typically decrease with increasing temperatures and increase with increased solar radiation. If water supply is limited, yields eventually decrease because of higher evapotranspiration. Precipitation will obviously have a positive effect when it reduces water stress but can also have a negative effect when, for example, it causes waterlogging.

In addition to CO_2, nitrogen (N) deposition is also expected to increase further (IPCC 2007) and it is known that increasing N supply frequently results in declining species diversity (Clark and Tilman 2008). In a long-term open-air experiment,

Table 8.2 Percentage increases in yield, biomass, and photosynthesis of crops grown at elevated CO_2 (550) in enclosure studies versus FACE (Free-air concentration enrichment) experiments (Long et al. 2006)

Source	Rice	Wheat	Soybean	C_4 crops
	Yield			
Kimball (1983)	19	28	21	–
Cure and Acock (1986)	11	19	22	27
Allen et al. (1987)	–	–	26	–
Enclosure studies	–	31	32	18
FACE studies	12	13	14	0[a]
	Biomass			
Cure and Acock (1986)	21	24	30	8
Allen et al. (1987)	–	–	35	–
FACE studies	13	10	25	0[a]
	Photosynthesis			
Cure and Acock (1986)	35	21	32	4
FACE studies	9	13	19	6

[a]Data from only 1 year (Leakey et al. 2006)

grassland assemblages planted with 16 species were grown under all combinations of ambient and elevated CO_2 and ambient and elevated N. Over 10 years, elevated N reduced species diversity by 16 % at ambient CO_2 but by just 8 % at elevated CO_2. Although the projected increase in atmospheric CO_2 and global warming may enhance food production to some extent in the temperate developed countries, it is likely to reduce both arable area and yield per crop in many less developed ones (Evans 2005).

The most likely scenario within which plant breeding targets need to be established, is the following:

- Higher temperatures, which will reduce crop productivity, are certain
- Increasing CO_2 concentration is certain with both direct and indirect effects
- Increasing frequency of drought is highly probable
- Increase in the areas affected by salinity is highly probable
- Increasing frequency of biotic stress is also highly probable

Given this scenario, and given that plant breeding has been a success story in increasing yield (Dixon et al. 2006), plant breeding may help in developing new cultivars with enhanced traits better suited to adapt to climate change conditions. These traits include field drought and temperature stress resistance—defined as higher and stable performance (=grain yield, forage yield, tuber yield, etc.) under below optimal moisture availability and above optimal temperature, resistance to pests and disease—which will increasingly cause crop losses (Oerke 2006; Newton et al. 2011), salinity, and waterlogging (Humphreys 2005).

Breeding for drought resistance has historically been one of the most important and common objectives of several breeding programs for all the major food crops in most countries (Ceccarelli et al. 2004, 2007, 2010). It has also been a major

investment, yet with no improved varieties developed, of molecular breeding and genomic technologies (Morrell et al. 2012).

As we will discuss in the second part of the chapter, breeding for the adaptation to climate changes will be fruitless if farmers do not adopt the varieties with the desirable traits, regardless of whether these traits have been assembled using conventional or DNA-based technologies.

One of the main opportunities for new cultivars with increased drought tolerance includes changes in phenology or enhanced responses to elevated CO_2.

Phenology is known to be a major determinant in drought tolerance allowing crops to complete the life cycle before the onset of drought (Baum et al. 2003) and therefore this will be one of the main traits in breeding for adaptation to climate changes.

Phenology has been shown in recent studies to be associated with yield under drought (Lakew et al. 2011, 2013), and it has been shown to have been modified in wild relatives of wheat and barley collected in Israel over a period of 28 years (Nevo et al. 2012). Genes controlling flowering time are among the top candidates controlling local adaptation in *Arabidopsis thaliana* (Gaut 2012).

Recent data based on a large number of studies (Wolkovich et al. 2012) show that warming experiments under-predict advances in the timing of flowering by 8.5-fold, compared with long-term observations and the experimental results did not match with the observational data in sign or magnitude. The observational data also showed that the species that flower earliest in the spring have the highest temperature sensitivities, but this trend was not reflected in the experimental data.

Root characteristics, generally poorly known, are expected to become more and more important as water availability becomes the main limiting factor.

With respect to water, a number of studies have documented genetic modifications in major crop species (e.g., maize and soybeans) that have increased their water-deficit tolerance (Drennen et al. 1993; Kishor et al. 1995; Pilon-Smits et al. 1995; Cheikh et al. 2000), although this may not extend to a wide range of crops. In general, too little is known currently about how the desired traits achieved by genetic modification perform in real farming and forestry applications (Sinclair and Purcell 2005).

Thermal tolerances of many organisms have been shown to be proportional to the magnitude of temperature variation they experience: lower thermal limits differ more among species than upper thermal limits (Addo-Bediako et al. 2000). A crop such as barley, for example, which has colonized a wide diversity of thermal climates, may harbor enough genetic diversity to breed successfully for enhanced thermal tolerance.

Soil moisture reduction due to precipitation changes could affect natural systems in several ways and therefore, indirectly, also the agricultural systems. There are projections of significant extinctions in both plant and animal species. Over 5,000 plant species could be impacted by climate change, mainly due to the loss of suitable habitats. By 2050, the extent of the Fynbos Biome (Ericaceae-dominated ecosystem of South Africa, which is an International Union for the Conservation of Nature and Natural Resources (IUCN) “hotspot”) is projected to decrease by

51–61 % due to decreased winter precipitation. The succulent Karoo Biome, which includes 2,800 plant species at increased risk of extinction, is projected to expand south-eastwards, and about 2 % of the family *Proteaceae* is projected to become extinct. These plants are closely associated with birds that have specialized in feeding on them. Some mammal species, such as the zebra and nyala, which have been shown to be vulnerable to drought-induced changes in food availability, are widely projected to suffer losses. In some wildlife management areas, such as the Kruger and Hwange National Parks, wildlife populations are already dependent on water supplies supplemented by borehole water (Bates et al. 2008).

With the gradual reduction in rainfall during the growing season, aridity in Central and West Asia has increased in recent years, reducing the growth of grasslands and ground cover (Bou-Zeid and El-Fadel 2002). The reduction of ground cover has led to increased reflection of solar radiation, such that more soil moisture evaporates and the ground becomes increasingly drier in a feedback process, thus adding to the acceleration of grassland degradation (Zhang et al. 2003). Recently, it has been reported that the Yangtze river basin has become hotter and it is expected that the temperature will increase by up to 2 °C by 2050 relative to 1950 (Ming 2009). This temperature increase will reduce rice production by up to 41 % by the end of the twenty-first century and maize production by up to 50 % by 2080.

The negative impact of climate changes on agriculture and therefore on food production is aggravated by the greater uniformity that exists now compared to 150–200 years ago, particularly in the agricultural crops of developed countries. The decline in agricultural biodiversity can be quantified. While it is estimated that there are ca. 250,000 plant species, of which about 50,000 are edible, in fact no more than 250 are used—out of which 15 crops provide 90 % of the calories in the human diet and three of them, namely wheat, rice and maize, provide 60 %. In these three crops, modern plant breeding has been particularly successful and movement towards genetic uniformity has been rapid—the most widely grown varieties of these three crops are closely related and genetically uniform (pure lines in wheat and rice and hybrids in maize—but hybrids are being promoted also in rice (Jahaiah 2002)).

The number of varieties covering large areas for the major crops is frighteningly small: 71 % of the area planted with maize is planted with 6 varieties, 75 % of the area planted with potato is planted with 4 varieties, 65 % of the area planted with rice is planted with 4 varieties, and so on (Secretariat of the Convention on Biological Diversity 2010).

The major consequence of the dependence of modern agriculture on a small number of varieties for the major crops (Altieri 1995) is that the main sources of food are more genetically vulnerable than ever before, i.e., food security is in danger. A number of plant breeders have warned that conventional plant breeding by continuously crossing between elite germplasm lines would lead to the extinction of diverse cultivars and nondomesticated plants (Vavilov 1992; Flora 2001; Gepts 2006; Mendum and Glenna 2010) and climate change may exacerbate the crisis. Gepts (2006) claims that the current industrial agriculture system is “the

single most important threat to biodiversity." Historically, there are several examples of the devastating effects of a narrow genetic base (Keneni et al. 2012). A recent example of this danger is the rapid spreading of diseases such as UG99 (a new race of stem rust of wheat caused by *Puccinia graminis triticii*, detected for the first time in Uganda in 1999). The new race is virulent to most wheat varieties and can cause complete loss of the crop (Pretorius et al. 2000; Singh et al. 2006). The danger of a narrow genetic base applies equally well to climate changes as the current predominant uniformity does not allow the crops to evolve and adapt to the new environmental conditions (see also Chapter 9). The expected increase of biofuel monoculture production may lead to increased rates of biodiversity loss and genetic erosion. Another serious consequence of the loss of biodiversity has been the displacement of locally adapted varieties, which may hold the secret of adaptation to the future climate (Ceccarelli and Grando 2000; Ceccarelli et al. 1992; Grando et al. 2001; Sarker and Erskine 2006; Rodriguez et al. 2008; Abay and Bjørnstad 2009; Ceccarelli 2012a).

One aspect of modern plant breeding in relation to traits important for adaptation to climate changes has been its reductionist approach in searching, for example, genes for drought resistance. Only recently it has been recognized that one of the key traits in relation to climate changes, namely drought resistance is too complex to be manipulated with biotechnological methods. In fact, those methods have so far failed to increase farmers yield in dry years or dry locations.

8.5 Agrobiodiversity and Plant Breeding

Plant breeding is one of the main causes for the reduction of agrobiodiversity quantified earlier and the evolution of plant breeding helps explain the progression of genetic erosion.

Selection started at the same time as domestication when the Neolithic men and women started intentional sowing, which applies strong, unconscious selection pressure (Zohary 2004). Alleles for nonshattering, lack of dormancy, reproductive determinacy, and increased fertility of formerly sterile florets are all favored by the sowing–harvesting–sowing cycle (Harlan et al. 1973). After domestication, farmers have continued to modify crops for millennia and have been largely responsible for the spreading of crops across the planet (Gepts 2002). As they migrated across continents, they brought with them their seeds and their animals, which both needed to adapt to the new environments, the new soil types and possibly to new uses. In the plant breeding done by farmers there was an emphasis on specific adaptation not only to the environment (climate and soil) but also to the uses so that it was obvious that the same farmer will select more than one variety of the same crop and that different farmers will select different varieties. An important aspect of farmers' breeding was that the selection environment and the target environment was the same, a situation that avoids the negative consequences of Genotype $\times$ Location

interaction on response to selection (Falconer 1981). Over thousands of years this process (farmers' breeding) led to the formation of landraces.

Therefore, long before Mendel and long before plant breeding as we know it today, farmers planted, harvested, stored, and exchanged seeds, and fed themselves and others, and in doing all this they built a considerable amount of knowledge about the crops, their characteristics and possible uses, and their interactions with the surrounding environment.

With the rediscovery of Mendel's work, two major changes took place, which profoundly affected the evolution of plants, particularly of domesticated crops and of their evolutionary potential (Ceccarelli 2009b). Firstly, plant breeding was moved from farmers' fields to research stations and from farmers to scientists. What was done by very many farmers in very many different places started to be done by relatively few scientists in a relatively few places (the research stations), which with time became more and more similar to each other. Secondly, selection for specific adaptation was replaced by selection for wide adaptation because of aiming at several target environments from few selection environments. Thirdly, plant breeding gradually went from publicly to privately funded—today even the CGIAR,[1] which were publicly funded till recently, have opened the door to private donors: as a consequence not all crops were treated equally, and some became "orphan crops," neglected by science. They include some important food crops such as banana, cassava, and yam that are central in the livelihoods of the poorest farmers (Bellon 2006) and of women farmers in particular (Howard 2003). In these changes, there is no evidence that any use was made of, or any attention was paid to, the local knowledge accumulated over thousands of years.

It is interesting to note that in the early part of the twentieth century a number of scientists were actually advocating an environmentally friendly type of plant breeding. In 1923, H. K. Hayes wrote, "The importance of plant breeding as a means of obtaining varieties which are adapted to particular environmental conditions is becoming more generally recognized." In 1925, F. L Engledow added, "We can no longer hope, as breeders once did, for the new form which everywhere and in all years will excel. Our hope is of breeding for every locality the form best adapted to the environment it offers."

However, the dominant breeding philosophy which eventually emerged as a consequence of what is known as the "Green Revolution" was based on "wide adaptation," i.e., the selection of varieties able to perform well in many different locations and countries, having lost photoperiod sensitivity and vernalization requirement.

The term Green Revolution was coined in March 1968 by William S. Gaud, the then director of the US Agency for International Development (USAID) to indicate the outcome of a development strategy based on (a) new crop cultivars, (b) irrigation, (c) fertilizers, (d) pesticides, and (e) mechanization. Within that strategy, the

[1] CGIAR is the new brand name of the Consultative Group of International Agricultural Research Center.

new varieties obtained by shuttle breeding were, as a collateral and unplanned effect, photoperiod insensitive and without vernalization requirement, hence widely adapted (Salvi et al. 2013). Not only was this exactly the opposite of what farmers had done for millennia, but the term wide adaptation was somewhat misleading because it indicates wide "geographical" adaptation rather than wide "environmental" adaptation (Ceccarelli 1989). In fact the agricultural environments in which these "widely adapted" varieties were successful were actually very similar (high rainfall and good soil fertility) or were made similar by adding irrigation water, fertilizers, and pesticides when farmers can afford them. This caused three major problems. First, the heavy use of chemicals soon began affecting the environment. Today it is estimated that about 25 % of N applied particularly in developing countries does not provide any additional yield increase but only increased pollution (Good and Beatty 2011). Second, the poorest farmers and particularly those living in marginal environments were bypassed because they could not afford to purchase the chemicals needed to create the right environments for the new varieties—not all scientists agree on this, but most of the poor farmers do. The father of the Green Revolution, Norman Borlaug, pointed out a few years ago, "despite the successes of the Green Revolution, about two billion people still lack reliable access to safe, nutritious food, and 800 million of them are chronically malnourished" (Reynolds and Borlaug 2006); these figures may well increase because of climate changes. Third, there was a dramatic decline in agricultural biodiversity because on one hand hundreds of genetically diverse local varieties selected by farmers over millennia for specific adaptation to their own environment and uses were displaced, and on the other hand the new varieties (despite having different names) were all very similar in their genetic constitution.

The trends towards uniformity has continued and today we see a dramatic contrast between, on one hand, the scientific literature showing how vital is biodiversity for our future on this planet and, on the other, the dramatic decrease of agrobiodiversity which is made even worse by the ever-increasing concentration of the seed market in the hands of a few seed industries (Fuglie et al. 2011).

A key issue in breeding for climate changes is to recognize that climate changes are a moving target and therefore the emphasis should not be so much on which trait to breed for but rather a drastic change in breeding strategies to have a highly dynamic and efficient system of variety deployment in farmers' fields.

8.6 Genotype × Environment Interactions and Breeding Strategies

One of the main consequences of the separation between the selection environment (the research station) and the target environments (the farmers' fields) is that a large amount of breeding material is discarded before knowing whether it could have been useful in the real conditions of farmers' fields, and the one which is selected is

likely to perform well in environments similar to the research stations, but not in environments which are very different. This is because of Genotype × Environment (GE) interactions which are one of the major factors limiting the efficiency of breeding programs when they cause a change of ranking between genotypes in different environments (crossover interaction).

Studies conducted in Australia (Pederson and Rathjen 1981; Cooper et al. 1997) to evaluate the relevance of research stations for their suitability as selection environments have found that, in many cases, the genetic correlations between the yield of breeding lines on the research station and yield under on-farm conditions were low in comparison with the genetic correlations between different on-farm experiments.

An example of crossover GE interactions between research stations and farmers fields is given in Fig. 8.2. In both cases there was much more similarity between research stations than between farmers' fields, and low or negative correlations between research stations and most of the farmers' fields. Another case is shown in Fig. 8.3. The five highest yielding barley lines in a farmer's field in Senafe (Eritrea) had a yield advantage over the local check of between 27 % and 30 %. However, when tested on-station the same lines showed a yield disadvantage of between 15 % and 87 % except entry 95 which had a yield advantage of only 4 %. Therefore, most probably they would have been discarded had the evaluation been done only in the research station.

In general, when different lines or cultivars of a given crop are evaluated in a sufficiently wide range of environments, GE interactions of crossover type seem to be very common (Ceccarelli 1996). We have argued (Ceccarelli 1989) that for crops grown in environments poorly represented by the research stations, this often results in useful breeding materials being discarded.

When GE interactions are significantly large, it is not possible to ignore them and the two remaining strategies are (1) to avoid them by selecting material that is broadly adapted to the entire range of target environments, or (2) to exploit them by selecting a range of material, each adapted to a specific environment (Ceccarelli 1989). The choice is based on a separate analysis of the two components of GE interactions, namely Genotype × Years (GY) and Genotype × Locations (GL), the first of which is largely unpredictable, while the second, if repeatable over time, identifies distinct target environments (Annicchiarico et al. 2005, 2006).

Selection for specific adaptation to each of the target environments is particularly important in breeding crops predominantly grown in unfavorable conditions such as those that will increasingly become more common with climate changes, because unfavorable environments tend to be more different from each other than favorable environments (Ceccarelli and Grando 1997). An example is shown in Fig. 8.4 where the total GE in the case of the two dry locations (left) was nearly 90 %, while in the case of the two high rainfall locations was less than 50 %.

Selecting for specific adaptation has the advantage of adapting cultivars to the physical environment where they are meant to be cultivated, and hence is more sustainable than other strategies, which rely on modifying the environment to fit new cultivars adapted to more favorable conditions (Ceccarelli and Grando 2002).

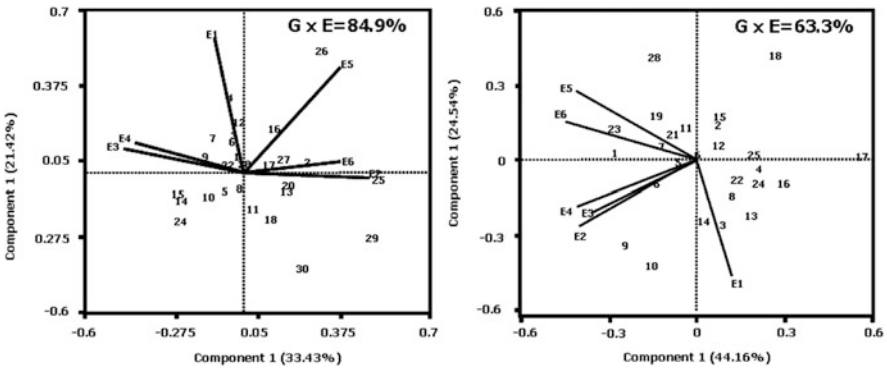

Fig. 8.2 Biplots of 30 barley genotypes grown in six locations in Morocco (*left*) including two research stations (E3 and E4) and four farmers' fields (E1, E2, E5, and E6) and of 25 barley genotypes in six locations in Tunisia (*right*) including two research stations (E5 and E6) and four farmers' fields (E1, E2, E3, and E4)

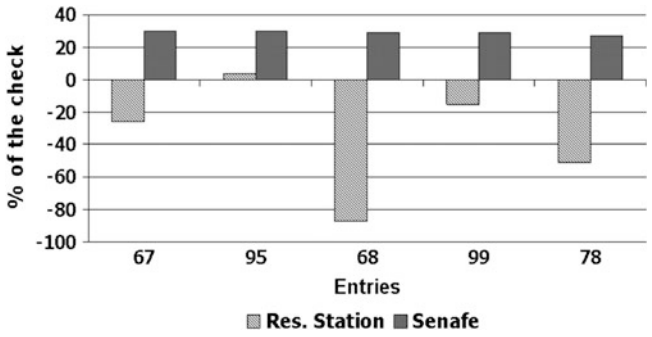

Fig. 8.3 Yield (in percent of the local check) of five barley lines in a farmer's field in Senafe (Eritrea) and in the research station at Halale (40 km south of Asmara)

Selection theory and experimental data (Annicchiarico et al. 2005) shows that selection for specific adaptation is more efficient because it exploits the larger heritabilities within each specific target environment.

The similarity between research stations observed in Fig. 8.2 and between high rainfall locations and years observed in Fig. 8.4 are likely to be also associated with the larger use of inputs (fertilizers, weed control, etc.) common to both research stations and high rainfall areas, which tend to smooth out differences between locations and years.

Selection for specific adaptation is based on direct selection in the target environment as farmers' did for millennia, which has also been defined as decentralized selection (Falconer 1981; Simmonds 1984, 1991). Murphy et al. (2007) have shown that selection for specific adaptation is important in organic agriculture (van Bueren Lammerts and Myers 2012).

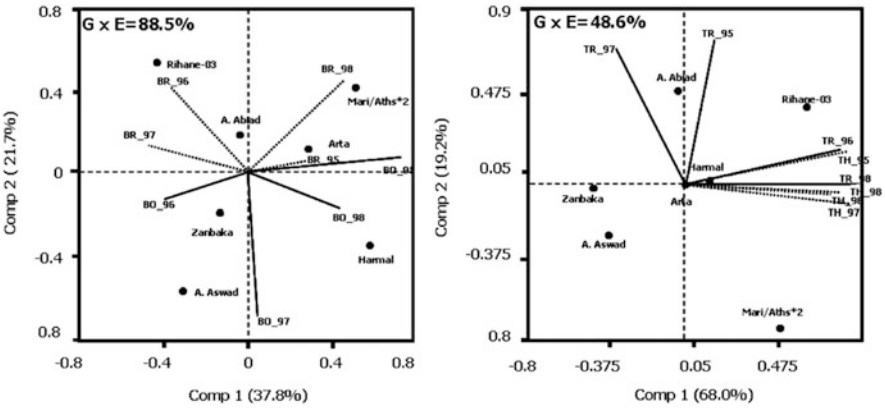

Fig. 8.4 Biplots of grain yield of seven barley cultivars grown for 4 years (1995–1998) in two dry locations, Bouider (BO) and Breda (BR) with a grand mean of 1.3 t/ha (*left*) and in two locations, Tel Hadya (TH) and Terbol (TR) with a grand mean of 3.5 t/ha (*right*)

Direct selection in the target environments is particularly important in the case of a moving target such as a gradual increase in temperature and a gradual decrease in rainfall, their interactions, and the interaction with biotic stresses and agronomic management, the likely scenario of climate changes. The advantages of selecting for specific adaptation to climate changes are the holistic approach and the fact that phenology, the plant attribute that we have seen as one of the major factors involved in adaptation to climate changes, is highly heritable. This should allow a cycle of selection for earliness on-station, which leaves enough genetic variability in the breeding material for adaptation to specific environmental conditions in the subsequent cycles of selection.

8.7 A New Paradigm

At this point, a number of questions can be asked such as:

- Would it have been possible to feed the world without depleting the resources of the planet?
- Given that plant breeding has been defined as "guided evolution," could we have "guided" the evolution of crops in a different direction?
- Would it be possible to harmonize the increase of agricultural production with agrobiodiversity conservation?
- In summary, would it be possible to organize agricultural research in general and plant breeding in particular, in such a way to increase agricultural production while at the same time respecting biodiversity, gender equity, the environment, and ultimately safeguarding human health?

Participatory research and participatory plant breeding (PPB) are addressing these specific questions while at the same time addressing some of the major global problems such as climate changes, biodiversity, and hunger.

8.8 Participatory Plant Breeding

In recent years, there has been an increasing interest towards participatory research in general, and towards participatory plant breeding in particular. Following the early work of Rhoades and Booth (1982), scientists have become increasingly aware that users' participation in technology development may in fact increase the probability of success for the technology.

The interest is partly associated with the perception that the impact of agricultural research, including plant breeding, particularly in developing countries and for marginal environments and poor farmers has been below expectations.

Three common characteristics of most agricultural research, which might help to explain its limited impact in marginal areas, are:

The research agenda is usually decided unilaterally by the scientists and is not discussed with the users;

Agricultural research is typically organized in compartments, i.e., disciplines and/or commodities, and seldom uses an integrated approach; this contrasts with the integration existing at farm level. The different technology needs of the users (influenced for example by socioeconomic, gender and cultural factors that might affect their agronomic practices, food preferences, crops and variety priorities) and their knowledge are rarely taken into account;

There is a disproportion between the large number of technologies generated by agricultural scientists and the relatively small number of them actually adopted and used by the farmers.

When one looks at these characteristics as applied to plant breeding programs, most scientists would agree that:

- Plant breeding has not been very successful in marginal environments and for poor farmers and has generally overlooked gender-based differences in crop and variety preferences and needs;
- It still takes a long time (about 15 years) to release a new variety as reported in the recommendations of Interdrought, Rome (2005) "While the support for and the capacity of plant biotechnology increased, the collaboration with plant breeding has been insufficient (with the exception of the private sector). This lack of collaboration resulted in slow delivery of biotechnology solutions to the user in the field. There is an explosive growth of information in genomics with a proportionally minute rate of application of this information to problem solving in farming under water-limited conditions"
- Many varieties are officially released, but few are adopted by farmers; by contrast farmers often grow varieties, which were not officially released

- Even when new varieties are acceptable to farmers, their seed is either not available or too expensive
- There is a widespread perception of a decrease of biodiversity associated with conventional plant breeding.

Conventional plant breeding seems therefore ill-suited to provide a dynamic and rapid adaptation to climate changes matched by a prompt adoption by farmers.

Participatory research, in general, defined as that type of research in which users are involved in the design—and not merely in the final testing—of a new technology, is now seen by many as a way to address the problems discussed in the first part of this chapter. PPB in particular, is defined as that type of plant breeding in which farmers, as well as other partners, such as extension staff, seed producers, traders, NGOs, etc., participate in the development of a new variety. PPB is expected to produce varieties, which are targeted (focused on the right farmers), relevant (responding to real needs, concerns, and preferences) and appropriate (able to produce results that can be adopted) (Bellon 2006).

In the next sections we will illustrate some of the characteristics of PPB using examples from projects implemented by the International Center for Agricultural Research in the Dry Areas (ICARDA) in a number of countries (Ceccarelli 2012b), from experiences in participatory plant breeding applied to organic agriculture (Desclaux et al. 2011) with an emphasis on the beneficial effects of PPB in relation to climate changes.

8.9 Plant Breeding and Plant Breeders

Plant breeding is an applied, multidisciplinary science based on the application of genetic principles and practices for the development of cultivars more suited to the needs of people; it uses knowledge from agronomy, botany, genetics, cytogenetics, molecular genetics, physiology, pathology, entomology, biochemistry, bioinformatics, and statistics (Schlegel 2003). The ultimate outcome of plant breeding is mainly improved cultivars. Therefore, plant breeding is primarily a science, which looks at the organism as a whole even though it is also suited to translate information at the molecular level (DNA sequences, protein products) into economically important phenotypes (Gepts and Hancock 2006).

As a science, plant breeding started soon after the rediscovery of Mendel's Laws at the beginning of the twentieth century. Before that, plant improvement had been done for several thousand years by farmers as described earlier.

There is evidence that hybridization also started before 1900 (as discussed by, for example, Strampelli 1944). Since then, plant breeding has evolved by absorbing approaches from different areas of science, allowing breeders to increase their efficiency and exploit genetic resources more thoroughly (Gepts and Hancock 2006). Over the years, it has put to productive use the progress in crop evolution, population and quantitative genetics, statistical genetics and biometry, molecular

biology, and genomics. Thus, plant breeding has remained a vibrant science, with continued success in developing and deploying new cultivars on a worldwide basis. On average, around 50 % of productivity increases can be attributed to genetic improvement (Fehr 1984). Despite differences between crops and between breeders, in all breeding programs it is possible to identify three main stages (Schnell 1982; Ceccarelli 2009a):

- Generating genetic variability. This includes making crosses (selection of parents, crossing techniques, and type of crosses), inducing mutation, and introducing exotic germplasm.
- Selection of the best genetic material within the genetic variability created in the first stage. In self-pollinated crops, this includes primarily the implementation of various methods, such as classical pedigree, bulk pedigree, backcross, hybridization, recurrent selection, or the F_2 progeny method. In self-pollinated tree crops, this includes progressive evaluation of individual plants. In cross-pollinated crops, synthetic varieties, open-pollinated varieties and hybrids are used, and in vegetatively propagated crops there are clones and hybrids. Marker-assisted selection (MAS) could be used in this stage.
- Testing of breeding lines. This includes comparisons between existing cultivars and the breeding lines emerging from Stage 2, and the appropriate methodologies to conduct such comparisons. These comparisons take place partly on-station (on-station trials) and partly in farmers' fields (on-farm trials).

As a consequence of Stage 1 and partly also due to selection during the first part of Stage 2, the amount of breeding materials generated is very large (from a few to several thousands). During Stages 2 and 3 the number of breeding lines decreases, the amount of seed per line increases and so does the number of locations where the material can be tested.

There are two other important stages in a breeding program: setting priorities; and dissemination of cultivars. These two steps have been discussed in detail by Weltzien and Christinck (2009) and by Bishaw and van Gastel (2009).

In a nonparticipatory program, all the decisions are taken by the breeder and by the breeding team, even in the case of on-farm trials.

An important characteristic of a breeding program is that it is a cyclic process in which each step feeds information and material into the subsequent step, and each breeding cycle feeds information into the next cycle (Fig. 8.5).

By breeding cycle we mean the period of time, usually 10–15 cropping seasons (assuming one generation per year), from making a cross to obtaining advanced lines or varieties, which in turn are used as parental material in the crossing program to start a new cycle, i.e., from cross to cross. In a breeding program, where crosses are made every year, several breeding cycles co-exist, each one year ahead of its successor. During this process, a tremendous amount of information is generated, and one of the major challenges in a breeding program is how to capture and store this information in a way that is sufficiently transparent for others (scientists and nonprofessionals) to use. In conventional (nonparticipatory) breeding programs (CPB), most of this information represents the "cumulative experience" or the

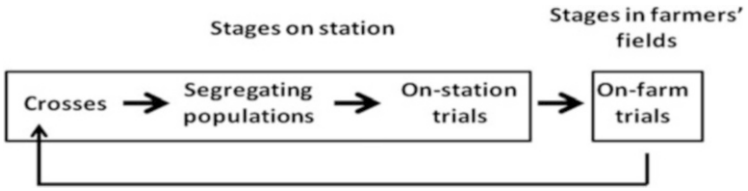

Fig. 8.5 Schematic representation of a typical centralized, nonparticipatory plant breeding (CPB) program that mostly takes place within a research station (the first three stages, which usually last more than 10 years), with all the decisions being taken by the breeder's team

"knowledge of the germplasm" that the breeder slowly accumulates over the years. Examples of the three main stages of a breeding program can be easily identified in the three major groups of crops, namely self-pollinated, cross-pollinated, and vegetatively (or clonally) propagated, and in the most common breeding methods used (Ceccarelli 2012b).

Alongside a definition of plant breeding it is also important to define who is a plant breeder.

The traditional definition of a plant breeder includes only those persons who have the full responsibility of a breeding program, made up of progressive cycles, as described earlier, to develop new cultivars and improved germplasm. However, many feel this definition should be expanded to include persons who contribute to crop improvement through breeding research (Ransom et al. 2006).

In this chapter, we will use the traditional definition of a plant breeder because we believe that only scientists who have the full responsibility for a breeding program can be successful partners of farmers in PPB programs.

8.10 Defining Participatory Plant Breeding

We define PPB as a dynamic and permanent collaboration that exploits the comparative advantages both of plant breeding institutions (national or international) that have the institutional responsibility for plant breeding, and of farmers and possibly other partners, as noted earlier. The definition does not imply preassigned roles, or a given amount of collaborative work (at one extreme, scientists may only supply germplasm, while at the other partners may only do field selection), nor imply that farmers and breeding institutions are the ONLY partners. This is because field experience in practicing PPB tells us that a true PPB program is a dynamic process in which both the roles of partners and the extent and the manner in which they collaborate change with time. Implicit in this definition is that farmer breeding, in which scientists or other stakeholders have no part, is not considered as a PPB program. This of course should not be interpreted as an underestimation of its value and importance. It is also important to mention that a truly participatory program is

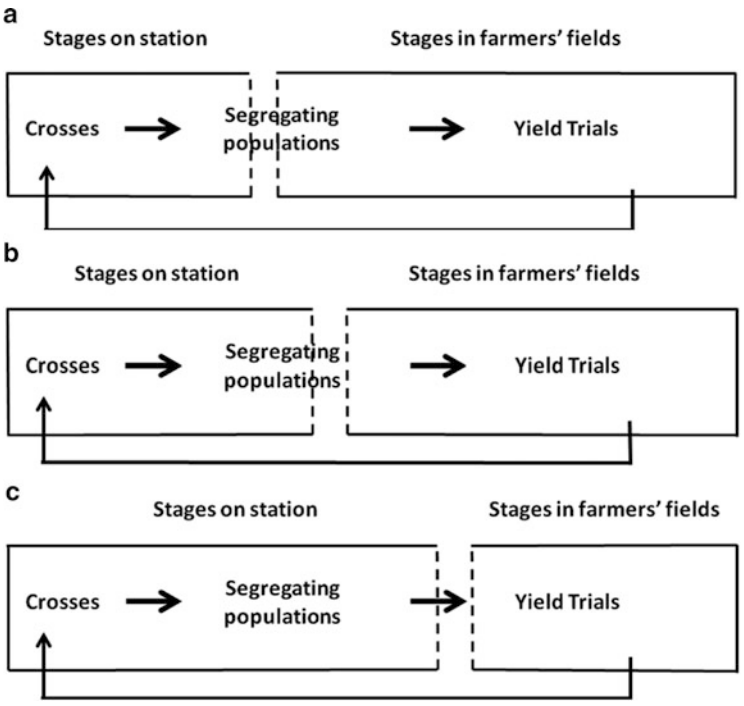

Fig. 8.6 Schematic representation of two types (A and B) of PPB program: the stages that take place within a research station are much less (the first and part of the second in A and the first and most of the second in B) than in a CPB program, with all the decisions being taken by the breeder's team together with the farmer community. If the decentralization takes place in the third stage (as in C) with a small number of lines the program becomes a Participatory Variety Selection program (discussed later)

necessarily inclusive in relation to gender and has, as we also see later, an empowering effect on the participants.

With regards to gender, while it is possible to conduct gender analysis and gender studies in a nonparticipatory context, the contrary is not true: in other words, a program that is not gender inclusive does not deserve to be defined as participatory.

A PPB program (Fig. 8.6) is similar to a CPB program in that it maintains the typical cyclic structure of a breeding program, but with three important organizational differences (Ceccarelli 2009c):

- Most of the program takes place in farmers' fields (i.e., is decentralized)
- The decisions are taken jointly by the breeder and the farmers and other partners
- The program, being decentralized, can be replicated in several locations with different methodologies and types of germplasm (Fig. 8.7)

Comparing Fig. 8.6 with Fig. 8.5, it will be noticed that there are no differences in the case of Stage 1; in Stage 2, the CPB program is conducted on-station, while in

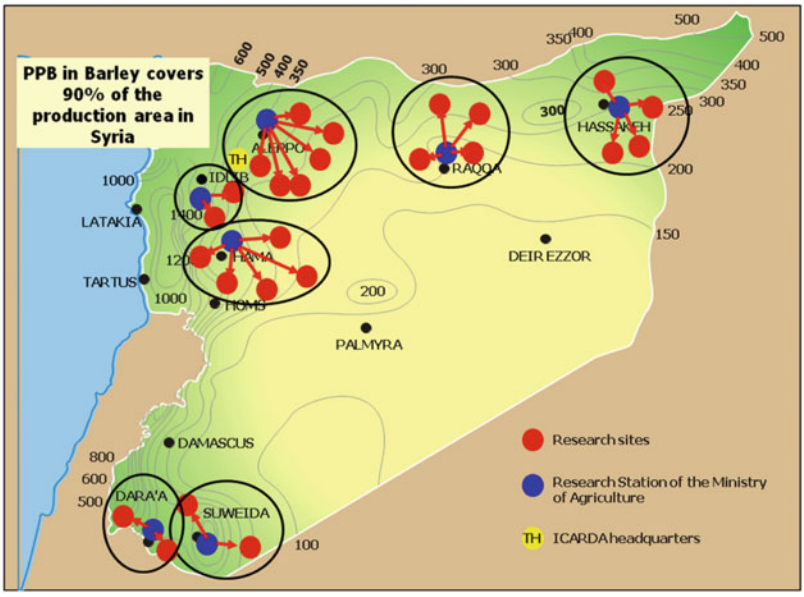

Fig. 8.7 The organization of a PPB program using the example of the barley PPB program in Syria

a PPB program it is conducted partly on-station and partly in farmers' fields; while in Stage 3, which in CPB programs is partly conducted on-station and partly conducted on-farm, in the case of a PPB program it is confined to farmers' fields. Figure 8.6C also represents the case of crops grown for the market (malting barley, wheat for industrial transformation, canola, groundnut, cassava, etc.), which need to possess a given expression of a suite of traits to be accepted by the market. These traits can be fixed, when possible with MAS, on-station, while traits associated with adaptation to different environments will be selected on-farm with the participation of farmers and other partners.

It is also possible for farmers to make crosses on-farm with the technical assistance of breeders. In these cases, the entire process takes place on-farm and the amount of variability can be increased by crosses coming from the station. These cases are not very frequent, as they require special skills and dedication.

8.11 Participatory Variety Selection

Participatory Variety (or Varietal) Selection (PVS) is a process by which the field testing of finished or nearly finished varieties, usually only a limited number, is done with the participation of the partners. Therefore, PVS is always an integral part of PPB, representing its final stages, but can also stand alone in an otherwise

nonparticipatory breeding program if, using Fig. 8.5 as an example, partners' opinion is collected and used during the final stage, i.e., the on-farm trials.

Involvement of partners during the last stage of an otherwise nonparticipatory breeding program has one major advantage and one major disadvantage: the advantage is that, if the partners' opinion becomes part of the release process which follows the on-farm trials, only the variety(ies) that partners like will be proposed for release, thus increasing enormously the speed and the rate of adoption; the major disadvantage is that because partners' opinion is sought at the very last stage of the breeding program there may be nothing left among the varieties tested in the on-farm trials that meets partner expectations. This disadvantage may induce the breeder to seek partner participation at an earlier stage of the breeding program, hence moving from PVS to PPB. PVS may also be used as a starting point, a sort of exploratory trial, to help partners in assessing properly the amount of commitment in land and time that a full-fledged PPB program requires.

8.12 A General Model of Participatory Plant Breeding

A general model of PPB as defined above is shown in Fig. 8.8a. In this model, the first step (generation of genetic variability) is often, but not necessarily always, the responsibility of the research institution. It should be noted that when the genetic variability is created by making crosses, there is a substantial difference between making crosses, choosing the parents, and designing the crosses. Making a cross is a purely technical operation, while choosing the parents and designing the crosses is a key decision in a breeding program. In a breeding program, a large part of the parental material used in crosses is represented by the best breeding material selected from the previous breeding cycle, and because in PPB the selection is done by both breeders and farmers, farmers do in fact participate in the choice of the parents to begin a new breeding cycle. Farmers may also explicitly choose parents by suggesting crosses to the research institution or learning to perform crosses themselves.

A number of stages of selection (four in this hypothetical example) are conducted in each farmers' field with the participation of men and women farmers and other stakeholders, with continuous interaction with the research institute (for example for the choice of appropriate experimental designs, data analysis, seed production, etc.) and with other farmers involved in the PPB program. The selection is conducted independently in each location. This generally leads to the selection of different entries in different locations but does not exclude selecting the same material (see for example in Fig. 8.8b variety A being selected in locations 1 and 3 and variety B being selected in locations 2 and 3).

The best breeding material produced after the four stages of selection can be used by farmers as varieties and by the research institute as parental material for crosses to begin a new breeding cycle. It is important to notice that different locations may receive different types of germplasm of the same crop and select

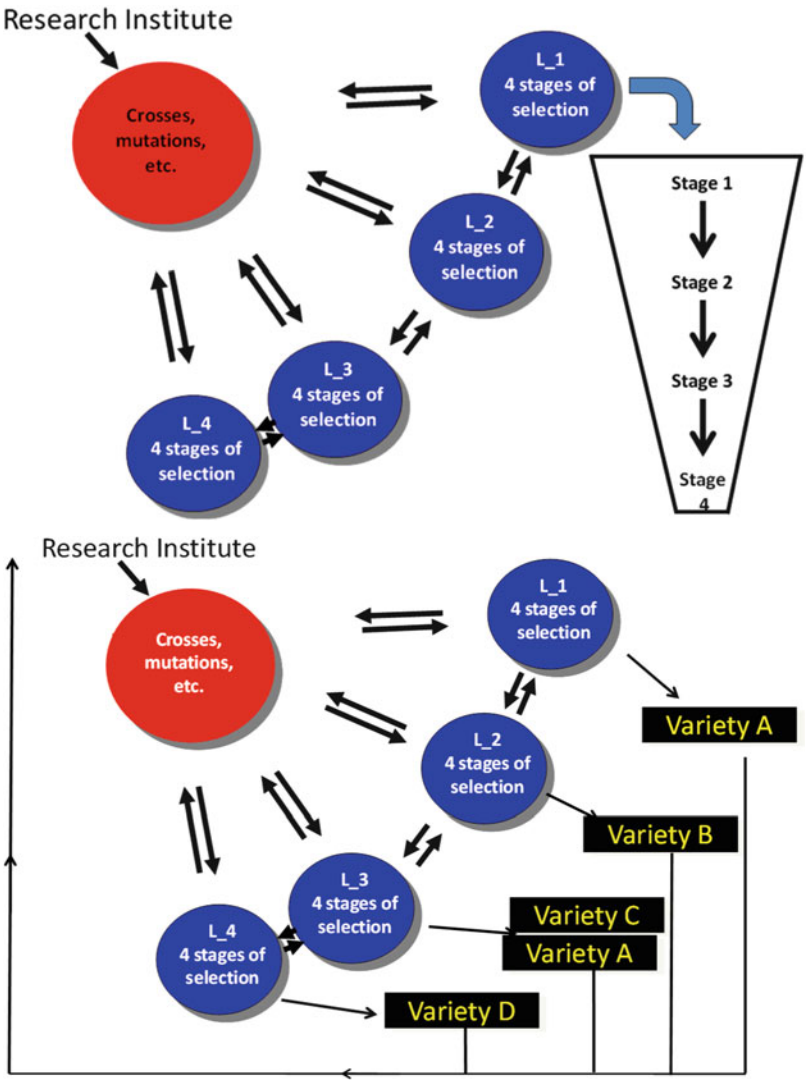

Fig. 8.8 (**a**) A schematic model of participatory plant breeding in four villages: from stage 1, grown by one farmer, participatory selection identifies the lines to be grown in the stage 2 trials by more farmers. The process is repeated to identify lines to be grown in stage 3 trials and in the stage 4 trials. The key aspect is that selection is conducted independently in each village. (**b**) As a result of the process described in (**a**), similar but more often different varieties are selected in each village. The varieties can go directly into cultivation, can be shared among farmers and go back to the research center for further cycles of recombination and selection. Hence, a participatory breeding program maintains the cyclic aspect of a breeding program

different varieties and that interaction among farmers may depend on their geographical location as well as communication technologies, language differences, etc.

A PPB program may lose a great deal of its potential effectiveness if the sample of both environments and users in which the program is implemented does not represent both the target environments and the target users. In order to do that, setting the criteria for identification of the target environments and users is a critically important step. This is even more important if one of the objectives of the program is to generate a continuous flow of varieties and/or population adapted to a moving target such as climate changes.

The most obvious criterion for the choice of the target physical environments is the representativeness of the combination of stresses, both biotic and abiotic the crops are likely to meet in the future. PPB has evolved mainly to address the difficulties of poor farmers in developing countries (Ashby and Lilja 2004), which have been largely bypassed by the products of CPB.

Once the target environments have been selected, the choice of which farmers in the communities and within the households to collaborate with is a key factor that affects the relevance of the improved varieties at ground level (Cornwall 2003; Guijt and Shah 2006). Targeting the right users is particularly critical for marginal areas where agriculture is characterized by wide spatial and temporal variability of agro-ecological conditions and by diverse socioeconomic needs resulting in complex stresses and high production risks (Aw-Hassan et al. 2008; Bellon 2006). Gender-sensitive targeting is important wherever socioeconomic needs vary within the community and the household. It is particularly critical in cases when men and women perform different agronomic activities that entail gender-differentiated skills, knowledge, needs, and trait preferences (Farnworth and Jiggins 2003; Pimbert 2006). For instance, those in charge of food processing might have preferences related to cooking quality that are different from those in charge of marketing who might prioritize customers' product requirements. Gender sensitive analysis and a careful and systematic observation in the field might help reveal gender-based agronomic roles, crop and variety preferences and overcome gender biases in the identification of farmers to collaborate with (Galiè et al. 2012). In cases when agricultural labor is not divided on the basis of gender, it might be worth assessing empirically how gender affects the preferences relative to farm activities. In Syria, for example, both men and older women are in charge of marketing the seed and straw (younger women are not involved in marketing). However, gender was found to affect their performance of marketing activities: men access more formal and wider markets than older women who mainly sell to other women in the village. This has consequences on their customers' requirements and therefore variety preferences (Galiè 2013c). Finally, the inclusion of gender concerns in PPB might also help identify crops that are considered important by farmers for their food security but usually neglected by crop improvement because they are considered less economically valuable.

Paris et al. (2008) argue that who participates in decision-making about crop improvement affects both the resulting varieties—because of the breeding priorities that are taken into account—and variety adoption—because involvement in variety trials and evaluations might affect final adoption. Effort to involve all household decision-makers in PPB seems a good strategy to ensure that the portfolio of PPB

varieties reflects the breeding priorities of all members, and are evaluated by them, all (Galiè et al. 2012). Also, selecting farmers who have knowledge, status, and decision-making power, and are well placed within the seed distribution network is helpful to increase the out-scaling of PPB varieties to villages not directly involved in the program. However, Farnworth and Jiggins (2003) argue that participants selected because of efficiency criteria only, might not be representative of the intended target group and most marginal individuals in the communities are likely to be excluded.

Empowerment of farmers is considered an important means to increase the participation of the most marginal farmers in agricultural research, to support their capacity to benefit from research results and to enhance locally adapted practices (De Schutter 2009; Skinner 2011). Research shows that PPB can have positive effects on the empowerment of farmers by, for example, enhancing their access to information, seed, and decision-making opportunities (Galiè 2013a). Equity concerns need to be taken into account when selecting the target farmers to ensure that the less vocal and more marginalized farmers are not excluded from opportunities for empowerment provided by the participation in PPB. In the case of Syria, for example, women's involvement in the participatory barley breeding program was discouraged at village level because barley was considered a male crop. Yet, when a gender-balanced participation of farmers was actively supported in the program, the sale of good PPB seed became an important income-generating activity for some women (as for the men) who had fewer opportunities than men to engage in nonagricultural paid work and mostly worked as on-farm unpaid labor (Galiè 2013b).

In the case of self-pollinated crops and when the breeding method is the pedigree method, the selection in farmers fields can start with the segregating populations (for example, F_2-derived F_3 families) after their number is reduced by selection (including MAS) on-station for disease resistance, for traits with high heritability (for example phenology), or for quality traits such as malting or culinary qualities. Distributing different segregating populations to different locations according to farmer preferences is an additional strategy to further reduce the amount of breeding material in any one farmer's field. When the breeding program uses the bulk-pedigree method, it is possible to start the field testing as early as the F_3 bulks. In both cases, the yield testing should continue for at least four consecutive cropping seasons to generate sufficient information on the stability and performance of the breeding material for farmers to make a decision about adoption and for the variety release process.

In the case of population improvement of cross-pollinated crops, the recombination phase corresponds with the creation of genetic variability, which can be done on-station while the selection and testing can be done in farmers' fields. In the case of hybrid development, the creation and enrichment of breeding populations can be done—and in fact is being done, for example in China—in farmers' fields (Song et al. 2006). The production of uniform inbred lines to use as parents of hybrid cultivars can equally well be done on-station or in farmers' fields. In the latter case, because of the lower yield of inbred lines, a farmer compensation scheme should be

envisaged. The advantage of developing inbreds in farmers' fields is that selection during the inbreeding process is done in the real production environment, making sure that field heterogeneity does not bias the selection. Similarly, in the case of test crosses, they can be more efficiently evaluated in farmers' fields. While the actual production of the hybrid seed can be done both on-station and in farmers' fields, the former has the advantage of not using farmers' land and farmers' labor. The field testing of the experimental hybrids has to be done for at least four cropping seasons, for the reasons given earlier. As in the case of self-pollinated crops, targeting germplasm to farmer preferences is an additional strategy to reduce the amount of breeding material under selection and testing at any one site.

In the case of vegetatively propagated crops after the initial crosses, all the subsequent generations are suitable for testing and selection in farmers' fields. As in the case of the pedigree method for self-pollinated crops, the number of clones can be reduced on-station by selecting for traits such as disease and or pest resistance, for traits with high heritability, and quality traits.

Other important features of the general model are summarized below.

- From Stage 1 to Stage 4 there is a progressive decrease in the amount of breeding material (entries) and an increase in the amount of seed available for each entry. This, as we will see later, affects the choice of the experimental design and the number of locations where the entries are tested.
- The decision on what to promote from one stage to the next is taken by the farmers in ad hoc meetings held between harvesting and planting, and is based on both farmers' visual selection during the cropping season and on the data collected by the researchers or by the farmers, or by both, after proper statistical analysis—as described later.
- In general, researchers have the primary responsibility for designing, planting and harvesting the trials, data collection, and data analysis. Farmers are responsible for everything else and make all the agronomic management decisions. However, as the program evolves, farmers can become responsible for planting, harvesting, and data collection.
- In terms of the farmer's time, the cost of participation ranges from 2 days to 2 weeks annually, depending on the level of participation.
- A back-up set of all the materials tested in Stages 1 to 4 is also planted at the research station to purify the bulks if pure lines are required in the case of self-pollinated crops, but, more importantly, to produce the seed needed for the trials and to insure against the risk of losing the trials to drought or other climatic events.
- In some countries, the farmers who are hosting trials are compensated (in kind) for the area used for the trials with an amount of seed equivalent to the production expected in an average year.

Seed cleaning machinery is supplied to some villages to assist in the multiplication and dissemination of selected varieties following the fourth year of farmer selection.

Screening for diseases and insect pests is carried out on-station before the first stage of yield testing on farmers' fields to avoid the spreading of new diseases or pests, as PPB has been criticized (for example, in Syria) for the danger of spreading new diseases, yet interestingly in Syria, most of the wheat and barley varieties released through CPB are disease susceptible.

The approach is flexible enough to accommodate biotechnological techniques, specifically MAS, after the first year of farmer selection (PPB should be able to provide reliable information on desirable traits that could later be evaluated via MAS should this be available and deemed desirable by farmers).

One of the consequences of a PPB program is that the number of varieties it generates and the turnover of varieties are both higher than with CPB, thus increasing both spatial and temporal agrobiodiversity.

Also, it is not unusual that more varieties are adopted and cultivated within a region at any given time. While this is of course highly positive in terms of both agricultural biodiversity conservation and enhancement, and of protection against pests and diseases, it poses a number of challenges to seed production and for studies on the impact of PPB programs.

8.13 Farmers' Selection, Selection Criteria, and Data Collection

At the time of selection, farmers are provided with field books to register both qualitative and quantitative observations. Farmers' preferences are usually recorded from 0 (discarded) to 4 (most preferred plots) by between 10 and 30 farmers including (in some countries) women, occasionally assisted by scientists (or literate farmers) to record their scores. Breeders collect quantitative data on a number of traits indicated by farmers as important selection criteria (such as growth vigor, plant height, spike length, grain size, tillering, grain yield, biomass yield, harvest index, resistance to lodging and to diseases and pests, cold damage, etc.), as usually done in the MET in a CPB. If the testing environment has been properly chosen, these data will provide information on differences in adaptation to abiotic stresses together with farmers' preferences.

It is at this stage that a PPB program can accommodate the collection of data on those traits that could be associated with adaptation to climate changes and discussed earlier in this chapter.

The data are processed (see under Sect. 8.14) and the final decision of which breeding lines to retain for the following season is made jointly by breeders and farmers in a special meeting and is based on both quantitative data and visual scores.

The process is repeated at each stage and in each cycle of selection, and this continuous association with the breeding material has both an enormous empowering effect and is what it is driving adoption.

8.14 Experimental Designs and Statistical Analysis

One widespread criticism towards PPB is that it is not science-based, and in fact several PPB programs do suffer from lack of suitable experimental designs and of statistical analysis.

Two experimental designs which are suitable in the first stage, where there is only one host farmer in each location, are (a) the unreplicated design with systematic checks every ten or every five entries arranged in rows and columns or (b) the partially replicated design in which about 20–25 % of the entries are replicated twice and all the others are present once. In Stage 1, when the total number of new entries tested vary (in our projects) between as few as 50 to as many as 160 at each location, a compromise must be sought between the plot size and the number of locations. This compromise is reached by sacrificing replications in favor of locations, as done in most CPB in the initial stages (Portmann and Ketata 1997) in recognition that in this stage of the breeding program ranking of genotypes is more important than predicting their yields (Kempton and Gleeson 1997) and the G×E variance is larger than the experimental error variance.

In the second, third, and fourth stages of the PPB program, as in CPB, the number of lines progressively decreases because of the selection, while the amount of seed available for each entry increases. Another characteristic of the second, third, and fourth stage of trials is that they usually contain different entries in each of the different locations in which the PPB program is conducted and in which the Stage 1 trials were planted. This is a consequence of the selection being conducted independently at each location, which usually results in different Stage 1 entries being selected in different locations. Another difference is that, while there is usually only one Stage 1 trial in each location, it is advisable to have at least three Stage 2, 3, and 4 trials at each location. This allows capturing differences within each location between agronomic practices, soil physical characteristics, uses of the crops, farmers' preferences, etc., and allows genotype × farmer interaction analysis. In stages 2, 3, and 4 seed is usually nonlimiting and therefore it is possible to use progressively larger plot size; this has the additional advantage of providing a large seed supply of the lines that will eventually be adopted at the end of the cycle.

The data are subjected to different types of analysis such as the spatial analysis of unreplicated or replicated trials (Singh et al. 2003). The environmentally standardized Best Lineal Unbiased Predictors (BLUPs) obtained from the analysis are then used to analyze Genotype × Environment Interactions (GE) using the GGEbiplot software (Yan et al. 2000).

Therefore, the PPB trials generate the same quantity and quality of data as that generated by the MET in a CPB with the additional information on farmers' preferences usually not available in the conventional MET. As a consequence, varieties produced by PPB are eligible for submission to the official variety release process that in several countries, including many in the developing world, is the legal prerequisite for commercial seed production.

8.15 Time to Variety Release

In a typical breeding program of a self-pollinated crop and following a classical pedigree method, it takes normally about 15 years to release a variety. With the method described in the previous section the time is reduced by half and this advantage is particularly important in the case of climate changes, because it assures a dynamic turnover of progressively better adapted varieties. However, the comparison is biased because of the difference in the genetic structure of the material being generated, i.e., pure lines in one case and populations in the second.

If populations are not acceptable by the variety release authorities, and the model includes pure line selection within the superior bulks, it can be shown that the time to variety release in the PPB program is still 3–4 years shorter than the CPB based on the pedigree method, and again the comparison is biased because the CPB does not generate the information on farmers' preferences which is one of the main characteristics of a PPB program.

This characteristic of a PPB program is important to cope with climate changes because it ensures a rapid and continuous turnover of varieties.

The method is therefore very flexible because it can generate populations, pure lines, and eventually mixtures of pure lines. Similarly, when applied to cross-pollinated crops, PPB can be used to produce hybrids, populations, and synthetics.

8.16 Effect on Biodiversity

One of the main benefits expected from PPB, of particular importance to maintain and improve adaptation to climate changes, is an increase in crop biodiversity because of the joint effect of decentralized selection and of the farmers' participation. The effect on biodiversity is illustrated using the data of the 2001–2004 breeding cycle in Syria (Table 8.3). As indicated earlier, in each village the starting point of the breeding cycle in farmers' fields are the initial yield trials with 165 genetically different entries: the number of entries tested in the subsequent trials decreases to about 17 in Stage 2, to 7 in Stage 3, and to 3 in Stage 4. The number of trials per village varies from 1 in the case of Stage 1, to about 3 in the case of the other trials. The number of lines selected by between 8 to 10 farmers per village was on average 17, 8, 3.5, and between 1 and 2.

Because different germplasm is tested in different villages, the total number of genetically different entries tested in the various trials was 412 in Stage 1, 238 in Stage 2, 51 in Stage 3, and 19 in Stage 4. In the case of Syria, the number of different entries at the end of a breeding cycle in farmers fields is higher than the number of lines the Syrian National Program tests at the beginning of its on-farm testing which usually ends with one or two recommended varieties across the country (Ceccarelli et al. 2013).

Table 8.3 Flow of germplasm, selection pressure, number of farmers participating in the selection, and number of lines in initial adoption in one cycle of participatory plant breeding on barley in Syria

	Stage 1	Stage 2	Stage 3	Stage 4
Entries tested per village	165	17.3	7	3
Trials per village	1	3.2	3.4	2.8
Entries selected per village	17	8	3.5	1–2
Farmers selecting	9–10	8–9	8–9	8–9
No. of different entries per village	412	238	51	19

8.17 Variety Release and Seed Production

The potential advantages of PPB, such as the speed with which new varieties reach the farmers helping them face the challenges of climate changes, the ability to address gender-based crop and variety preferences vis-à-vis climate change and the increasing feminization of agricultural labor, the increased adoption rate and the increased biodiversity within the crop due to the selection of different varieties in different areas, will not be achieved if the seed of the new varieties does not become available in sufficient amounts to the entire farmer community. In many countries this is associated with, and depends on, the official recognition of the new varieties. This process, called variety release, is usually the responsibility of a committee (the variety release committee) nominated by the Minister of Agriculture, which decides whether to release varieties based on a scientific report on the performance, agronomic characteristics, resistance to pests and diseases, and quality characteristics of the new variety. The process suffers from several drawbacks: (1) it takes a long time, (2) testing sites are poorly chosen, (3) the trial management is often not representative, (4) the trial analysis is biased against poor environments, (5) traits important to the farmers are not included, (6) farmers' opinion is not considered, (7) there is often lack of transparency in sharing the information, and (8) the trials are often conducted using obsolete experimental design and statistical analysis.

As a consequence there are several cases of varieties released which have never been grown by any farmer and also of varieties grown by farmers without being released. In these cases, the considerable investment made in developing the new variety and in producing its seed has no benefits.

One of the most important advantages of PPB is associated with reversing the delivery phase of a plant breeding program (Fig. 8.9). In a CBP, the most promising lines are released as varieties, their seed is produced under controlled conditions (certified seed) and only then do farmers decide whether to adopt them or not; therefore the entire process is supply-driven. In many developing countries the fact that very few of the varieties released by conventional breeding are actually adopted by farmers is explained with the reluctance of farmers to change. As breeders are rewarded based on the number of varieties released, they have no reason to test the hypothesis that lack of adoption may have different reasons. With

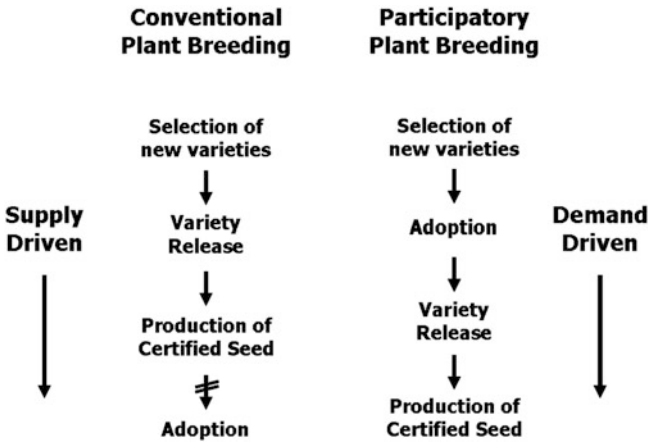

Fig. 8.9 In conventional plant breeding new varieties are released before knowing whether the farmers like them or not and the process is typically supply-driven. In participatory plant breeding the delivery phase is turned upside down because the process is driven by the initial adoption by farmers at the end of a full cycle of selection and is therefore demand-driven

PPB, it is the initial farmers' adoption which drives the decision of which variety to release, and therefore the process is demand-driven. Adoption rates are higher (showing that farmers are not reluctant to changes), and risks are minimized, as an intimate knowledge of varietal performance is gained by farmers as part of the selection process. Last but not least, the institutional investment in seed production is nearly always paid off by farmers' adoption.

The implementation of a PPB program implies not only a change in the process of variety release but also assumes changes in the seed sector. CPB and the formal seed sector have been successful in providing seeds of improved varieties of some important staple or cash crops to farmers in favorable areas of developing countries. However, the policy, regulatory, technical, and institutional environment under which these institutions operate limits their ability to serve the diverse needs of the small-scale farmers in marginal environments and remote regions. In other words, to capture the potential benefits of PPB in relation to climate changes the seed legislation needs to be changed also because it does not have any biological justification.

Gender concerns also need to be included in systems for seed delivery that take into account gender-based restrictions on seed access. For example, informal seed distribution channels might be supported to provide seed to women farmers—given that the presence of women in public agricultural spaces (e.g., agricultural retailers, or extensions) is often discouraged—and to farmers who are located in areas not reached by the formal system (Galiè 2013b).

The full advantages of PPB could be captured and scaled up with the inclusion of small seed companies as participants in the process. Small seed companies can cover a limited area and within that area they could be instrumental in spreading the

continuous flow of PPB varieties of various crops to a wider community of farmers sharing the benefits with those farmers participating in the selection process.

In those countries where most of the seed used is produced by the informal seed system, the model can provide the informal system with quality seed of improved varieties.

8.18 An International Decentralized Participatory Plant Breeding

International plant breeding programs such as those of the CGIAR aim to assist national programs to increase agricultural production by developing superior cultivars. This is traditionally done through very large breeding programs, which develop fixed or semifixed lines with an average good performance across many environments (often high input research stations).

This type of interaction between international and national plant breeding programs has been largely a one way, "top-down" process (Simmonds and Talbot 1992) where international programs develop germplasm, distribute it as "international nurseries," and national programs test it, and eventually release selections as cultivars. This "top-down" approach has often excluded the use of locally adapted germplasm, which is specifically adapted to particular conditions and often performs poorly in the favorable conditions of research stations, and has, in fact encouraged its displacement.

The distribution of germplasm from CGIAR centers to national breeding programs has indeed historically also included segregating populations. However, such segregating populations, obtained from crosses designed by the international breeders, are the same for all the countries, and they are not usually targeted to a specific environment.

To exploit specific adaptation fully and make positive use of GE interactions, international breeding programs can decentralize most of the selection work to national programs by gradually replacing the traditional international nurseries with targeted segregating populations with the possible addition of specific genetic stocks. The distribution of segregating populations reduces the danger of useful lines being discarded because of their relatively poor performance at some selection sites (Ceccarelli et al. 1994). It also a way to capitalize from the extensive training programs on plant breeding conducted by CGIAR.

An example of what CGIAR centers could do to contribute to biodiversity is the decentralization of the ICARDA's barley breeding program, which started in 1991 with the distribution of targeted segregating populations first to Morocco, Algeria, Tunisia, and Libya (Ceccarelli et al. 1994), and later to Iraq in 1992, to Egypt in 1995, and gradually to other countries.

The term decentralization is used here to mean decentralized selection, i.e., selection in the target environment(s).

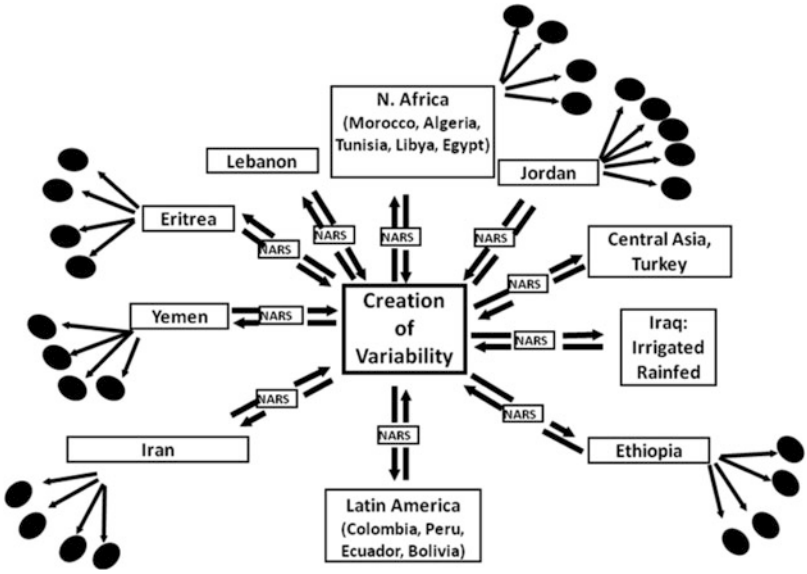

Fig. 8.10 An example of an international decentralized participatory breeding program: an international breeding program such as those of the CGIAR Center creates genetic variability in the form of targeted segregating populations which are distributed to specific National Agricultural Research Systems (NARS). NARS multiply the material to have sufficient seed to test the material in farmers' (*filled ovals*) fields with farmers' participation. The model is based on an efficient feedback process (*bidirectional arrow*) between farmers and NARS and NARS and CG centers

While the national programs accepted decentralization very positively, we started recognizing that decentralization per se will not necessarily respond to the needs of resource-poor farmers in less favored areas, if it is only a decentralization from the research station(s) of a CG center to the research stations of a national program, and if the research stations of the national programs do not represent, as is often the case, the environments where the crop is predominantly grown. To exploit the potential gains from specific adaptation to low input conditions, breeding must be decentralized from research stations to farmers' fields (Fig. 8.10) following the methodology described earlier. An essential component of the system is the continuous feedback that could allow breeders in the CGIAR centers to continuously improve the targeting of the germplasm.

Although decentralization and farmer participation are unrelated concepts, decentralization to farmers' fields almost inevitably (except in Australia, where decentralization has not been followed up by participation) leads to the participation of farmers in the selection process.

A scheme such as the one shown in Fig. 8.10, integrated with the development of evolutionary populations, if applied by all CGIAR centers to the main food crops and in the poorest countries, could provide a major contribution to the enhancement of biodiversity and therefore to adaptation to climate changes and to food security.

Table 8.4 Number of varieties selected and adopted by farmers in the PPB programs in five countries

Country	Crop(s)	Varieties
Syria	Barley	93
Jordan	Barley	1 (submitted)
Egypt	Barley	5
Eritrea	Barley	3
Yemen	Barley	2
	Lentil	2
Ethiopia	Barley	3

8.19 Impact of Participatory Plant Breeding

By 2011 the model shown in Fig. 8.8a, b was fully implemented in Syria, Jordan, Algeria, Egypt, Ethiopia, Eritrea, and in Iran. PPB programs based on the methodology described above have also been implemented in Tunisia and Morocco (Ceccarelli et al. 2001), and Yemen. These PPB projects had four main types of impact

Variety development: a number of varieties have already been adopted by farmers even though the program is relatively young in breeding terms (Table 8.4). In Syria adoption is taking place for the first time in low rainfall areas (<250 mm annual rainfall) (Table 8.5).

Institutional: in several countries, the interest of policy-makers and scientists in PPB as an approach, which is expected to generate quicker and more relevant results, has considerably increased.

Farmers' skills and empowerment: the cyclic nature of the PPB programs has considerably enriched farmers' knowledge, improved their negotiation capability, and enhanced their dignity (Soleri et al. 2002); PPB affected positively the recognition of women as farmers; their access to and control of relevant seed; and their decision-making about variety development (Galiè 2013a).

Enhancement of biodiversity: different varieties have been selected in different areas within the same country, in response to different environmental constraints and users' needs. In Syria, where this type of impact has been measured more carefully, the number of varieties selected after three cycles of selection is 4–5 times higher than the number of varieties entering the on-farm trials in the CBP.

An economic analysis of the PPB barley breeding program in Syria shows that PPB increases the benefits to resource-poor farmers. The total estimated discounted research induced benefits to Syrian agriculture were estimated at US$21.9 million for conventional breeding and US$42.7 million to US$113.9 million for three different PPB approaches (Lilja and Aw-Hassan 2003).

Using case studies on different crops, Ashby and Lilja (2004) have shown that:

- The use of participatory approaches improves the acceptability of varieties to disadvantaged farmers by including their preferences as criteria for developing, testing, and releasing new varieties. A survey conducted on over 150 PPB

Table 8.5 Varieties adopted from the PPB program by farmers in Syria in various rainfall zones

Pedigree	Name	Location	Rainfall[a]
H.spont.41-1/Tadmor	Raqqa-1	Bylounan	212.4
Arta//H.spont.41-5/Tadmor	Raqqa-2	Bylounan	"
Zanbaka/JLB37-064	Karim	Bylounan	"
Tadmor/3/Moroc9-75/ArabiAswad//H.spont.41-4	Akram	Bylounan	"
Mo.B1337/WI2291//Moroc9-75/3/SLB31-24	Suran-1	Suran	383.7
ChiCm/An57//Albert/3/Alger/Ceres.362-1-1/4/Arta	Suran-2	Suran	383.7
ER/Apm//Lignee131/3/Lignee131/ArabiAbiad/4/Arta	Suran-3	Suran	383.7
Hml-02/5/..Alger/Ceres362-1-1/4/Hml	Nawair-1	Suran	"
Hml-02/5/..Giza 134-2L/6/Tadmor	Nawair-2	Suran	"
SLB03-10/Zanbaka	Yazem	J. Aswad	226.4
Tadmor//Roho/Mazurka/3/Tadmor	Salam	J. Aswad	"
ArabiAswad/WI2269/3/ArabiAbiad/WI2291//Tadmor/4/Akrash//WI2291/WI2269	Ethiad	J. Aswad	"

[a]Annual rainfall in mm in the period 2000–2005

projects showed that (a) PPB improved program's effectiveness in targeting the poor, (b) by consulting women and involving them in varietal evaluation, there was a better acceptability and faster adoption of the varieties, and (c) involvement of women farmers in the development of maize seed systems in China resulted in a broadened national maize genetic base, improved maize yield, and strengthened women's organizations.

- PPB improves research efficiency. A case study conducted using the PPB program in Syria (Ceccarelli et al. 2000, 2003) found that farmers' selections are as high yielding as breeders' selections. Another study found that by introducing farmer participation at the design stage, a 3-year reduction was achieved in the time taken from initial crosses to release. In another example, breeders concluded that it was faster, less expensive, and more reliable to involve farmers directly in the identification of promising accessions for use in the breeding program. Efficiency gains depend also on the extent to which farmer involvement enables the breeding program to minimize its investment in the development of varieties, which, after release, turn out to be of little if any interest to farmers.
- PPB accelerates adoption. The incorporation of participatory approaches consistently enables breeding programs to "break through" adoption bottlenecks caused by low levels of acceptability of new varieties by poor farmers. In addition to the examples given in Table 8.4, other examples are Ethiopia, where out of over 122 varieties of cereals, legumes, and vegetables which had been released, only 12 were adopted by farmers, Brazil, where after years of nonadoption, the implementation of PPB led to the adoption of several clones of cassava which were both resistant to root rot and highly acceptable to farmers,

and Ghana, where maize breeders had released several modern varieties (MVs) which had poor acceptability and poor adoption, while with farmers' participation the overall adoption of MVs increased to over two-thirds.

- Finally, there is increasing evidence that one of the most widespread impacts of participatory plant breeding, and possibly of participatory research in general, is of a psychological and ethical nature: when farmers are asked which benefits they believe they receive from PPB, they state that their quality of life has improved, that they feel happier as a consequence of changing their role from passive receivers to active protagonists, that their opinion is valued, and that, as an Eritrean farmer said, they have taken science back into their own hands.

8.20 Conclusions

The results presented in this chapter indicate that it is possible to organize a plant breeding program in a way that addresses not only those plant characteristics that maximize yield and stability over time in a given physical environment, but also the preferences of the users, by developing varieties, which are specifically adapted to different physical and socioeconomic environments and gender needs. Such an objective can be achieved by using a decentralized participatory approach, which needs to be extended also to seed production aspects. A breeding program organized according to these principles will have the advantages of producing environmentally friendly varieties and of maintaining or even enhancing biodiversity.

The main objections to participatory plant breeding are usually that (1) plant breeding is "plant breeders' business," and if plant breeders do their job properly there should not be the need for participatory plant breeding, (2) it is not possible for seed companies to cope with the multitude of varieties generated by participatory plant breeding, and (3) varieties bred through participatory plant breeding do not meet the requirements for official variety release (Ceccarelli and Grando 2007).

With regard to the first objection, circumstantial evidence suggests that while plant breeding has been a success story in climatically, agronomically and economically favorable areas, and in areas where the agronomic environment could be modified to create near-optimum growing conditions, it has been much less successful in less-favorable areas. In those areas where it has been successful, plant breeding has raised both environmental concerns due to high levels of chemical inputs required by modern varieties, and biodiversity concerns because of the narrowing of the genetic basis of agricultural crops. More recently, there is widespread concern about the use of the improperly called genetically modified organisms (GMOs) which, regardless of other considerations, represent yet another type of top-down technology. For these reasons, it may be useful to explore alternative avenues of plant breeding where the same science can be used in a different way.

The second objection assumes implicitly the need to breed taking into account the requirements of the seed companies rather than the interest of the farmers, the consumers, and society at large. It also ignores the fact that in the case of the major food crops and in developing countries, farmers and not seed companies are the main suppliers of seed with over 90 % of the seed, which is currently planted: participatory plant breeding can introduce new varieties directly into the most efficient seed system currently operating.

Against the third objection, the chapter has shown that it is possible to organize a participatory breeding program in such a way that it generates the same quantity of information of the same (or even better) quality than a conventional breeding program. In addition to the usual data set on agronomic characteristics, a participatory breeding program also generates information on farmers preferences (which is missing in the data set generated in a conventional breeding program), and therefore it makes the process of variety release more efficient and effective.

The third objection usually addresses also the genetic structure of the varieties produced by PPB. It assumes that varieties produced by PPB are inevitably genetically heterogeneous, unstable and not distinct and therefore not suited for release. On this issue there are three points to make. Firstly, the majority of cultivars still grown in marginal environments are genetically heterogeneous, and in several cases their seed is multiplied officially by the same authorities which deny the right of populations to be released; secondly, it is disputable how wise it is to replace them with genetically uniform material and it has been recently shown (Di Falco and Chavas 2006) that crop genetic diversity can increase farm productivity and can reduce the risk of crop failure; thirdly, we have shown that PPB, like conventional plant breeding, is flexible and can be used to produce varieties with different genetic structure including pure lines and hybrids.

Therefore, the most frequent objections to PPB are unfounded; they ignore the fact that farmers have domesticated the crops that feed the world, and that they have continued to modify these crops for millennia. In this process they have planted, harvested, exchanged seed, introduced new crops and new varieties, fed themselves and others and in so doing they have accumulated a wealth of knowledge that modern science tends to ignore. Participatory plant breeding is one way of recognizing farmers' knowledge and to merge it with modern science.

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Chapter 9
Evolutionary Breeding and Climate Change

Kevin M. Murphy, Arron H. Carter, and Stephen S. Jones

Abstract The genetic uniformity within, and typified by, most monocultural cereal-based systems has been shown to limit the crops' capacity to evolve in response to adverse environmental conditions, thereby leading to a possible decrease in the yield stability of the cropping system. Deployment of significantly increased crop diversity across the global landscape has the potential to reduce the progress of crop epidemics, optimize yield stability, and positively enhance crop resilience in the ever-changing visage of climate-induced stress. One method of increasing genetic diversity within cereal crop populations is through evolutionary breeding (EB). In EB populations of self-pollinating cereals, natural selection acts upon the heterogeneous mixture of genotypes over generations and across environments and traits positively correlated to reproductive capacity increase over time. Crop populations with enhanced genetic diversity mimic natural ecological communities, which are better equipped to adapt to future unpredictable temporal climate shifts than are monocultures. Evolutionary participatory breeding merges the EB method with farmer selection to develop high-yielding, disease-resistant cultivars while maintaining a high degree of genetic variation to allow for adaptability to fluctuations in environmental conditions. The EB method can contribute to the development of cropping systems with greater resilience and yield stability in the climate change era.

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9.1 Introduction

9.1.1 Climate Change and Crop Resilience

There is much reported unpredictability in future global agriculture due to the considerable variability within and between countries, with some areas potentially benefiting, and others suffering from, climate change (Jones and Thornton 2003; Gregory et al. 2009). Crops that are grown in regions that reach their maximum temperature tolerance, particularly in low-latitude dryland farming systems, are expected to experience yield decreases with even minimal changes in climate (Parry et al. 2005). The distribution of the effects of climate change will almost certainly be uneven, with the livelihoods of subsistence farmers likely being the most negatively affected and the risk of hunger increasing predominantly in the most marginalized economies (Rosenzweig and Parry 1994; Parry et al. 2005). In China, it is clear that the climate has warmed significantly since 1960, with increased frequency of heat waves. However, both geographic and annual variability in water resources prevents an accurate understanding of the impact of climate on China's agriculture (Piao et al. 2010). Regional (within countries rather than across countries) climate simulations and management strategies should be developed to better understand potential crop response to climate change (Piao et al. 2010).

Many complex crop × climate interactions are influenced by crop pathogens and insect pests. Each year, plant diseases account for global harvest losses of approximately 10–16 % (Oerke 2006), and disease resistance in cultivars of wheat, oats, rice, tobacco, and sunflower have been shown to be differentially affected by temperature (Gregory et al. 2009). For example, differential resistance expression has been shown at 10 °C and 25 °C against isolates of *Puccinia recondita* among cultivars of wheat, and similar temperature sensitivities have also been reported to *P. striiformis* in wheat, *P. tritici* in oats, and to *Xanthomonas oryzae* pv. *oryzae* in rice (Martens et al. 1967; Dyck and Johnson 1983; Gerecheter-Amitai et al. 1984; Jones 2003; Garrett et al. 2006). The effectiveness of currently deployed resistance genes has been shown to be compromised, over- or underexpressed when faced with more extreme and variable climatic conditions (Gregory et al. 2009). Resistance genes in barley have been shown to lose gene expression due to drought stress and salt stress as well as to drought stress relief and cold stress relief (Newton and Young 1996; Barker 1998; Stewart 2002; Goodman and Newton 2005). Aphids, one of the most important pests in agriculture throughout the globe, may be able to exploit the changing conditions particularly well due to their short generation time, low developmental threshold temperature, and significant dispersal abilities (Sutherst et al. 2007).

It is impossible to predict annual fluctuations in rainfall and temperature in any given location, much less across locations, thereby making proper varietal selection somewhat of a guessing game. This could become progressively more problematic in the face of increasingly unpredictable environmental fluctuations of a potentially

larger magnitude due to climate change. One way to combat this issue is to deploy inter- and intraspecific crop diversity across the landscape, thereby reducing the progress of crop epidemics and optimizing yield stability (Gregory et al. 2009). Increased crop diversity should positively enhance crop resilience in the ever-changing face of climate-induced stress, resulting in improved crop performance (Newton et al. 2009) and enhanced food security.

9.1.2 Intraspecific Genetic Diversity

Because of the predominance of monocultures in cereal crops throughout much of the world, crop diversity occurs at scales where individual genotype unit areas (GUAs) (Mundt and Browning 1985) are often many square kilometers (Newton et al. 2009). The genetic uniformity inherent in monocultures can restrict the crops' ability to tolerate diverse abiotic environmental stresses and pests and diseases, thereby leading to a potential decrease in the stability of the cropping system (Hooper et al. 2005; Hughes et al. 2008).

Niche segregation in the form of light gradient and exposure, soil moisture availability, and rooting depths for mineral uptake often increase complementarity while simultaneously reducing competition (Silverton 2004). Cultivar mixtures, multilines and isogenic lines have the capacity, to varying degrees, to increase the intraspecific genetic diversity in the field, and make small to potentially extreme reductions in GUAs. The use of multilines has been shown repeatedly to effectively reduce yield losses due to disease (Wolfe 1985; Garret and Mundt 1999; Zhu et al. 2000). For example, the multiarchitectural canopy types present in intentional grain mixtures have the capacity to reduce or prevent infection by splash-dispersed pathogens, including Septoria tritici blotch (*Mycosphaerella graminicola*) and glume blotch (*Phaeosphaeria nodorum*) on wheat (Jeger et al. 1981a; Cowger and Mundt 2002) and *R. secalis* infection on barley (Jeger et al. 1981b). Likewise, the severity of barley yellow dwarf virus in oats decreased in mixtures compared to pure lines (Peltonensaino and Karjalainen 1991; Karjalainen and Peltonensaino 1993). In wheat, it has also been shown that only 10–20 % of the variation in yield was attributable to disease in mixtures compared to 52–58 % in monocultures (Finckh and Mundt 1992). This complementation effect among varieties in a mixture aids in a more extensive exploitation of the major limiting resources, and decreases between-plant competition (Doring et al. 2011).

In addition to complementarity, facilitation—a positive interaction in which plants enhance the environment of their neighbors—has also been shown to be augmented through increased genetic diversity in a system (Vandermeer 1989; Long et al. 2007). Facilitation has been reported to be of greatest significance under abiotic stress (Cheng et al. 2006). However, positive effects have been found in high-input systems as well (Hauggaard-Nielsen and Jensen 2005). In barley, direct facilitation among genotypes in mixtures has been shown to impact levels of *Rhynchosporium secalis* infection through microclimatic differences

resulting from the modification of the canopy (Hoad and Wilson 2006). Indirect facilitation occurs as well; for example, mixtures of wheat were shown to significantly enhance control of *Polymyxa graminis*, the vector for wheat soil-borne mosaic virus, thus reducing the incidence of this disease (Hariri et al. 2001). Variety mixtures can also aid in disease control by increasing the physical distance between susceptible genotypes with the resistant plants acting as barriers to these foliar pathogens (Garret and Mundt 1999).

9.1.3 Towards Global Crop Resilience and Yield Stability

Yield stability across contrasting environmental conditions will be of paramount concern throughout the climate change era, particularly in the low latitude regions of the world and those areas with marginalized economies and/or dependent on rainfed cropping systems. Historical trends clearly point to evidence of seasonally and regionally altered precipitation distributions, which will have implications on changes in cropping systems and pathogen and pest incidence and severity (Barnett et al. 2006; Chakraborty and Newton 2011). Varietal mixtures typically stabilize grain yield and minimize disease-induced yield losses as well as yield losses due to abiotic stresses (Finckh et al. 2000). In comparison with monocultures, spring barley mixtures were found to be more stable for both actual yield and for yield ranking across 17 different environments in Denmark (Ostegaard et al. 2005). Using biplot analysis methods and variance models to assess yield stability, Cowger and Weisz (2008) found that mixtures of winter wheat were more stable than their individual components. Smithson and Lenne (1996) showed that yield stability of mixtures increased with the number of genotypes. Yield stability in mixtures is often due to the compensatory effect found when the loss of performance of one or more genotype in a mixture is compensated by the improvement in performance of other genotypes (Yachi and Loreau 1999; Swanston and Newton 2004; Newton et al. 2009; Doring et al. 2011).

Chakraborty and Newton (2011) suggest that strategies for establishing greater resilience and yield stability in crops should focus on the introduction of increased genetic variability, both within and between cultivars, into agricultural systems. Crop populations with this added genetic diversity can then mimic natural ecological communities, which are better equipped to adapt to future unpredictable temporal climate shifts than are monocultures. Genetically uniform cultivars have been shown to lack the ability to adjust and adapt to highly unpredictable, in direction and range, environmental fluctuations and novel stress factors (Verboom et al. 2010). As compared to monocultures, in which the use of major genes for resistance to pathogens often leads to strong selection on pathogen populations to overcome these genes (Pangga et al. 2011), genetically diverse, heterogeneous cropping systems can potentially adapt to evolving pathogen threats resulting in durable disease resistance and stable, resilient farming systems (Huang et al. 1994; Allard 1999; Chakraborty and Newton 2011).

9.2 Evolutionary Breeding

Many decades before warnings of climate change emerged in the scientific literature, Harry Harlan, a plant explorer and cereal geneticist of the United States Department of Agriculture (USDA) constructed his first barley composite cross populations (Harlan et al. 1940). For example, Barley Composite Cross II (CC II), one of the most widely studied of Harlan's composite cross populations, was created in 1929 through the hybridization of 30 diverse barley cultivars from around the world in all possible cross combinations (Harlan and Martini 1929). CC II and other composite cross populations were grown annually, first at the University of California at Davis and later in other environments, under the typical agronomic conditions of the time period, and harvested at maturity without any targeted selection by the researchers (Suneson 1956; Ramage 1987; Murphy et al. 2005). These populations were subjected to natural selection through temporal and spatial fluctuations in rainfall and temperature, similar to, though perhaps of less magnitude than, the climatic fluctuations of the present day.

9.2.1 Natural Selection and Fitness in Heterogeneous Populations

Natural selection acts upon a heterogeneous mixture of competing genotypes that undergo continual recombination and subsequent segregation and selection over many generations. Jain (1961) describes three quantifiable components of fitness in self-pollinating, annual cereals (1) the ability of a plant to germinate and emerge, (2) the survival of the plant to the reproductive growth stage, and (3) the number of seeds produced per plant. Natural selection in the barley composite cross population was shown to favor high-yielding genotypes as a result of the relationship between the yield capacity of an individual plant and its fitness components (Allard 1999). Traits positively correlated to the reproductive capacity of a self-pollinating cereal, including spike weight, number of seeds per plant and seed yield, have all been shown to increase over time through the effects of natural selection found in evolutionary breeding of barley populations (Allard 1990). For example, reproductive capacity over 50 generations of evolutionary breeding of CC II consistently kept yield performance within 95 % of the most current highest yielding varieties (Allard 1990).

Coefficients of variability are an indicator of yield stability across years and locations. In comparison with the high-yielding cultivar Atlas, Suneson (1956) reported a coefficient of variability across environments almost twice as low in CCII over an 18-year time period (1937–1955). CCII progressed from F_{11} to F_{29} during this time period. Newer composite crosses in the same study, including CCV (F_{15}), CCXII (F_{14}), and CCXIV (F_{12}) had yields similar to Atlas in 1955. Similarly, in a study comparing seed yields of lima bean composite cross populations, pure

lines and seed mixture over 4 years in California, the CC populations outproduced both the mixtures and the pure lines (Allard 1961). To minimize the effects of genotypic variation, both the mixtures and populations were developed using the pure lima bean lines in this study as parents. Suneson (1956) estimated that 15 generations of natural selection in barley was sufficient time for the composite crosses to have improved fitness traits compared to the parent genotypes.

Natural selection must favor genotypes with superior agronomic performance, otherwise the composite populations will not reach optimal fitness levels (Phillips and Wolfe 2005). Jain and Qualset (1975) suggested that stabilizing natural selection was the driving force for many traits, including seed size, spike length, days to heading and spike density, whereas directional selection was the primary selective force influencing seed number per plant. Evolutionary breeding is clearly most effective in increasing grain yield when selection pressures are constant and directional (Degago and Caviness 1987), although disruptional selection can also be effective in increasing yield. For example, even under conditions that fluctuated significantly in rainfall, temperature and day length, segregating bean populations showed a mean yield gain of 2.5 % over a 17-year time period when compared to the mean of the parents (Corte et al. 2002). In fact, the utilization of different sites with contrasting, disruptional selection pressures has been recommended as an effective method to maintain genetic diversity of disease-resistant genes within a population, thereby increasing the overall fitness of the population (Paillard et al. 2000; Phillips and Wolfe 2005).

9.2.2 Yield of Composite Cross Populations in Marginal Environments

Reduced temporal predictability of biotic and abiotic stresses in marginal environments coupled with low-input systems can make it difficult to accurately select pure line cultivars that will perform best from year to year in these challenged agronomic systems (Phillips and Wolfe 2005). These marginal environments are perhaps where the deployment of CC populations would be most effective. Many marginal environments lack a buffering or adaptive capacity, and it is the countries, regions, and societal groups with limited adaptive capacity that face the most significant threat to food security (von Braun 2007).

CCII (F_{15} to F_{37}) was shown to be equal to the yield of the best control cultivars in marginally productive environments in Montana, USA, but when grown in highly productive environments in Montana and California, was susceptible to disease and lodging (Hockett et al. 1983). Additionally, bulk F_2 populations of wheat were found to yield more than the parent cultivars by up to 26 %, but only when grown in marginal conditions (Qualset 1968). Several generations of CCV in barley were shown to have superior yields during periods of drought when compared to commercial cultivars when grown over an 18-year period in Cambridge,

UK. However, the reverse was true under the favorable agronomic conditions, which predominated during that time span (Danquah and Barret 2002). Soliman and Allard (1991) found that barley composite crosses showed significantly more yield stability across a range of marginal environments when compared to commercial cultivars. When grown in favorable agronomic environments, however, these same commercial cultivars yielded significantly higher than the composite crosses.

Because of the various resources that trigger plant-to-plant competition in cereals, including light, moisture, and nutrients, Frey and Maldonado (1967) suggested that the benefits of heterogeneous cereal populations are more clearly seen in resource-limiting environments. Likewise, Danquah and Barret (2002) established that advanced generations of barley composite cross populations were responsive and well suited to marginal, fluctuating, and stressed environments. Yield increases of 57 % over 6 years, or an average of 9.5 % per year, were found in a barley population derived from the mixing of 6,000 entries from the world barley collection and grown under late planting induced stress (Rasmusson et al. 1967).

9.3 Evolutionary Participatory Breeding

On the basis of the limitations inherent in reliance solely upon natural selection within heterogeneous populations, researchers have suggested the utilization of artificial selection within composite cross populations to drive each population in the desired direction for nonfitness-related traits of interest (Mak and Harvey 1982; Patel et al. 1987). This artificial selection may be carried out by breeders on research stations and farmers' fields, as well as by farmers in their own fields.

In regard to the latter option, farmer participatory breeding has been shown to be effective in selecting varieties of major cereal crops, including barley, maize, wheat, and rice (Sthapit et al. 1996; Bänziger and Cooper 2001; Ceccarelli et al. 2001; Witcombe et al. 2003; Thapa et al. 2009; Bachmann 2010; Medina 2012). In fact, farmers have been shown to be as capable as plant breeders in selecting high-yielding varieties on research stations, and when selection occurred on farmers' individual fields, more proficient than plant breeders in selecting high-yielding varieties (Ceccarelli et al. 2000). Evolutionary participatory breeding (EPB) merges the evolutionary breeding method described above with farmer participatory breeding to develop high-yielding, disease-resistant cultivars of desired quality while maintaining a high degree of genetic variation to allow for adaptability to fluctuations in environmental conditions (Murphy et al. 2005). For a complete review of the EPB process, please refer to Murphy et al. (2005). Below is a case study that will illustrate EPB in wheat.

9.3.1 Case Study of EPB in Wheat

In 2002, Lexi Roach, an 8th grader at Kahlotus, WA middle school, drove two hours from her family farm in Kahlotus, WA to Pullman, WA, with her grandfather Jim Moore. Jim and his family grow winter wheat on approximately 10,000 acres of farmland in a low-rainfall, rain-fed environment (~200–250 mm precipitation per year) in South-Central Washington State. In this environment, it typically takes 2 years of moisture to raise one crop of winter wheat. The only nonirrigated rotation in the area is winter wheat in Year 1 followed by fallow (tillage or chemical) in Year 2.

Lexi and Jim were traveling to Pullman to make crosses between varieties of wheat that did well on their farm, and took the initial step in the EPB process. Working with Kerry Balow in the winter wheat program, three crosses were successfully completed. F_1 seed was obtained and advanced to the F_2 in the greenhouses at Washington State University (WSU) in Pullman. This seed was then planted on their farm using small, plot-scale breeding equipment. In the F_3 to F_8, seed from each population was planted each year in the late summer, subjected to natural selection and to farmer selection by Lexi and Jim, and then harvested in bulk, subsampled, and replanted.

The seed was typically planted 5–7″ deep in order to reach available moisture, thereby selecting for genotypes with strong emergence qualities, including longer coleoptiles and perhaps faster germination and shoot initiation. Each summer, individual plants that were susceptible to yellow rust were pulled out of the population by Lexi and Jim and the farm crew.

By 2009, one of the populations, now called Lexi II, proved to be the highest yielding and was included as "WA 8094" in the WSU Statewide Variety Testing program and grown in yield trials along with 59 of the most promising varieties from 11 regional breeding programs at over 16 locations in high, medium, and low rainfall regions across the state. In the low rainfall zone, six locations including Connell, Harrington, Horse Heaven, Lind, Ritzville, and St. Andrews, were represented. When compared to the top five varieties (by acreage) grown in Washington State in 2009–2010, WA8094 yielded significantly lower than "Xerpha," the highest yielding of the dominant varieties (Table 9.1). However, WA8094 yielded the same as the most widely grown variety statewide in dry areas, "Eltan," and yielded significantly higher than "ORCF-102," "WB-528," and "Madsen" (ranked 2, 4, and 5 respectively in Washington acreage) when averaged across the six low rainfall locations (Table 9.1). In this same year, at the St. Andrews location, WA8094 was the top yielder surpassing all of the 59 other varieties.

In 2010–2011, WA8094 yielded lower than Xerpha and ORCF-102, and was statistically equal to Eltan, WB-528, and AP 700 CL (which had replaced Madsen as the 5th most widely grown soft white winter wheat variety in Washington State, when averaged across all six locations (Table 9.2).

Although WA8094 has a very high yield potential under this selection criteria, there are other disease factors that need to be taken into account. New shifts in

Table 9.1 2010 Soft white winter wheat variety yield (bu/a) trial

Variety	WA Acreage Rank (2010)	Connell	Harrington	Horse Heaven	Lind	Ritzville	St. Andrews	Average yield
Xerpha	3	67	59	46	51	64	66	59
Eltan	1	65	50	38	45	68	57	54
WA 8094	na	59	50	38	45	55	75	54
ORCF-102	2	54	53	37	44	59	48	49
Madsen	5	47	49	34	45	63	43	47
WB-528	4	46	46	42	39	51	50	46
lsd ($P = 0.10$)		7	9	4	5	9	14	4

Table 9.2 2011 Soft white winter wheat variety yield (bu/a) trial

Variety	WA Acreage Rank (2011)	Connell	Harrington	Horse Heaven	Lind	Ritzville	St. Andrews	Average yield
Xerpha	3	56	64	69	53	88	68	66
ORCF-102	1	71	64	67	43	77	63	66
Eltan	2	56	61	62	48	77	82	64
WB-528	4	67	63	64	39	75	50	60
WA 8094	na	53	56	65	43	74	63	59
AP 700 CL	5	69	58	55	38	75	57	59
lsd ($P = 0.10$)		10	9	10	11	11	20	5

pathogen races warrant the need for selection each year. During the F_3 to F_8 stages when WA8094 was selected on farm, it was resistant to local races of yellow rust. Two years after commercial production on the farm, a new race entered the area which is virulent on WA8094. Currently, fungicide applications are needed to protect the yield potential of this line. Similarly, a long, cool growing season in 2011 caused aphid populations to increase, and resulted in a severe case of the aphid transmitted barley yellow dwarf virus (BYDV). Since this growing region seldom sees aphid problems, WA8094 was highly susceptible to BYDV, a problem attributed mainly to changing climate variables. As climate change will not only change the agronomic growing conditions but also pest populations, concurrent production and selection fields are needed to maintain identification of high-yielding adapted lines with excellent resistance to changing pest populations.

The fact that a bulk population with recurrent, farmer imposed, natural and intentional selection could rank high in an elite yield nursery demonstrates with some clarity the potential for this method. Continued annual selection under changing environmental and disease pressures will maintain populations of adapted and resistant material. As our climate becomes less predictable we will be well served to not only increase the diversity in our fields but also in the approaches that we take towards crop improvement.

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Chapter 10
Bioinformatics Tools to Assist Breeding for Climate Change

David Edwards

Abstract Major advances have been made in the various "omics" fields in recent years, driven by new genomics technology, particularly DNA sequencing and high-throughput genotyping. The ability to interrogate the genome of diverse organisms enables the characterization of allelic variants underlying important agronomic traits. Advances in bioinformatics support the analysis of the genomic data flood, and applied genome informatics has grown rapidly over recent years in parallel with the expansion of genomics. As we face the perfect storm of increasing population and increasing climatic variation, advances in genomics, phenomics and related research, supported by bioinformatics, offer the greatest potential for accelerating food production and sustainability. An increasing number of crop genomes are being sequenced, and large-scale diversity studies, performed at the whole genome level, are being undertaken for the major crops. The analysis of this data is facilitated by customized databases, statistical methods and visualization tools. Through the integration and interrogation of the various "omics" data and the application of these resources for crop improvement, we have the potential to maintain and improve food security during a period of increasing climate variability.

10.1 The Growth of Bioinformatics

Bioinformatics is a relatively new field of research, which has evolved with the rapidly increasing requirement to manage and interrogate the massive amounts of biological data being delivered by a range of technological advances. Bioinformatics means many different things to different people and in the context of this chapter

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I will refer to the broader definition of "structuring biological information to enable logical interrogation." At the same time, while acknowledging the role of bioinformatics in protein and metabolite studies, I will limit my discussion to the genetics and genomics aspects of bioinformatics that are likely to have the greatest impact on breeding for climate change.

10.2 Application to Breeding for Climate Change

Traditional breeding has made major and consistent improvements in yield and quality of many important commercial crops. However, as evidenced by increasing food prices and global food insecurity, there is a constant challenge to continue this improvement. This challenge may become overwhelming in the face of climate instability combined with peak phosphate and oil which is used for the production of nitrate fertilizer production. The fields of genomics and applied bioinformatics offer tools to help address the challenge of increasing food yield and quality through the development and application of advanced breeding techniques. Together, genomics and bioinformatics may provide breeders with the knowledge they need to make more rapid selections and apply advanced breeding strategies to produce climate-resilient crops. There remains much potential to improve the germplasm of many crops through selections and the introduction of novel alleles from wild relatives, and with the advances in our understanding of genomics, this potential is likely to be realized over the coming decades, providing secure food for future generations.

10.3 Data Explosions

The rapid growth of bioinformatics has been driven by the explosion in data production. Nowhere has this been more evident than in the DNA sequencing revolution. The introduction of next-generation DNA sequencing and the continued growth of this field have revolutionized modern biology. While this technology continues to advance, other "omics" technologies have been developed for high-throughput analysis of proteins, metabolites and importantly for crop breeding; phenotypes. As biologists and bioinformatics researchers struggle to integrate the explosion in the diversity and volume of data, the sequencing revolution continues unabated with the prospect of third-generation sequencing becoming common in the next few years.

10.4 DNA Sequencing Technology

The biggest technology driver in biology today is the ever-increasing throughput and decreasing cost of DNA sequencing technology. Traditional Sanger-based sequencing was the basis of genomics for several decades. The arrival of pyrosequencing just over a decade ago started a revolution which continues with the sequencing of single DNA molecules.

The first commercially available pyrosequencing system was commercialized by Roche (Basel, Switzerland) as the 454 GS20, capable of sequencing over 20 million base pairs in just over 4 h (Margulies et al. 2005). This was replaced in 2007 by the GS FLX model, capable of producing over 100 Mbp of sequence in a similar amount of time. The current system, the GS FLX+ can produce around 700 Mbp of data with read lengths of up to 1 kbp with multiplexing of samples (http://www.my454.com). The Roche 454 FLX system performs amplification and sequencing in a high-throughput picolitre format. Emulsion PCR enables the amplification of a DNA fragment immobilized on a bead, generating sufficient DNA for the subsequent sequencing reaction. Beads are distributed onto the plate. DNA sequencing then involves the sequential flow of both nucleotides and enzymes over the plate, which converts chemicals generated during nucleotide incorporation into a chemiluminescent signal that can be detected by a CCD camera. The light signal is quantified to determine the number of nucleotides incorporated during the extension of the DNA sequence. The output is in the form of "flow space," which is converted to the traditional ACGT nucleotide sequence format. Because of the difficulty in calling mononucleotide strings in a single flow, the main error types are additional or reduced numbers of nucleotides around these mononucleotide strings.

The SOLiD System from Life Technologies (Applied Biosystems) enables parallel sequencing of amplified DNA fragments linked to beads. The method uses sequential ligation of dye-labelled oligonucleotides, and the latest 5500xl system produces 20–30 Gbp of data per day, with read lengths of up to 75 bp (http://www.appliedbiosystems.com/). SOLiD data features a two-base encoding mechanism that interrogates each base twice providing a form of built-in error detection for single nucleotide polymorphism (SNP) discovery when comparing reads to a reference.

The Illumina sequencing platforms are currently the most popular in use globally. They use sequencing by synthesis reversible terminator chemistry to generate up to 600 Gbp of sequence data per run, the greatest volume of data from any current next-generation sequencing platform. Sequencing templates are immobilized on a flow-cell surface, and amplification generates clusters of up to 1,000 identical copies of each DNA molecule. Sequencing uses fluorescently labelled nucleotides to produce reads of up to 150 bp in length, though 100 bp is more common. Reads can be produced as pairs. The use of paired reads improves the accuracy of reference mapping, overcoming many of the limitations of short read lengths such as inaccurate resolution of repeats, indels and structural rearrangements. By using the distance between a read pair to infer an insertion or deletion in the reference or sample and to

resolve repeats in de novo assembly, higher assembly accuracy is achieved. In contrast to several other technologies, insertion deletion errors are rare and nucleotide substitutions are the main error types. Because of the high accuracy of the data, Illumina sequencing is now becoming the platform of choice for resequencing, SNP discovery, whole-genome shotgun (WGS) sequencing and de novo assembly (Imelfort and Edwards 2009; Imelfort et al. 2009a; Williams-Carrier et al. 2010; Shulaev et al. 2011).

Ion Torrent is a relatively new technology and uses a novel high-density array of semiconductor micro-reaction chambers (http://www.iontorrent.com). Changes in pH are recorded as a result of the release of a hydrogen proton during the incorporation of a nucleotide during DNA synthesis. This produces reads of 100–200 bp, with up to 1 Gbp of data per run. The error profile of this system is yet to be published, but the technology has potential for cost-effective re-sequencing and variant discovery with fast runs of 2 h. Although relatively new, this technology is undergoing rapid evolution with the release of the replacement Ion Proton machine which is capable of greater throughput using larger chips.

Pacific Biosciences is one of the first "third-generation" sequencing systems to go on the market, and applies a novel single-molecule sequencing technique called SMRT™ (Single Molecule Real Time) technology. Read lengths of several thousand nucleotides have been reported (http://www.pacificbiosciences.com/). As with the Ion Torrent system, little is known about the error profile of the system, but missing or added bases, and hence indel calling would be a likely challenge with this technology.

Next-generation sequencing technologies continue to evolve at an incredible rate and new technologies such as the Oxford Nanopore system are likely to push the market forward over the coming years. While not as yet released, the Oxford Nanopore GridION and MinION promise further revolutions in data volumes, cost and usability, with the USB MinION being able to generate sequence data from raw samples such as whole blood in a device small enough to plug into a computer USB port.

10.5 Genome Sequencing

While the revolution in DNA sequencing is advancing gene expression studies, its chief impact has been in the area of genome sequencing (Edwards and Batley 2010). Significant advances in genome sequencing have been made in the last few years due mainly to the availability of next-generation DNA sequencing. Initial genome sequencing projects applied bacterial artificial chromosome (BAC) by BAC approaches, where the genome was dissected into regions of around 120 kbp, cloned as BACs. While some genome projects continue with this approach, the greatest advances have been made where WGS sequencing methods have been combined with next-generation sequencing technologies. This shift has been due to both the rapid improvements in sequencing technology and the

development of advanced bioinformatics tools that are capable of manipulating and assembling the vast quantities of data. With the decreasing cost of sequencing and rapid improvements in both data quality and read length from next- and third-generation technologies, WGS sequencing projects are likely to be established for many additional species.

Arabidopsis thaliana was the first plant species to have a sequenced genome (Arabidopsis_Genome_Initiative 2000). Since this milestone, the number of plant genomes being sequenced continues to increase. While several species with small genome sizes, as well as model species are now complete, genome researchers are now starting to tackle some of the larger and more complex genomes (Berkman et al. 2011, 2012b; Edwards and Wang 2012). *Brassica* species share extensive synteny with *Arabidopsis thaliana*, enabling comparative mapping and exploitation of the *Arabidopsis* genome sequence for Brassica crop improvement. Among the six cultivated species of *Brassica*, *B. rapa* (syn. *campestris*, AA, $n = 10$), *B. juncea* (AABB, $n = 18$) and *B. napus* (AACC, $n = 19$) are agronomically important oilseeds, whereas *B. oleracea* (CC, $n = 9$) is valued as leafy vegetables (e.g., broccoli, cauliflower, cabbage, khol-khol, etc.). The other two species, *B. nigra* (BB, $n = 8$) and *B. carinata* (BBCC, $n = 17$) are largely valued as condiments. Proprietary AA, CC, and AACC genomes were sequenced in 2009 (http://tinyurl.com/brassicagenome), and recently the Multinational Brassica Genome Project (MBGP) published the first public *B. rapa* genome (Mun et al. 2010; Wang et al. 2011; Edwards and Wang 2012). It is expected that further Brassica genomes will be published during 2013, with applications for Brassica crop improvement (Hayward et al. 2012a, b; Tollenaere et al. 2012).

Rice has the smallest genome size among major cereal crops, estimated at 430 Mbp (Goff et al. 2002) and the genome sequences of rice provide a basis for integrating and comparing biological information from rice and related cereal crops (Goff et al. 2002; Yu et al. 2002). *Brachypodium* was sequenced by the US Department of Energy Joint Genome Institute (DOE JGI) to provide a genomic bridge between rice and other agronomically important cereals (International Brachypodium Initiative 2010), and the subsequent sequencing of the sorghum genome opened opportunities for comparative studies to be carried out in the grass family between rice, sorghum and *Brachypodium* (Appleby et al. 2009). The draft release of the maize genome (Schnable et al. 2009) was sequenced using a minimum tiling path of BACs ($n = 16{,}848$) and fosmid ($n = 63$) clones derived from integrated physical, genetic and optical maps. The release of the maize genomes has opened up research areas characterizing diversity across maize germplasm and understanding how modern breeding has shaped the genomes of current elite varieties (Chia et al. 2012; Hufford et al. 2012; Jiao et al. 2012).

The size of the wheat (*Triticum aestivum*) genome is approximately 17,000 Mbp, much larger than related cereal genomes such as barley (*Hordeum vulgare*, 5,000 Mbp), rye (*Secale cereale*, 9,100 Mbp), and oat (*Avena sativa*, 11,000 Mbp). The size and hexaploid nature of the wheat genome create significant problems in elucidating its genome sequence (Edwards 2011). A pilot sequencing project was initiated in 2004 to assess BAC fingerprinting of the largest hexaploid

wheat chromosome 3B. A total of 68,000 BAC clones of a 3B chromosome-specific BAC library (Safar et al. 2004) have been fingerprinted, and a minimal tiling path is currently being sequenced. To complement these activities, individual chromosome arms are being sequenced using Illumina shotgun sequencing (Berkman et al. 2011, 2012b; Hernandez et al. 2012). While these do not produce a finished genome, the assemblies and syntenic builds, generated by comparison with related cereals, provide access to genomic sequences for all genes, while placing the majority of genes within an approximate order and orientation. Currently, only data for chromosome 7 is publicly available at http://www.wheatgenome.info (Lai et al. 2012a).

Legumes represent the third largest plant family and are the second most important crop family (Cannon et al. 2006). *Medicago truncatula* is an annual diploid with eight chromosomes. Six chromosomes have been sequenced in a US project and two additional chromosomes were sequenced by partners in Europe. In addition, there is the *Medicago* HapMap Project, which aims to deep-sequence whole genomes of 30 inbred *Medicago* lines using the Illumina platform, and use the reference genome to determine SNPs and Indels. A current release of the genome (Mt3.5) is available at (http://www.medicagohapmap.org). *Lotus japonicus* is a diploid self-fertile perennial pasture legume, with six chromosomes and a genome sequence of around 450 Mbp. The variety Miyakojima MG-20 has been sequenced using a shotgun approach (Sato et al. 2008) and data is available at http://www.kazusa.or.jp/lotus/. The genome sequences of *Medicago truncatula* and *Lotus japonicus* provide evolutionary insights into these and other species such as *Arabidopsis* (Schlueter et al. 2008; Bertioli et al. 2009). Soybean (*Glycine max*) is a major crop that accounts for 70 % of the world's edible protein. Its 1.1-Gbp genome was sequenced using a WGS approach (Schmutz et al. 2010), and the sequence is available through the phytozome database (http://www.phytozome.net/soybean). Resequencing of cultivated and wild varieties has enabled a detailed characterization of genome variation in this species (Lam et al. 2010). Early and current studies on the evolution and domestication of soybean have shown a loss of genetic diversity as a result of domestication (Hyten et al. 2006).

The sequencing of the tomato genome marks the first step in bringing together genetic maps and genomes of all Solanaceae and related plants, including potato, eggplant, pepper, petunia and coffee. The variety Heinz 1706 was selected as BAC resources were already available. A minimal tiling path of BAC clones was constructed and BAC clones were individually anchored to a genetic map based on a single, common *L. esculentum* × *L. pennellii* F_2 population. In 2009, WGS approaches were used to improve assemblies and an assembly was published in 2012 (The Tomato Genome Consortium 2012) and current builds are available at (http://solgenomics.net/). Potato is an economically important food crop with 330 million tons produced globally in 2009 (http://www.fao.org). The potato genome sequencing consortium (PGSC) sequenced the potato genome using a BAC-by-BAC approach (Xu et al. 2011) and data are available at (http://solgenomics.net/organism/Solanum_tuberosum/genome).

The monkey flower *Mimulus guttatus,* has become a model system for studying ecological and evolutionary genetics due to its diverse phenotypes, which include

adaptations to desert and aquatic environments, selfing and outcrossing, annual and perennial forms and varied floral morphology. The DOE Joint Genomes Institute (JGI) commenced sequencing *Mimulus guttatus* in 2006 using a WGS approach. Additionally, WGS sequencing of IM62 inbred lines is ongoing. A draft release of the genome is available on phytozome (http://www.phytozome.net/mimulus).

The papaya sequencing project was founded by the Centre for Genomics, Proteomics and Bioinformatics Research Initiative (CGPBRI) at the University of Hawaii in 2004. Papaya (*Carica papaya*) was the first fruit species and commercially important transgenic plant to be sequenced (Ming et al. 2008). A WGS approach was applied, with a total of 2.8 million reads assembled into contigs containing 271 M band scaffolds spanning 370 Mb. Cocoa (*Theobroma cacao*; $2n = 2x = 20$) was sequenced by the International Cocoa Genome Sequencing consortium (ICGS), using a WGS approach with Sanger and Roche 454 technologies (Argout et al. 2011). The sequencing of cocoa enabled the comparison of the grape, soybean, poplar and *A. thaliana* genomes with cocoa revealing 682 gene families (2,053 genes) unique to the cocoa genome.

Genetic diversity within the Rosaceae necessitated the use of several model species as references for comparative analysis in this family. Model species identified for this purpose, include strawberry (*Fragaria vesca*), peach (*Prunus persica*) and apple (Shulaev et al. 2008). Because of the complexity of the octoploid cultivated strawberry *F.* × *ananassa*, ($2n = 8x = 56$), the sequencing of its diploid progenitor, the woodland strawberry *F. vesca* ($2n = 2x = 14$), was undertaken (Shulaev et al. 2011). Sequencing of the apple genome (Velasco et al. 2010) followed a similar approach to that used to sequence the highly heterozygous grape genome *Vitis vinifera* cv. Pinot Noir (Zharkikh et al. 2008) using a combination of paired end reads produced by Sanger sequencing and unpaired reads produced by sequencing by synthesis.

10.6 Next-Generation Genotyping

Deciphering the genome sequence of a crop is only the first step towards using genomics for crop improvement (Berkman et al. 2012a). Perhaps of greater importance is knowledge of the variation in this genetic code between individuals. This genome variation is assayed as molecular genetic markers, and the DNA sequencing revolution is driving a revolution in molecular marker technology. Molecular genetic markers represent one of the most powerful tools for genome analysis and permit the association of heritable traits with underlying genomic variation. Two forms of sequence-based marker, simple sequence repeats (SSRs), also known as microsatellites, and SNPs are the most commonly used markers currently applied in modern genetic analysis. These are supplemented with anonymous marker systems such as amplified fragment length polymorphisms (AFLPs) (Vos et al. 1995), and diversity array technology (DArT) (Jaccoud et al. 2001). The reducing cost of DNA sequencing has led to the availability of large sequence data sets that enable the

mining of sequence-based markers, such as SSRs and SNPs, that may then be applied for genome characterization and breeding.

During the past two decades, several molecular marker technologies have been developed and applied for genome analysis. However, due to the relatively high cost associated with marker development, these methods have only been applied to a limited number of species, by a few researchers predominantly in developed countries. Even in these situations, the application of molecular markers has tended to focus on a small number of important diseases or high value traits. The increasing application of association mapping and genomic selection highlights the requirement to be able to identify and screen large numbers of markers, rapidly and at low cost. Bioinformatics systems that improve marker identification help to broaden the uptake of markers to more diverse species and for a greater variety of traits.

10.6.1 SNPs

SNPs are often considered as the ultimate form of molecular genetic marker, because an SNP represents a single nucleotide difference between two individuals at a defined location (Edwards et al. 2007a). SNPs are also the most abundant form of genetic polymorphism and may therefore provide a high density of markers near a locus of interest (Edwards et al. 2007b). There are three different categories of SNPs: transitions (C/T or G/A), transversions (C/G, A/T, C/A, or T/G) or small insertions/deletions (indels). SNPs are direct markers as the sequence information provides the exact nature of the allelic variants. Furthermore, this sequence variation can have a direct impact on the heritable phenotype. SNPs at any particular site could in principle be bi, tri or tetraallelic, but in practice they are generally biallelic (Chagné et al. 2007). SNPs are evolutionarily stable, not changing significantly from generation to generation, and their low mutation rate makes them excellent markers for studying complex genetic traits and for advanced breeding applications. SNPs are used routinely in crop breeding programs (Gupta et al. 2001), for genetic diversity analysis, cultivar/breed identification, phylogenetic analysis, characterization of genetic resources and association of genetic loci with valuable traits (Rafalski 2002; Batley and Edwards 2007). The high density of SNPs makes them valuable for genome mapping, and in particular they allow the generation of ultra-high density genetic maps and haplotyping systems for genes or regions of interest, and map-based positional cloning.

10.6.2 SSRs

SSRs, also known as microsatellites, are short stretches of DNA sequence occurring as tandem repeats of mono-, di, tri, tetra, penta and hexanucleotides. Perfect SSR repeats are without interruptions, imperfect repeats are interrupted by non-repeat

nucleotides, while compound repeats are cases where two or more SSRs are found adjacent to one another. Combinations of these are also found, for example imperfect compound repeats (Weber 1990). SSRs are widely and ubiquitously distributed throughout eukaryotic genomes (Toth et al. 2000; Katti et al. 2001; Mortimer et al. 2005). They are one of the most powerful genetic markers as they are highly polymorphic due to mutation affecting the number of repeat units. SSRs provide several advantages over some other molecular markers, namely that multiple SSR alleles may be detected at a single locus using a simple PCR-based screen, very small quantities of DNA are required for screening, and analysis is amenable to automated allele detection and sizing (Schlotterer 2000). SSRs also demonstrate a high degree of transferability between species, as PCR primers designed to an SSR within one species frequently amplify a corresponding locus in related species. This transferability makes them suitable for genetic diversity and comparative genomic analysis. SSRs are applied to a wide range of applications, including genetic mapping, the molecular tagging of genes, genotype identification, analysis of genetic diversity, phenotype mapping and marker-assisted selection (Tautz 1989; Powell et al. 1996).

10.7 Computational Molecular Marker Discovery Methods

10.7.1 In Silico SNP Discovery

Traditionally, the implementation of SNPs and SSRs has been limited by the initial cost of their development. The discovery of SSR loci previously required the construction of genomic DNA libraries enriched for SSR sequences, followed by DNA sequencing of the clones and analysis of the sequence for the presence of SSRs (Edwards et al. 1996). This was both time consuming and expensive due to the large amount of specific sequencing required. In silico methods of SNP and SSR discovery are now routinely applied, providing cheap and efficient marker identification (Batley et al. 2007b; Batley and Edwards 2009b). Vast quantities of sequence data are being generated by next-generation sequencing projects and this provides a valuable resource for the mining of molecular markers (Duran et al. 2009b; Azam et al. 2012).

In one of the first examples of SNP discovery from next-generation sequence data, a total of 36,000 maize SNPs were identified in data from a single run of the Roche 454 GS20 DNA sequencer (Barbazuk et al. 2007). WGS is the most robust method to identify the great variety of genetic diversity in a population and gain a greater understanding of the relationship between the inherited genome and observed heritable traits. The continued rapid advances in genome sequencing technology will lead to whole-genome sequencing becoming the standard method for genetic polymorphism discovery (Lai et al. 2012b).

The dramatic increase in the number of DNA sequences submitted to databases makes the electronic mining of SNPs possible without the need for additional specific allele sequencing. The identification of sequence polymorphisms in assembled sequence data is relatively simple. However, the challenge of in silico SNP discovery is not SNP identification, but rather the ability to distinguish real polymorphisms from the often more abundant sequencing errors. High-throughput sequencing remains prone to inaccuracies, Sanger sequencing produces errors as frequent as one in every one hundred base pairs, whilst some of the next-generation technologies are even less accurate with errors as frequent as one in every 25 bp. These errors impede the electronic filtering of sequence data to identify potentially biologically relevant polymorphisms and several sources of sequence error need to be addressed during in silico SNP identification.

The frequency of occurrence of a polymorphism at a particular locus provides a measure of confidence in the SNP representing a true polymorphism, and is referred to as the SNP redundancy score. By examining SNPs that have a redundancy score equal to or greater than two (two or more of the aligned sequences represent the polymorphism), the vast majority of sequencing errors are removed. Although some true genetic variation is also ignored due to its presence only once within an alignment, the redundancy within the data permits the rapid identification of large numbers of SNPs without the requirement of sequence quality files. However, while redundancy-based methods for SNP discovery are highly efficient, the non-random nature of sequence error may lead to certain sequence errors being repeated between runs around locations of complex DNA structure. Therefore, errors at these loci would have a relatively high SNP redundancy score and appear as confident SNPs. In order to eliminate this source of error, an additional independent SNP confidence measure is required. This can be obtained by measuring the co-segregation of SNPs defining a haplotype. True SNPs that represent divergence between homologous genes co-segregate to define a conserved haplotype, whereas sequence errors do not co-segregate with a haplotype. Thus, a co-segregation score, based on whether a SNP position contributes to defining a haplotype is a further independent measure of SNP confidence. By using the SNP score and co-segregation score together, true SNPs may be identified with reasonable confidence. Four tools currently apply the methods of redundancy and haplotype co-segregation; autoSNP (Barker et al. 2003; Batley et al. 2003), SNPServer (Savage et al. 2005), autoSNPdb (Duran et al. 2009a, c; Lai et al. 2012b) and SGSautoSNP (Lorenc et al. 2012b). The increased production of next- or second-generation sequence data provides an additional source of valuable SNP information and new tools and approaches are being developed to make the most of these data types (Imelfort et al. 2009b; Duran et al. 2010b; Edwards et al. 2012a).

10.7.2 In Silico SSR Discovery

The availability of large quantities of sequence data make it economical and efficient to use computational tools to mine for SSRs, and methods are now being developed for the discovery of SSRs from next-generation DNA sequence data (Jiang et al. 2012; Nie et al. 2012). The flanking DNA sequence may then be used to design suitable forward and reverse PCR primers to assay the SSR loci. Furthermore, when SSRs are derived from expressed sequence tags (ESTs), they become gene-specific and represent functional molecular markers. These features make EST-SSRs valuable markers for the construction and comparison of genetic maps and the association of markers with heritable traits. Several computational tools are available for the identification of SSRs in sequence data as well as for the design of PCR amplification primers. Because of redundancy in EST sequence data, and with datasets often being derived from several distinct individuals, it is now also possible to predict the polymorphism of SSRs in silico.

The MIcroSAtellite (MISA) tool (http://pgrc.ipk-gatersleben.de/misa/) identifies perfect, compound and interrupted SSRs. It requires a set of sequences in FASTA format and a parameter file that defines unit size and minimum repeat number of each SSR. The output includes a file containing the table of repeats found, and a summary file. MISA can also design PCR amplification primers on either side of the SSR. The tool is written in Perl and is therefore platform independent, but it requires an installation of Primer3 for the primer search (Thiel et al. 2003). MISA has been applied for SSR identification in moss (von Stackelberg et al. 2006) and coffee (Aggarwal et al. 2007). The tool SSRIT (Simple Sequence Repeat Identification Tool) (http://www.gramene.org/db/searches/ssrtool) uses Perl regular expressions to find perfect SSR repeats within a sequence. It can detect repeats between 2 and 10 bases in length, but eliminates mononucleotide repeats. The output is a file of SSRs in tabular format. A Web-based version is available that will take a single sequence, and a stand-alone version is also available for download. SSRIT has been applied to rice (Temnykh et al. 2001). RepeatFinder (Volfovsky et al. 2001; http://www.cbcb.umd.edu/software/RepeatFinder/) finds SSRs in four steps. The first step is to find all exact repeats using RepeatMatch or REPuter. The second step merges repeats together into repeat classes, for example repeats that overlap. Step three merges all of the other repeats that match those already merged, into the same classes. Finally, step four matches all repeats and classes against each other in a non-exact manner using BLAST. The input is a genome or set of sequences, and the output is a file containing the repeat classes and number of merged repeats found in each class. RepeatFinder finds perfect, imperfect and compound repeats, and was not designed specifically to find SSRs so can find repeats of any length. It runs on Unix or Linux and has been used to identify SSRs in peanut (Jayashree et al. 2005). Sputnik is a commonly used SSR finder as it is fast, efficient and simple to use. It uses a recursive algorithm to search for repeats with length between 2 and 5, and finds perfect, compound and imperfect repeats. It requires sequences in FASTA format and uses a scoring system to call each SSR.

The output is a file of SSRs in tabular format. Unix, Linux and Windows versions of sputnik are available from http://espressosoftware.com/pages/sputnik.jsp and http://cbi.labri.fr/outils/Pise/sputnik.html (PISE enabled version). Sputnik has been applied for SSR identification in many species including *Arabidopsis* and barley (Cardle et al. 2000). The SSR identification tool Tandem Repeat Occurrence Locator (TROLL) (Castelo et al. 2002; http://wsmartins.net/webtroll/troll.html) draws a keyword tree and matches it with a technique adapted from bibliographic searches, based on the Aho-Corasick algorithm. It has drawbacks in that it does not handle very large sequences and cannot process large batches of sequences as the tree takes up large amounts of memory. Tandem Repeats Finder (TRF) (Benson 1999; http://tandem.bu.edu/trf/trf.html) can find very large SSR repeats, up to a length of 2,000 bp. It uses a set of statistical tests for reporting SSRs, which are based on four distributions of the pattern length, the matching probability, the indel probability and the tuple size. TRF finds perfect, imperfect and compound SSRs, and is available for Linux. TRF has been used for SSR identification in Chinese shrimp (Gao and Kong 2005) and cowpea (Chen et al. 2007).

Some SSR finders combine previous methods to produce extended output. SSRPrimer (Robinson et al. 2004; Jewell et al. 2006) combines Sputnik and the PCR primer design software Primer3 to find SSRs and associated amplification primers. The scripts take multiple sequences in FASTA format as input and produce lists of SSRs and associated PCR primers in tabular format. This Web-based tool is also available as a stand-alone version for very large datasets. SSRPrimer has been applied to a wide range of species including shrimp (Perez et al. 2005), citrus (Chen et al. 2006), mint (Lindqvist et al. 2006), strawberry (Keniry et al. 2006), Brassica (Burgess et al. 2006; Batley et al. 2007a; Hopkins et al. 2007; Ling et al. 2007), Sclerotinia (Winton et al. 2007) and *Eragrostis curvula* (Cervigni et al. 2008). SSRPoly (http://www.appliedbioinformatics.com.au/index.php/SSRPoly) is currently the only tool which is capable of identifying polymorphic SSRs from DNA sequence data. The input is a file of FASTA format sequences. SSRPoly includes a set of Perl scripts and MySQL tables that can be implemented on UNIX, Linux and Windows platforms.

10.8 Data Storage

Large-scale discovery projects are uncovering vast quantities of marker data. As the data size increases, the storage and logical organization of the information becomes an important challenge (Edwards et al. 2009; Lee et al. 2012; Marshall et al. 2010). Marker databases vary between centralized repositories that integrate a variety of data for several species, to small specialized databases designed for very specific purposes (Duran et al. 2011; Lorenc et al. 2012a). The larger repositories tend to lack detailed analysis tools, while the smaller systems may include further species-specific data integration (Lai et al. 2012c). dbSNP is becoming the default repository for SNP data, and there are a wide variety of additional marker databases

specific to particular species. The most commonly used marker databases are detailed below.

Gramene is an online comparative mapping database for rice and related grass species (Ware et al. 2002a, b; Jaiswal et al. 2006). Gramene contains information on cereal genomic and EST sequences, genetic maps, relationships between maps, details of rice mutants, and molecular genetic markers. The database uses the sequenced rice genome as its reference and annotates this genome with various data types. As well as the genome browser, Gramene also incorporates a version of the comparative map viewer, CMap. This allows users to view genetic maps and comparative genetic mapping information and provides a link between markers on genetic maps and the sequenced genome information. GrainGenes integrates genetic data for Triticeae and Avena (Matthews et al. 2003; Carollo et al. 2005). The database includes genetic markers, map locations, alleles and key references for barley, wheat, rye, oat and related wild species. Graingenes also provides access to genetic data using CMap.

Wheatgenome.info (Lai et al. 2012a) is a genetics and genomics resource for bread wheat and provides public access to wheat chromosome 7 assemblies annotated with candidate functional genes and large numbers of SNP molecular markers (Edwards et al. 2012b).

The Arabidopsis Information Resource (TAIR) (http://www.arabidopsis.org/) provides an extensive Web-based resource for the model plant *Arabidopsis thaliana* (Huala et al. 2001; Rhee et al. 2003; Weems et al. 2004). Data includes gene, marker, genetic mapping, protein sequence, gene expression and community data within a relational database.

MaizeGDB (Lawrence et al. 2004; Lawrence 2007) combines information from the original MaizeDB and ZmDB (Gai et al. 2000; Dong et al. 2003) repositories with sequence data from PlantGDB (Dong et al. 2004, 2005; Duvick et al. 2008). The system maintains information on maize genomic and gene sequences, genetic markers, literature references, as well as contact information for the maize research community.

AutoSNPdb implements the autoSNP pipeline within a relational database to enable mining for SNP and indel polymorphisms (Duran et al. 2009a). A Web interface enables searching and visualization of the data, including the display of sequence alignments and SNPs. All sequences are annotated by comparison with GenBank and UniRef90, as well as through comparison with reference genome sequences. The system allows researchers to query the results of SNP analysis to identify SNPs between specific groups of individuals or within genes of predicted function. AutoSNPdb is currently available for barley (Duran et al. 2009c), rice, wheat (Lai et al. 2012b) and *Brassica* species and is available at: http://autosnpdb.appliedbioinformatics.com.au/.

10.9 Data Visualization

The effective visualization of large amounts of data is as critical an issue as its storage (Batley and Edwards 2009a). Increasing volumes of data permit researchers to draw, with increasing confidence, comparative links across the genome to phenome divide. Visualization tools, combined with the ability to dynamically categorize data, allow the identification of trends and relationships at varying tiers of resolution (Duran et al. 2009d). Current visualization techniques for molecular markers broadly fall into two categories: graphical map viewers and genome browsers. Map viewers display markers as a representation of a genetic linkage map. Genome browsers generally host a greater quantity of annotation data and may be linked to related genetic map viewers.

Graphical Map Viewers. The NCBI map viewer (http://www.ncbi.nih.gov/mapview) uses sets of aligned maps to visualize molecular genetic markers, genome assemblies and other annotations (Wheeler et al. 2008). It allows users to show multiple levels of annotation in tandem for a given chromosomal segment. As well as allowing users to view the map graphically, NCBI also provides a function to download raw mapping data in a tabular format.

CMap is a tool for viewing and comparing genetic and physical maps and has been applied successfully for the comparison of maps within and between related species (Jaiswal et al. 2006). CMap was originally developed for the Gramene project (http://www.gramene.org/) and has since been applied for the comparison of genetic maps of Brassica (Lim et al. 2007), sheep, cattle, pig and wallaby (Liao et al. 2007), honeybee, grasses (Somers et al. 2004; Jaiswal et al. 2006), peanut (Jesubatham and Burow 2006), Rosaceae (Jung et al. 2008) and legumes (Gonzales et al. 2005). The database specification dictates that a given species may have one or more "map sets," where a map set represents a collection of maps. The CMap database was designed with flexibility in mind, allowing the database to be used for a wide variety of mapping applications. For example, in genetic mapping studies, a map set is most likely to represent a particular organism, where the contained maps will probably be the genetic linkage groups. To generate genetic linkage maps, software such as Joinmap (Van Ooijen and Voorrips 2001), MapMaker (Lander et al. 1987) or Map Manager QTX (Manly et al. 2001) are frequently used. Once the maps are generated, they are parsed into the CMap database using a Perl script provided with the CMap software package. The comparative relationships, or correspondences, between maps are generated once the maps are added to the database, using a combination of automated tools and manual curation. The automated tools included with CMap create correspondences on the basis of common feature names. If maps are generated with a consistent marker-naming strategy, this can reduce the manual curation required and provide a firm basis for further curation. Curation is performed by parsing a tab-delimited file with the correspondences using a custom Perl module included in CMap.

As an extension to CMap; CMap3D allows researchers to compare multiple genetic maps in three-dimensional space (Duran et al. 2010a). CMap3D accesses

data from CMap databases, with specifications defined by the Generic Model Organism Database (GMOD) (http://www.gmod.org/wiki/CMap). The viewer is a stand-alone client written in Processing (http://www.processing.org) and is available for Windows, OSX and Linux. Information from the CMap repository enables CMap3D to generate appropriate external Web links for features of a given map. By clicking on a feature in the viewing space, the user will be taken to the relevant Web page. Compatibility with current CMap databases has been preserved by taking a server/client approach. The client is a user-side application that connects to servers hosting an existing CMap database known as a repository. The server is a Web service that hosts a variety of scripts that communicate directly with a CMap MySQL database. The Cmap3D client first connects to a centralized repository listing server, which provides the client with a list of available repositories and their details. The client then communicates directly with the repository server to request and retrieve the required data. Data transfer uses the HTTP protocol for data transfer, to minimize institution network security conflicts.

10.10 Applying Bioinformatics for Employing Genomics for Combating Climate Change

Genetic markers, genome sequencing and bioinformatics have all played a major role in our understanding of heritable traits, however while great advances have been made in characterizing genomes and genetic markers, the ability to link these genomic differences with heritable phenotypic traits remains a bottleneck. In the current genomics era, molecular genetic markers, with the help of bioinformatics, are gradually bridging the divide between traits and increasingly available genome sequence information (Edwards and Batley 2004, 2008; Edwards 2007). With the expansion of next-generation sequencing technologies, there will be a rapid growth in associated marker information and the use of these markers for applications including the breeding of crops for increased climate resilience. These new technologies make it possible to speed up the breeding process. For example, a desired trait may only be observed in the mature plant, but marker-assisted selection allows researchers to screen for the trait at a much earlier growth stage (Raman et al. 2012). Further advantages of molecular markers are that they make it possible to select simultaneously for many different plant characteristics. They can also be used to identify individual plants with a defined resistance gene without exposing the plant to the pest or pathogen in question. In order to increase throughput and decrease costs, it is necessary to eliminate bottlenecks throughout the genotyping process, as well as minimize sources of variability and human error to ensure data quality and reproducibility. These new technologies may be the way forward for the discovery and application of molecular markers and will enable the application of markers for a broader range of traits in a greater diversity of species than currently

possible, accelerating crop improvement to meet the challenge of climate-related global food insecurity.

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Chapter 11
Facilitation of Future Research and Extension Through Funding and Networking Support

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Abstract The breeding of climate-resilient crops is as complex as it is promising. Given these scientific complexities and the scale of impending climate change, research in this area will demand international collaboration in order to succeed in addressing critical challenges. This is particularly important for developing countries, which will bear the brunt of the impacts of climate change but may lack the physical and human resources to address the requirements for targeted breeding of more resilient crop varieties. Collaboration is required at many levels in genomics, germplasm collection and conservation, germplasm exchange, and breeding technologies such as phenotyping and genotyping methods. Establishing functional collaborative research networks is central to such a coordinated global approach. Because the benefits that climate-resilient crops convey to producers are often complex and stochastic in nature, facilitating the adoption and diffusion of these crops also requires strong collaboration and creativity in extension efforts, including the coordination of public and private sectors. We argue that support for networking and funding of collaborative research and extension activities will directly shape the success of global efforts to develop and disseminate climate-resilient crops and the improvements in food security they should provide.

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11.1 Introduction

The effective application of advances in genomic technology in providing security for food supply in the face of the challenges of climate change requires the concerted application of the best science globally. Networking of researchers is essential, as is funding that supports international collaboration.

Population growth and growing per capita consumption of food due to economic development is driving strong global growth in demand for food. Efforts to develop climate-resilient crops to ensure sustainable food security require substantial improvements in both crop varieties and their crop management systems and technologies. Diversification of food production systems to include a wider range of cultivars, and/or species may also provide a complementary strategy for improving food security globally.

Climate change is predicted to have greater effects in many developing countries as compared to the developed countries (Lobel et al. 2008). However, in many cases, agroecosystems in developing countries are more likely to be under pressure from the impacts of climate variability and change, as the demands for food security have intensified cropping cycles but the use of inputs such as fertilizers has become limited by costs and environmental concerns.

Many collaborative networks involving developing countries have been maintained for years—thanks largely to development assistance funding. This funding, which has usually been provided through time-bound projects involving collaboration between research organizations of developed and developing countries, has focussed on capacity building as well as on the development of climate-resilient germplasm, particularly with an emphasis on drought tolerance.

11.2 Sizing Up the Challenges: Developing and Disseminating Climate-Resilient Crops: Research Challenges

11.2.1 Advances in Genomics Technologies

Genomics technologies have advanced rapidly in the last few years due to the rapid development and deployment of new technologies for DNA sequencing (Henry 2012). These technology developments provide new opportunities for rapid developments in biology (Castiglioni et al. 2008). Analysis of whole-genome diversity in populations of wild crop relatives may reveal how plants adapt to climate variation in nature (Fitzgerald et al. 2011; Shapter et al. 2012) providing clues as to how we can adapt them to be more climate resilient in agricultural systems. New sequencing approaches allow rapid characterization of domestication genes in wild crop relatives (Malory et al. 2011) providing improved tools to accelerate domestication of genera and species.

11.2.2 Need for Cooperation in Access to Genetic Resources

Key areas of collaboration are in the exchange of germplasm (seed) materials and genomic and phenotypic data on this germplasm. Stocks of seed and accompanying data are held in many different countries and global advancement in crop improvement is directly facilitated by assess to these resources. International networking is required to provide access to these resources for effective crop improvement to support food security. Provision of funding is essential to enable networking of research.

11.2.3 Collaboration in Collecting Resources

Wild crop relatives and other genetic resources (Henry 2005) for major and minor crop relatives remain poorly collected in many cases (Dillon et al. 2007) and international collaboration provides an important means to their improved collection. Seedbanks enable ex situ conservation of genetic resources and allow exchange of genetic resources globally in the form of seed. While most seedbanks are already networked globally, further enhancements of collaboration will advance response to future food security needs.

The best option for conservation of genetic resources for many species is protection of wild populations in situ. International support can be essential to conservation especially when species are found in countries with limited resources or expertise for management of these resources. Conservation of agriculture and nature may compete for land use in many cases and the importance of reserving some land for in situ conservation of the wild biodiversity, on which the agricultural system depends, needs careful communication (Henry 2010).

An active crop wild relatives group (CropWildRelativesGroup@yahoogroups.com) has been formed to network scientists with an interest in crop wild relatives internationally.

11.2.4 Access to Biotechnology Patents

Assess to enabling intellectual property is essential for global crop improvement. Innovative strategies need to be used to provide this access to support food security in key areas. The Public Intellectual Property Resource for Agriculture (PIPRA) supports access to critical intellectual property rights for public sector research and development in support of crop improvement globally (Chi-Ham et al. 2012). This is an effective mechanism established by the Rockefeller Foundation in 2004 to facilitate access to freedom to operate with plant transformation technology. This

type of arrangement might also benefit exchange of other technologies and information globally.

11.2.5 Biodiversity Convention

The International Convention on Biological Diversity (1992) provided a new framework for control of exchange of genetic resources at an international level. The international Treaty on Plant Genetic Resources for Food and Agriculture (2001) establishes a basis for free exchange of genetic resources of important food crops. A standard Material Transfer Agreement has been developed for this purpose. The Nagoya Biodiversity Agreement (2010) provided agreement to protect natural environments protecting wild plant genetic resources and support in in situ conservation.

11.2.6 Role of DNA Banks

DNA banks facilitate conservation research and exchange of genetic resources at the DNA level (Rice et al. 2006a, b). Networking of DNA banks provides opportunities for exchange of genetic material and information internationally to support crop improvement. DNA banks have been proposed for some time, however they have been limited in development because of the need for improved international collaboration and networking. An international network of DNA banks was established in 2007, but expansion of the coverage of crops in these collections is of international importance. The role of DNA banks is changing as technology advances. While early banks provided DNA for PCR-based analysis, DNA banks are now required to retain samples for whole-genome analysis by next-generation sequencing.

11.2.7 Sequence Data Collections

Collections of DNA sequence data are the most easily shared internationally, as they can obviously be exchanged by electronic means while seed and DNA exchange may be much more restricted. Increased storage and improved access to these data repositories is a key way to advance global crop improvement and research. Sequence data information continues to grow rapidly. Linking of DNA sequence data to germplasm and DNA collections will deliver greatly enhanced tools for effective germplasm utilization. Databases need to link directly information on DNA sequence to corresponding samples in the germplasm collection and possibly to the DNA samples in the DNA bank. Data collection efforts should now

aim to ultimately provide whole genome sequence data for each sample held in collections. This goal will become more realistic as methods for sequencing continue to improve, reducing costs further.

11.3 Extension and Adoption of Challenges

Given how daunting the above research challenges can be, it can be tempting to focus entirely on bridging these "upstream" gaps and implicitly adopt a "build it and they will come" mentality. Decades of research into the determinants of technology adoption suggest the fallacy of framing agricultural production problems as purely technical matters (see Foster and Rosenzweig (2010) and Feder et al. (1985) for surveys of this research). As climate-resilient crops emerge from labs, ready to be tested on farm and ultimately released for broader adoption, a different but similarly complex set of challenges arise. These challenges stem from farmers' perspectives on changing varieties, technologies and practices, but have important implications for both public and private extension services. In this section, we highlight these perspectives and discuss these challenges.

11.3.1 Seeing Climate-Resilient Crops from Farmers' Perspective

Farmers with different production systems and wealth levels and in different policy and market contexts can have dramatically different perspectives on new technologies. These differing views reflect substantial heterogeneity in farmers' capacity, willingness and incentives to experiment and ultimately adopt these technologies. Specific features of climate-resilient crops imply that appreciating farmers' perspective on these new technologies is crucial to understanding "downstream" adoption and extension challenges.

To appreciate the importance of farmers' perspective on climate-resilient crops, we should consider a few key dimensions of heterogeneity that are likely to shape differing perspectives. First, farmers tend to be part of households, and the broader economic activities, constraints and resources of the household can directly shape farmers' perspectives. Thus, a farmer whose broader household relies heavily on a few crops for their livelihood will approach new technologies differently than one whose household activities are diversified across different crops and other off-farm pursuits.

Second, the degree of control farmers have over production outcomes—which may largely reflect reliability of their access to inputs such as irrigation, improved seeds, fertilizers, and pesticides—can hamper their ability to trial new technologies and practices. With some technologies and in some settings, farmers can effectively

learn from demonstration plots, controlled trials, or neighboring farmers with greater input control (e.g., Foster and Rosenzweig 1995; Conley and Udry 2010). This may or may not be true for climate-resilient crops that deliver a more nuanced set of benefits.

Third, farmers' access to output and other markets can dramatically change their perspective and production approach. Whether a farmer is well integrated with output markets not only changes production strategies but can also enable farmers to specialize in ways that changes how they manage their operations. Farmers who are poorly integrated into other food markets tend to subsist on their own production, which changes the kinds of tradeoffs they are willing to make between risk and return.

Finally, limited access to financial services can seriously constrain farmers' willingness to experiment with new technologies. Credit and insurance, when available in the right forms and at the right times, can directly support farmers' uptake of new techniques. Farmers who lack access to either formal or informal financial services tend to see new technologies as much riskier than those with access to these services.

11.3.2 Learning and Adoption

The differences in farmers' perspectives described above can have obvious implications for technology adoption. These connections have been widely studied by social scientists in recent decades, beginning with the diffusion of hybrid corn in the US (Griliches 1957) and continuing with the studies of Green Revolution technologies in the 1970s and 1980s (see Feder et al. (1985) for a review of this work). While learning by doing or learning from others has figured in this line of work, learning may play a particularly important role in the case of climate-resilient crops. Such learning from experience will be important with the highly heterogeneous agricultural production of smallholder agriculture in developing countries—the target of much of the current discussion of climate-resilient crops.

As described in Lybbert and Bell (2010), these crops tend to offer a different risk and return tradeoff than previous crop improvements, which focused on increasing yields under favorable conditions. In particular, these climate-resilient crops typically aim to be risk-reducing rather than yield-increasing. This complicates on-farm experimenting and learning because it implies that compelling differences between an old variety and a climate-resilient one are only evident during production seasons with particular features (e.g., certain levels of drought). Whether a farmer can actually discern these differences when they arise depends on some of the differences discussed above. Marginal farmers—who typically face poor soils, erratic weather, and limited or no access to irrigation and other inputs—often lack the control required to perceive subtle differences between competing varieties.

11.3.3 Implications for Extension

These differences in farmers' perspectives on new technology and the potentially important role learning will play in the diffusion of climate-resilient crops poses some unique extension challenges. The research challenges outlined above should be explicitly informed by these looming downstream challenges.

The first challenge related to extension is that there are now few functional and effective public extension systems, especially in much of the developing world (see Anderson and Feder 2004). In many countries, the private agroservices sector is increasingly playing an important extension role. This trend similarly provides an important backdrop to any discussion of extension challenges that are specific to climate-resilient crops.

Where they function well, public agricultural extension systems take into account differences across farmers in different agroecological zones when formulating training and making recommendations. Climate-resilient crops may make this an even more important feature of public extension, particularly since the productivity and profitability of these crops is likely to vary dramatically across these zones both within and across years. In other words, the fact that the performance of climate-resilient crops is dependent on drought stress and that the level of stress is likely to vary based on farm-specific factors such as soil moisture and topography as well as other broader and "shared" factors, for example rainfall, will present some new challenges for extension services.

A primary implication of the challenges for extension services is their role in facilitating learning about the benefits of climate-resilient crops and varieties relative to familiar crops and varieties. These state-contingent relative benefits might be easier for farmers to perceive if experiences are formally and informally shared across agroecological zones. This may pose a new challenge to the "demonstration plot" approach to extension, which is still utilized in a number of countries.

Another potential extension challenge comes from the fact that reaping the full benefit of climate resilience may require not just specific germplasm improvements but also changes in production practices (e.g., seeding rates and practices, nutrient management, interaction with other crops, and water control). In this sense, effective extension focused on climate-resilient varieties must move beyond the Green Revolution model in which providing the seed was providing the technology, which worked in the favorable irrigated environments in South and Southeast Asia. Given that climate-resilient crops will usually target a more diverse set of unfavorable environments and require a broader set of changes to agronomic practices, extension in many settings will face greater demands on dissemination.

11.4 Some Current Collaborative Research Networks and Support

11.4.1 Potential Advantages of Networks

Research networks have advantages for both the developing and developed countries, especially with recent evidence of a decrease in the rates of genetic gains from cereal breeding (Fischer and Edmeades 2010). International collaboration in the development of climate-resilient crops often can provide access to a broader range of genetic resources than would otherwise be possible, including wild progenitors that may have performed in stressed environments (Hodson and White 2010). It also enables breeders to thoroughly explore the environmental (climate, hydrology, soils) impacts on field performance of lines, which has been highlighted by Reynolds et al. (2012) and others. This leads to better targeting of germplasm from using a wide range of environments through conduct of multilocational trials in areas where a range of climate stresses emerge. There are benefits not only from increased capacity for conducting resource-intensive variety and agronomic trials, but also access through local collaborators to local understanding of agronomic systems and practises. Researchers and breeders can also be connected with unconventional partners such as NGOs and farmer groups. Overall, collaboration can lead to better coordination and reduced duplication of research efforts, also supporting the communication and dissemination both of research results and improved germplasm.

Networks have provided the critical mass for multilocation assessment of genotype performance across different environments, high-throughput phenotyping and identification of donor genes and quantitative trait loci (QTLs) for drought tolerance. Research partnerships have also provided access to broader skills, such as natural resource management and social sciences, and the opportunity for integration with the commercial seed and input supply systems. Regional research networks such as the IRRI-led Consortium for Unfavorable Rice Environments ensure that new germplasm is agronomically appropriate for the target environments (Serraj et al. 2011).

As resources in developing countries increase, “home grown” networks for breeding are also emerging. For example, in early 2011, the Indian Council for Agricultural Research established the “Indian National Initiative for Climate-Resilient Crops.” Even in the case of international donor-funded projects, the recent trend has been for these projects to be led by developing country organizations, for example, the Bill and Melinda Gates and Buffett Foundation-funded project, Water Efficient Maize for Africa, is led by the African Agricultural Technology Foundation (http://www.aatf-africa.org), based in Nairobi, as well as partners such as CIMMYT and Monsanto as well as several Southern African government partners. It has a focus on conventional and molecular breeding for drought tolerance.

11.4.2 Role of the CGIAR Consortium and Centers

Unlike "virtual" programs that come and go with funding cycles, the CGIAR centers, while themselves often coordinators of, or participants in networks, have a long-term physical presence. The trigger for their formation was the recognition of the threat of famine in parts of South and South East Asia during the 1960s. Several of the CGIAR centers were established to facilitate the delivery of new crop germplasm as global public goods. Together with collections held by National Agricultural Research Centers, the CGIAR centers have typically been the repositories of crop germplasm. Although their focus is on delivering research for development (poverty reduction) outcomes for developing countries, many developed countries have also shared in the benefits. For example Australia, which shares the challenges of significant climatic variability with many temperate developing countries, has benefited significantly from the investment in the CGIAR to develop climate-resilient crops (Brennan and Fox 1998; Brennan et al. 2003).

CGIAR research has had a significant focus on the development of climate-resilient crops through collaborative networks involving both advanced research institutes and developing country partners. CGIAR centers have had a role in introducing molecular techniques into these collaborations, to support the identification and selection of genes controlling stress tolerance and for introgression of desirable traits from wild crop relatives. Some areas of recent emphasis within the CGIAR include the development of over 50 varieties of drought-tolerant tropical maize through collaboration between the International Maize and Wheat Improvement Center (CIMMYT) and partners in subSaharan Africa. By using networks of farmer groups, field testing has been carried out at nearly 150 stress-prone sites. CIMMYT scientists are identifying areas of the maize genome that are linked to drought tolerance. In West Africa, researchers at the International Institute of Tropical Agriculture (IITA) have made significant progress in developing early and extra-early maturing maize varieties that can grow in regions with short rainy seasons.

With rice, the New Rices for Africa (NERICA), developed by the Africa Rice Center and partners have drought escape characteristics. Early maturing NERICA varieties enable more intensive cropping and can escape the intermittent droughts that can occur at critical stages in crop development. IRRI researchers and collaborators have utilized the rice gene *Sub1*, which allows plants to survive completely submerged for up to 2 weeks (Septiningsih et al. 2009). The "water-proofing" trait has been transferred into a popular rice variety in Bangladesh, and the improved version is providing high yields while protecting harvests against flooding.

Barley breeders at the International Center for Agriculture in the Dry Areas (ICARDA) have demonstrated how drought tolerance in this crop can be markedly improved through combination of genomic approaches (Lakew et al. 2010) and farmer participation. Cassava performs well even in drought-prone areas and infertile soils. Researchers at IITA are evaluating cassava in semi-arid regions of

East and West Africa in an effort to determine what mechanisms enable the crop to withstand dry spells. The results should make it possible to identify genes for this trait and further enhance the drought tolerance of cassava. Using molecular tools, researchers at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) have isolated and are employing genes for the so-called "stay-green" trait in millet and sorghum. By delaying the death of leaves and plants and enhancing grain development, these genes enhance drought tolerance. The International Center for Tropical Agriculture (CIAT) has succeeded in breeding drought-tolerant higher yielding common beans, while IITA has incorporated tolerance to drought and heat into cowpea.

In earlier times, the International Wheat Improvement Network (Reynolds and Borlaug 2006) was one of the first formal international networks to collaborate on germplasm exchange for crop breeding. Established in the 1950s, in recent years it has been coordinated by CIMYYT and involves distribution of international nurseries targeted to different agroecosystems and breeding "hubs" that address a range of priorities including both abiotic and biotic stresses. The Africa Trial sites network (http://www.africats.org) facilitates variety evaluation across a range of crops and a range of stressed and favorable sites.

The Generation Challenge Program was created by the CGIAR in 2003 to employ genetic diversity and advanced plant science to strengthen breeding programs for developing countries, particularly those that targeted variable climates—drought-prone and other harsh environments. It is a large network of CGIAR, university and NARS programs. While the first 5-year phase focused on "orphan" crops to enable molecular breeding approaches to be used in these often-neglected species, its more recent emphasis has been on strengthening drought tolerance in nine major crops. A breeding-friendly genotyping platform has been developed for high-throughput low-cost applications using sets of publicly available single nucleotide polymorphisms. As of 2011, the research activities of the GCP were incorporated into a range of new CGIAR Research Programs.

The importance of "virtual collaboration" and information exchange is increasing. For example, the Integrated Breeding Platform (http://www.integrated-breeding.net) describes itself as a "Web-based one-stop shop for information, analytical tools and related services to design and carry out integrated breeding projects" with the aim to "boost crop productivity and resilience for smallholders in drought-prone environments by exploiting the economies of scale afforded by collective access to cutting-edge breeding technologies and information." The platform was coordinated by the CGIAR Generation Challenge Program (http://www.generationcp.org) as its service delivery components. It includes information sources such as germplasm and trait databases and provides advice and capacity building for developing country breeders on breeding strategies, especially those involving molecular breeding.

In 2011–2012, a number of new CGIAR Research Programs were initiated to align the research of the 15 CGIAR Research Centers and their partners into efficient, coherent, multidisciplinary programs. Of the programs, the CGIAR research programs on wheat, maize, rice, roots, tubers and bananas, grain legumes

and dryland cereals all have major components that involve the development of climate-resilient crops, in most cases with a significant dependence on molecular techniques (http://cgiarfund/cgiarfund/crp). The overarching CGIAR research program on Climate Change and Food Security has also developed a global database of crop trials that address climate-resilience issues (http://www.agtrials.org).

11.4.3 Philanthropic Funds and Donors

The inaugural Asian biotechnology network for crop development was the Rockefeller Foundation Rice Biotechnology Network, followed by the related Asian Rice Biotechnology Network. Between them, the programs ran from 1984 until 2002. The Rockefeller Foundation Drought Tolerance Research program in China, India, and Thailand (Pray et al. 2011) had a more direct focus on climate resilience in rice breeding. The initial emphasis was drought tolerance and it was soon recognized that this was a much more difficult trait to improve than disease resistances; the program therefore employed a number of approaches including marker-assisted selection and transgenics in rice improvement. A 2011 analysis of program achievements revealed that progress had been slow, and the modest improvements in yield that had been able to be demonstrated were only shown thus far on experimental stations rather than in farmers' fields.

The Asian Maize Biotechnology Network (AMBIONET) ran from 1998 to 2005, and involved CIMMYT and research organizations from China, India, and several Southeast Asian countries. Supported by the Asian Development Bank, CIMMYT and USAID, it aimed to strengthen the capacity of the participating scientists and institutions in the development of maize varieties that were higher yielding, disease resistant, and tolerant to abiotic stresses. After 7 years, progress was made in development of lines with greater disease resistance but less progress was made with drought tolerance, again indicating the greater complexity of this challenge.

While not being a central focus, the development of climate-resilient germplasm also forms a part of a number of other agricultural and environmental research and development networks. For example, the Forum for Agricultural Research in Africa (FARA, http://www.fara-africa.org), provides networking support functions to enhance access to knowledge and technologies, strengthen capacities and facilitate development of partnerships and strategic alliances. With Japanese government (JICA) funding, the AGRA (Alliance for a Green Revolution in Africa, http://www.agra-alliance.org) has supported a new rice network. The fact that rice consumption has been expanding in recent years in Africa but that this demand has been filled by increasing exports has motivated the formation of this network and others such as the Coalition for African Rice Development. With the majority of rice in Africa being produced under rainfed conditions, development and dissemination of high-yielding drought-tolerant varieties remains critical.

Cassava is widely utilized for both food, feed and starch production, and is often grown under dryland conditions on poor soils. The Global Cassava Partnership for

the 21st Century (GCP21, http://www.danforthcenter.org/GCP21) is an international alliance of research institutions dedicated to increasing research and research funding on cassava, and to transferring modern technologies to enable crop improvement breakthroughs. As an informal organization it fosters rather than funds collaboration, and supports information exchange. A major focus of their recent efforts has been on cassava improvement to counter climatic variability (Hyman et al. 2012).

Although a number of donors support agricultural research and several have fostered collaborative partnerships, one donor, The Australian Centre for International Agricultural Research (ACIAR, http://www.aciar.gov.au), which is part of that government's development assistance program, has a specific focus on facilitating and funding collaborative agricultural research partnerships, usually between Australian and/or CGIAR center scientists and research collaborators in developing countries. In many cases the projects and program build networks rather than simply bilateral partnerships.

Several of ACIAR's programs support networks that use genomics to address development and selection of climate-resilient crops. "Improving the adaptive capacity to climate change of rice production systems in the Mekong Delta Region" is a partnership between the IRRI, Vietnamese and Australian research centers, local authorities and farmers. The $20 million, 4-year Sustainable Intensification of Maize–Legume Farming Systems in eastern and southern Africa program, is managed by CIMMYT in collaboration with the University of Queensland, Murdoch University and African and Australian research agencies. The Indo-Australian program on marker-assisted wheat breeding is a co-funded program using marker-assisted selection for faster development of wheat germplasm for farmers. Several of the program's activities address climate resilience, such as water use efficiency through root architecture and crop establishment, waterlogging, micronutrient stresses, sodic soils. It is planned to extend these gains in bioinformatics to allele mining and functional genomics.

11.4.4 Some Developed Country Networks

Research at the Australian Center for Plant Functional Genomics (ACPFG, http://www.acpfg.com.au) focuses on the identification of genes and gene networks efficiency underpinning a range of abiotic stress-tolerance traits, in Australia's two most important field crops, wheat and barley. This includes work to improve water use efficiency and enhance responses to drought, salinity, and nutrient toxicities. It has been established as a network, with a central node at the University of Adelaide with research nodes at the Universities of South Australia, Queensland, and Melbourne. Once again, the long-term nature of investment has been important in the Center's success—it was established in 2002. Recently, the center has formed a commercial alliance with DowAgrosciences to undertake collaborative research and licence new traits for use in crop improvement. This also builds on other

international collaborations with INRA (France), CIMMYT, ICARDA, and international universities.

The main research approaches of the ACPFG are to identify genetic mechanisms that control tolerance to specific stresses (such as drought tolerance in wheat, Fleury et al. 2010) and compare these with mechanisms that control broad range tolerance to abiotic stresses. Genome-wide analyses to define key cellular processes (including regulatory networks) that enable adapted plants to withstand abiotic stress are undertaken. Ways of manipulating these networks are then identified, through existing genetic diversity or through functional genomics technologies, to deliver new, better adapted germplasm. Recently, ACPFG, in collaboration with CSIRO, identified an ancestral sodium transporter gene that when present in near-isogenic lines, significantly improved wheat yields on saline soil (Munns et al. 2012).

11.4.5 Sharing of Genomics Resources: International Climate-Resilient Crop Genomics Consortium (http://www.climatechangegenomics.org)

The application of genomics to tackle the challenge of increased climate resilience was behind the recent formation of an International Climate-Resilient Crop Genomics Consortium (ICRCGC).

Through collaboration, genomics-based breeding and transgenic approaches should result in a better understanding of crop performance in a changing climate while supporting crop improvement programs. The Consortium will provide better coordination of international research efforts to better define and faster advance the priority objectives.

11.5 Delivery Innovations and Public–Private Dissemination Efforts

11.5.1 Bundled Innovations

On the basis of the learning challenges described in Lybbert and Bell (2010), there are several innovations that might serve to facilitate adoption of climate-resilient crops. Germplasm innovations that confer benefits that are not "state-contingent" may be particularly important. Generalized gains in water use efficiency and early maturation, for example, can confer relative benefits across a broader range of climate stresses and are therefore easier for farmers to appreciate and discern.

11.5.2 Facilitating State-Contingent Learning

Innovative extension systems in both the public and private sector and in hybrid public–private systems can facilitate the diffusion of climate-resilient crops by facilitating how farmers learn about state-contingent benefits. Local demonstration plots and farmer field days may need to be augmented in specific ways to enable farmers to learn from experiences of nearby farmers who faced different climate stresses in a particular growing season. In this sense, creative extension agents might leverage the natural agroecological and climatic heterogeneity in a region to ensure that climate stresses isolated to one part of the region present a learning opportunity for farmers throughout the region. While some of this learning happens naturally in some settings through social networks, there is likely to be more that extension systems could do to facilitate this kind of spatial learning by enabling farmers to share experiences across a wider set of fellow farmers.

11.5.3 Public–Private Collaboration

In many developing country settings, state-contingent relative benefits make seed pricing particularly important. If climate-resilient crops are priced at a premium, farmers—especially poor farmers—will be much less likely to experiment and learn about any benefits they might confer. This raises an important opportunity for upstream collaboration of private and public sector research, in particular, collaboration that involves the segmentation of profitable and humanitarian markets with royalty-free licensing agreements (Lybbert 2002) might help keep prices down. Fortunately, these kinds of collaborations are already operative in the case of drought tolerance in Africa.

For example, the Drought Tolerant Maize for Africa (DTMA, http://dtma.cimmyt.org/) project—which is led by CIMMYT and IITA and funded by a mix of private foundations and official development agencies—coordinates public research and the private sector to develop and disseminate drought-tolerant maize varieties and hybrids in 13 African countries. The other major drought-tolerance initiative in Africa—the Water Efficient Maize for Africa (WEMA) project, which is led by the African Agricultural Technology Foundation (AATF) and involves a partnership with Monsanto—shares these broad objectives but embraces marker-assisted breeding and biotechnology as well as conventional breeding techniques.

Experiences from these existing collaborating initiatives should inform future research collaborations. These research collaborations will almost surely generate important delivery and extension benefits, which will be needed to successfully meet the challenges of disseminating climate-resilient crops to vulnerable farmers.

11.6 Conclusions and Prospects

11.6.1 Challenges with Networks

While this chapter describes the achievements of several successful networks, it is clear that the potential for use of networks in breeding for climate resilience has not been achieved in many cases. There is still inadequate characterization of the production constraints and environments in the most stressed areas as they are almost always the most poorly resourced and the geographic coverage of field trials that aim to assess the performance of climate-resilient crops is typically limited.

From an agronomic perspective, networks that are funded for the short term (3–5 years) can only provide very limited information on long-term performance and climate resilience of crops as they usually only provide 3 years of trial data at best—so practice impacts can be limited. There remains the need to have mechanisms that can provide for long-term networking on assessing and mapping yield gaps and that provide systematic analysis of the adaptation of genotypes. While molecular breeding approaches are widely used in developed countries and the private sector, usage in developing countries has been constrained through the shortage of trained scientists, poor infrastructure for high-throughput screening, limited access to information management systems and constraints in the translation of the results from molecular breeding to field germplasm development programs (Delannay et al. 2012).

The development of abiotic stress resistance and climate-resilient crops has also proven much more challenging than breeding for other characteristics such as disease resistance or processing quality, largely because of the genetic complexity of plant response to stresses (multiple gene involvement) and the strong interactions with other stress responses, such as those to biotic stresses (Atkinson and Urwin 2012). Many of the collaborations, especially those with developing countries have had more tangible achievements in capacity development of the scientists involved rather than production of new varieties.

The private sector has emerged as a powerful player in the development of climate-resilient crops. Monsanto's DroughtGard™ maize is expected to be released in 2013 as the first commercially available transgenic drought-tolerant crop. Hybrid seed sold under this trademark will contain a novel transgenic trait based on the bacterial *cspB* gene for "cold shock protein B" (CSPB) from *Bacillis subtilis*. CSPB act as an RNA chaperone, and is thought to help maintain normal physiological performance during stress events by binding and unfolding tangled RNA molecules so that they can function normally. Dupont, Syngenta, and Pioneer HiBred are also investing in climate-resilient re-engineered maize, but their significant resources enable these companies to undertake much of the development of these crops in-house. However, there have been some notable collaborations involving these companies and public sector partners. For example, the USDA SNP project involves these companies, USDA and international public sector partners in the analysis of wheat genetic variation, both for disease resistance and

stress tolerance. In 2012, USAID provided new funding for the development of climate-resilient cereals using advanced molecular tools, through private public alliances under the "Feed the future" initiative (http://www.feedthefuture.gov).

Although it is tempting to frame the research challenges associated with climate-resilient crops as purely technical in nature, it is critical that the research community take "downstream" adoption, extension and diffusion considerations seriously even while these technical challenges are being addressed. Ignoring these behavioral and contextual considerations and proceeding with a "build it and they will come" mentality will produce disappointing results. While this is true of technocentric approaches in general, nuances of climate-resilient crops—often the same ones that make "upstream" research more challenging—make these considerations particularly relevant in the case of these technologies.

In this chapter, we have made some suggestions for how the research and extension communities might address these adoption challenges. Of these suggestions, one is worth reiterating here: The collaborative research networks we propose should be explicitly informed by "downstream" adoption and diffusion challenges and should tap into social science research communities focused on these important perspectives.

11.6.2 Challenges for Funding Agencies

The nature of public sector research, including that for the development of climate-resilient crops, is that the extent of formation of and effort through collaborative networks is very dependent on external funding. While there have been some notable exceptions, and the focus on climate variability and change over the last decade has increased the priority of climate resilience in the eyes of many funding bodies, the high risk nature of funding work to address what are usually complex and multigene characters presents a challenge. Funding climate-resilient crop development is also a long-term commitment, and even when crop varieties with established climate resilience are available to farmers, depending on the traits, the uptake of these crops can be mixed. Often, seed companies will promote—and farmers will grow—the varieties that produce the highest yields under favorable conditions. Few farmers plant their crops expecting a drought, and unfortunately some of the drought-tolerant varieties that have been developed are not the highest yielding under favorable conditions.

"Donor badging" also presents a challenge in supporting developing country partnerships. It is difficult to get other donors to contribute funding to a network that has been initiated by another donor. But the new CGIAR Donor Fund and Consortium, which has established large CGIAR research program networks, is intended to get around this challenge.

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Chapter 12
Climate Change and Intellectual Property: Regulatory Issues

Michael Blakeney

Abstract The international IPR regime based upon the World Trade Organization (WTO) Agreement on Trade-related Aspects of Intellectual Property Rights (TRIPS Agreement) establishes a global intellectual property (IP) regime, which obliges states to provide legal protection among other things for newly developed plant varieties and patents for inventions. This IP regime facilitates the patenting of stress-tolerant DNA in the 157 member states of the WTO. As a matter of practice, most of this patenting is confined to a relatively small group of life-sciences companies. This is resulting in a market concentration, which has important agricultural policy implications particularly for developing countries. At the same time, plant breeding methods are becoming the subject of patent protection, whereas formerly this was exclusively the subject of laws based on the international convention dealing with plant variety protection (UPOV Convention). The germplasm from which useful biological material is extracted either for patenting or the development of new plant varieties is also the subject of an evolving international regime dealing with authorized access and benefit sharing.

This chapter analyzes these issues, concluding that the impact of IP rights upon food security is becoming as significant as its impact upon access to medicines.

12.1 Introduction

Climate change is imposing significant stresses upon agriculture at a time when more food is required for an increasing world population. The breeding of stress-resistant crops or their genetic engineering are possible responses to these difficulties. Intellectual Property Rights (IPRs) have been identified as a means for incentivizing agricultural innovations. The creation of plant patents and plant

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breeders' rights dates back to the beginning of the twentieth century when Carl Correns rediscovered Mendel's plant breeding experiments. As early as in 1906 a Bill was introduced into Congress providing for the protection of plant patents. The later development of recombinant DNA technology provided the technological basis for the patenting of DNA. Climate change concerns has led to the identification and patenting of stress-tolerant genes.

The global commercial significance of climate technologies is assured by the global reach of the international intellectual property (IP) regime. Members of the World Trade Organization (WTO), which include some 153 countries as of February 10, 2011, are obliged to introduce IP laws, which implement the norms prescribed by the WTO Agreement on Trade-Related Aspects of Intellectual Property Rights ("TRIPS Agreement"). Key provisions in the present context are Article 23.1, which provides that "patents shall be available for any inventions, whether products or processes, in all fields of technology, provided that they are new, involve an inventive step and are capable of industrial application." This provision requires also that "patents shall be available and patent rights enjoyable without discrimination as to the . . . field of technology." The effect of this provision is to establish a patenting regime, which extends to all WTO Members. Additionally, Article 27.3(b) of the TRIPS Agreement requires that WTO Members "shall provide for the protection of plant varieties either by patents or by an effective *sui generis* system or by any combination thereof."

Although the TRIPS Agreement does not prescribe a *sui generis* system for the protection of plant varieties, most countries have adopted the 1991 version of the International Convention for the Protection of New Varieties of Plants (UPOV). Thus to January 15, 2011 the UPOV Convention has 71 signatories, with 41 of those joining after January 1, 1995. One of the reasons why countries have tended to adopt UPOV 1991, rather than to craft a *sui generis* alternative, is that the IPR chapters in the free trade agreements ("FTAs") signed since the 1990s by the USA and the EU with their various bilateral partners includes the obligation to subscribe to the 1991 version of UPOV. UPOV provides for the protection of new plant varieties, which are "distinct," "uniform" and "stable." Excepted from protection under the 1978 version of UPOV, was propagating material, which had been harvested by farmers and retained for further planting or for sale. Article 15(2) of the 1991 version of the UPOV Convention confined this seed saving exception to the use of saved material for propagating purposes on farmers' own holdings and in reasonable quantities. UPOV 1991 also permits the use of protected varieties for the purpose of breeding new varieties. As is indicated below, where a new variety can be patented, the seed saving and breeding exceptions become irrelevant.

12.2 Patenting of DNA

The modern biotechnological revolution has enabled the engineering of desirable genetic traits from useful local species. These include (1) pest control traits such as insect, virus, and nematode resistance as well as herbicide tolerance; postharvest traits such as delayed ripening of spoilage prone fruits; (2) agronomic traits such as nitrogen fixation and utilization, restricted branching, environmental stress tolerance, male and/or seed sterility for hybrid systems; and (3) output traits such as plant color and vitamin enrichment. The production of transgenic plants has become possible through the development of a number of enabling and transformation technologies.

A key issue around the patenting of genetic resources (GR) was whether a DNA sequence could be characterized as an "invention." In the early history of patent law an invention was thought to involve some kind of technical innovation and a distinction was drawn between patentable inventions and nonpatentable discoveries. The US Supreme Court in its 1980 determination, *Diamond v. Chakrabarty* (447 US 303 (1980)) held in a 4:3 majority decision that a bacterium genetically engineered to degrade crude oil was an invention. This decision provided the legal underpinning for the US biotechnology industry. The European Parliament's belated response in 1998 was its Biotechnology Directive which provided in Article 3.2 that "biological material which is isolated from its natural environment or produced by means of a technical process is deemed to be an invention even if this material previously occurred in nature."

The patentability of genetic materials and gene fragments, such as expressed sequence tags (ESTs) and single nucleotide polymorphisms (SNPs), as well as enabling gene-based technologies led to what has been described as a "genomic gold rush" in the 1990s as vast numbers of gene-based patent applications were filed, particularly in the USA (Joly 2003). Significant misgivings were expressed by numerous commentators. Probably the most influential among these were Heller and Eisenberg (1998) who suggested that genetic research tool patents could create a "tragedy of the anticommons" in which multiple patent owners would tie-up genetic materials in a thicket of IP patent rights. This was perceived by Correa (2009) to be a particular problem for the genetic improvement of crops since this is an incremental process and each new patent would constrain the "freedom to operate" particularly of public agricultural research institutes.

Arguably, this gold rush was brought to an end, at least in the US, by 2005 in the *In* re *Fisher* decision of the US Court Of Appeals for the Federal Circuit (421 F.3d 1365 (Fed. Cir. 2005)), which upheld a ruling by the US Board of Patent Appeals and Interferences refusing to allow patent applications made on behalf of Monsanto Co on five ESTs encoding protein and protein fragments in maize plants grown by the Asgrow Seed Company of Des Moines, Iowa. Joly (2006) suggested that that the *Fisher* case was used by Monsanto Co, a significant downstream user of research tools, to urge upon the court a higher patentability standard in order to eliminate the thousands of research tool patents which were cluttering research

efforts. Mainly for this reason, the case attracted amicus briefs filed by academic institutions as well as major biotechnology and pharmaceutical companies. The Board of Patent Appeals and Interferences was unable to identify any "substantial utility" or usefulness in the application for patentability of the ESTs. The Appeal Court agreed with this approach stating that that claimed inventions "ought to have a specific and substantial utility" to satisfy the requirements of the US patent statute. The Court observed that the application comprised asserted uses based upon "merely hypothetical possibilities," which had not yet been achieved in the real world. As the applicant did not identify the function for the underlying protein-encoding genes, the Court held that "the claimed ESTs have not been researched and understood to the point of providing an immediate, well-defined, real world benefit to the public meriting the grant of a patent."

Although this decision imposed a higher patent standard, which might result in the invalidation of previously granted patents over research tools, this was not specifically addressed by the Court. However, Joly (2006) optimistically suggested that "academic researchers, as well as a considerable portion of the biotechnology and pharmaceutical industry will be satisfied by this judgment as it should reduce the number of parasite patents on gene sequences, in the United States."

Two recent US cases have raised the very question of the patentability of genetic material. In *Association for Molecular Pathology v. USPTO* (94 USPQ2d 1683 (S. D.N.Y. March 29, 2010)) a Judge of the United States District Court for the Southern District of New York delivered a summary judgment which invalidated patents related to the BRCA 1 and 2 breast and ovarian cancer susceptibility genes, which had been held by the company Myriad Genetics. He ruled that the claims to DNA sequences in isolation were held to be insufficiently distinct from naturally occurring genes in the body and were thus products of nature rather than inventions. He observed that DNA represents the physical embodiment of biological information, distinct in its essential characteristics from any other chemical found in nature and that DNA in an "isolated" form alters neither this fundamental quality as it exists in the body not the information it encodes.

This decision was successfully appealed to the US Court of Appeals for the Federal Circuit (CAFC) in Washington, DC, which published its decision in August 2011. The Appeal Court considered that the District Court, Judge had fallen into error in considering not whether the isolated DNAs were markedly different from naturally occurring DNAs, but rather whether they had the same informational content as native DNA sequences. Nevertheless, the CAFC considered that the District Court was correct in holding that Myriad's claims directed to comparing and analyzing gene sequences were not patentable, as these claims contained no transformative steps and covered only patent ineligible abstract steps.

This reasoning was considered recently by the US Supreme Court in *Mayo Collaborative Services v. Prometheus Laboratories, Inc.* No. 10–1150. Decided March 20, 2012) which concerned patents obtained by Prometheus which instructed doctors in the use of thiopurine drugs to treat autoimmune diseases. Mayo had developed its own diagnostic test which Prometheus claimed infringed its patents. Justice Breyer, delivering the opinion of the Court, noted the long held view of the

Supreme Court that laws of nature, natural phenomena, and abstract ideas are not patentable. He quoted from the Court's decision in *Diamond v. Chakrabarty* that "a new mineral discovered in the earth or a new plant found in the wild is not patentable subject matter." (on p. 309). The Court held that Prometheus' process was not patent eligible because the laws of nature recited by Prometheus' patent claims, i.e., the relationships between concentrations of certain metabolites in the blood and the likelihood that a thiopurine drug dosage will prove ineffective or cause harm, were not themselves patentable.

The opponents of the patents in the Myriad Genetics litigation have claimed that the Prometheus decision calls into question the Appeal Court decision in that case whereas the supporters of the Appeal Court draw a distinction between the method claims in that case and the composition-of-matter claims in the Prometheus suit (Frankel 2012). In any event on March 26, 2012 the Supreme Court remanded to Appeal to it in the Myriad Genetics litigation to the Court of Appeals for the Federal Circuit for further consideration in light of its decision in Prometheus.

12.3 DNA Patenting and Agriculture

The cultivation by farmers of genetically modified (GM) crops has on occasion led to IP liability, where GM seed is patented and the cultivation of that seed by the patentee is unauthorized. The cases are divided between those where farmers knowingly cultivate patented GM seed and those where the cultivation of patented seed is apparently inadvertent, for example, where crops are apparently pollinated by wind or insect-borne pollen.

An example of the first category of case is *Monsanto Co. v. Scruggs* (342 F. Supp 2d 584 (2004)) which concerned Monsanto's patented Roundup Ready ("RuR") glyphosate-tolerant seeds. This was licensed to seed companies, who were obliged to sell the seed to growers who signed technology license agreements acknowledging Monsanto's patent and on condition that they could only be used by growers for a single commercial crop, i.e., growers could not save seed produced from a harvested crop for replanting during the following growing season. Scruggs, who had not signed a technology licensing agreement, purchased a small quantity of RuR soybeans and cotton seeds, which were cultivated and from which he saved seed for further plantings. The Court decided that Monsanto's patent had been infringed by Scruggs, rejecting his defense that neither Monsanto's biotechnology nor the plants in their fields were covered by the patent and that the first sale of the seed embodying the invention exhausted the patent rights of Monsanto. The Court noted that Monsanto never made an unrestricted sale of its seed technology, as it licensed its technology to seed companies with a proviso: subsequent sales of seed containing its transgenic trait must be limited to growers who obtained a license from Monsanto and for only a single growing season.

A recent variant of these facts occurred in *Monsanto Co v. Bowman* No. 10–1068, Fed. Circuit, September 21, 2011), where a farmer, Bowman,

purchased commodity seeds from a local grain elevator which were not subject to a technology agreement. Following the application of glyphosate to the crops grown from these seeds, Bowman identified those which were glyphosate resistant and these were saved and re-planted in subsequent years, which enabled Bowman to use glyphosate-based herbicide. Monsanto filed a patent infringement claim against Bowman and in September 2009, the district court in Indiana granted summary judgment on patent infringement for Monsanto. Bowman appealed to the Court of Appeals for the Federal Circuit. Bowman argued that Monsanto's patent rights were exhausted under the first sale doctrine in relation to all second-generation Roundup Ready soybean seeds that were present in the grain elevators. He cited the 2008 Supreme Court case of *Quanta Computer, Inc. v. LG Electronics, Inc.* (553 U.S. 617) (2008). In this case the Supreme Court held that sales of products that "substantially embody" the disputed patents will also be considered sales that exhaust the patent right. Bowman argued that the court should hold that subsequent generations of the seeds are "substantial embodiments" of the first-generation seeds, and thus the sales of these seeds would be exhausting sales. The appeal Court held that even if Monsanto's patent rights in the commodity seeds were exhausted, such a conclusion would be of no consequence because once a grower, like Bowman, planted the commodity seeds containing Monsanto's RuR technology and the next generation of seed developed, the grower had created a newly infringing article. It observed that "The fact that a patented technology can replicate itself does not give a purchaser the right to use replicated copies of the technology. Applying the first sale doctrine to subsequent generations of self-replicating technology would eviscerate the rights of the patent holder."

A case of apparently inadvertent infringement is illustrated by the Canadian litigation between Monsanto Canada, Inc and a farmer, Percy Schmeiser. Schmeiser grew canola commercially in Saskatchewan. He had never purchased Monsanto's patented RuR Canola nor did he obtain a license to plant it. Yet, in 1998, tests revealed that 95–98 % of his 1,000 acres of canola crop was made up of RuR plants. The origin of the plants is unclear. They may have been derived from RuR seed that blew onto or near Schmeiser's land. Monsanto brought an action for patent infringement. In finding patent infringement the trial judge ruled that the growth of the seed, reproducing the patented gene and cell, and sale of the harvested crop constituted taking the essence of Monsanto's invention, using it, without permission and in so doing infringed the patent. By a majority of 5:4 the Federal Court of Appeal ruled that Schmeiser's saving and planting seed, then harvesting and selling plants that contained the patented cells and genes appeared to the Court, on a common sense view, to constitute "utilization" of the patented material for production and advantage, within the meaning of s. 42 of the Canadian *Patent Act* (*Monsanto Canada, Inc. v. Schmeiser.* [2004] 1 S.C.R. 902, 2004 SCC 34). The argument that the infringing seed had merely grown, as the result of wind pollination or through the pollinating activities of birds and bees was rejected by the majority Judges as denying "the realities of modern agriculture." What was at stake in this case was sowing and cultivation, "which necessarily involves deliberate and careful activity on the part of the farmer." They noted that he had actively cultivated

RuR Canola as part of his business operations, thus in light of all of the relevant considerations, Schmeiser had used the patented genes and cells, and infringement was established.

12.4 Patenting of Stress-Tolerant Genes

Somvanshi in a 2008 study identified 30 patents relating to drought-tolerant genes (Somvanshi 2009). These included (1) patents related to proline biosynthesis; (2) patented dehydration responsive element binding factors (DREB) and C-repeat sequences binding factors (CBF); (3) patents related to protein kinases; (4) various patents awarded for transcription factors involved in improving drought stress tolerance in plants, and (5) patents related to miscellaneous drought-tolerance genes. A 2008 study by the ETC. Group identified 55 patent "families" or related patent applications and/or issued patents published in more than one country or patent office (ETC. 2008). A total of 532 patent documents were identified, which represented applications to patent offices by a group of biotechnology companies on so-called "climate-ready" genes around the world.

Its 2010 update of this study "examined patents containing claims concerned with abiotic stress tolerance (i.e. traits related to environmental stress, such as drought, salinity, heat, cold, chilling, freezing, nutrient levels, high light intensity, ozone and anaerobic stresses)" (ETC. 2010). It noted "a dramatic upsurge in the number of patents published (both applications and issued patents) related to 'climate-ready' genetically engineered crops from June 30, 2008 to June 30, 2010, identifying 262 patent families and 1,663 patent documents" (ETC. 2010, Appendix A).

The 2008 ETC. report was subjected to a close analysis by Dr. Carol Nottenburg (2009), the Principal of a US Patent firm and it is useful to examine the claims and counter-claims to identify the significant elements of the debate about the patenting of stress-tolerant genes, as her comments are equally applicable to the 2010 ETC. report. The ETC. report stated that the so-called "Gene Giants," exemplified by BASF, Bayer, DuPont, Monsanto, and Syngenta "are staking sweeping patent claims on genes related to environmental stresses" in patent offices around the world. Dr. Nottenburg points out that the patenting of gene sequences is not permitted in a number of developing countries, including Andean countries and an examination of the patents, which are identified in the 2008 report have been sought in Argentina, Brazil, and China, leaving more than 200 countries "in which these patent applications will never be pertinent." A similar argument was advanced by Attaran and Gillespie-White (2001), that patents did not stand in the way of access to HIV antiretrovirals in most African countries, but the political impact of the patents in a few of those countries, far outweighed their practical significance and brought about the first amendment of the TRIPS Agreement (Hestermeyer 2007).

Dr. Nottenburg also pointed out that the number of patent families is the better indicator of the incidence of the patenting of stress-tolerant genes, than patent

filings. This is certainly the case, as a number of filings are duplicated in different countries. The 2010 report identifies some 262 patent families, which is a considerable advance on the 55 identified in the 2008 report. However, it should be noted that even a small number of patent families can have a considerable political impact. For example, if the number of biopiracy incidents was totaled, they would probably not exceed around 20 causes celebres.

The 2008 report is critical of over-broad patent claims, but Dr. Nottenburg considers this to be a matter dictated by the "eye of the beholder" and in one case involved an error in the published patent document. She concludes that "visions of gene-grabbing and holding farmers hostage are unwarranted." A particular problem had been that patent applicants had been allowed to make bulk claims in relation to genetic material of which the use had not yet been identified. However, the 2010 report concedes that in 2001 the USPTO put a brake on "bulk claims" by issuing new guidelines requiring that claimed inventions must have "well-established" utility and in 2007 the USPTO limited bulk claims by notifying its patent examiners that they have the option of restricting claims to only a single nucleotide sequence in each patent application.

The 2010 report of the ETC. contrasts the ownership of 9 % patent families by public sector institutions (9 % of the total) with the private sector which holds 91 % of the total. As is the case with biotechnological patenting generally, proprietary biotechnologies are concentrated in the same few corporations (see also Lesser 1998). The 2010 report points out that "just three companies – DuPont, BASF, Monsanto – account for two-thirds (173 % or 66 %) of the total." This level of market concentration gives cause for concern for those who espouse the positive role of competition.

In addition to the possible adverse impacts this market concentration might have upon the vigor of competition, the market dominance of these private corporations also has an important influence upon the sort of biotechnological research, which is undertaken. For example, to what extent will the dominance of private corporations in biomedical and agricultural research direct that research towards Northern concerns away from Southern food priorities (Alston et al. 1998). It has been estimated that only 1 % of research and development budgets of multinational corporations is spent on crops of interest that could be useful in the developing world (Pingali and Traxler 2002). Almost entirely neglected by these corporations are the five most important crops of the poorest, arid countries—sorghum, millet, pigeon pea, chickpea, and groundnut (Human Rights Council 2008).

12.5 Patenting of Plant Varieties

The development of new plant varieties is protectable in most countries as a species of intellectual property right (IPR) derived from the International Convention for the Protection of New Varieties of Plants (UPOV). Countries which are members of the World Trade Organization (WTO) are obliged by Article 27.3(b) of the WTO

Agreement on Trade Related Aspects of Intellectual Property Rights (TRIPS) to "provide for the protection of plant varieties either by patents or by an effective *sui generis* system or by any combination thereof." The TRIPS Agreement does not specify which "*sui generis* system" will meet its requirements, but most of the Members of the WTO have promulgated domestic legislation based upon the 1991 version of UPOV.

UPOV allows the protection of new varieties of plants which are distinct, uniform, and stable. A variety is considered to be new if it has not been commercialized for more than one year in the country of protection. A variety is distinct if it differs from all other known varieties by one or more important botanical characteristics. A variety is uniform if the plant characteristics are consistent from plant to plant within the variety. A variety is stable if the plant characteristics are genetically fixed and therefore remain the same from generation to generation, or after a cycle of reproduction in the case of hybrid varieties. The 1991 version of UPOV recognizes the right of breeders to use protected varieties to create new varieties. However, this exception is itself restricted to such new varieties as are not "essentially derived" from protected varieties. The drafters added this restriction to prevent second-generation breeders from making merely cosmetic changes to existing varieties in order to claim protection for a new variety. From the perspective of farmers, probably the most contentious aspect of the 1991 Act is the limitation of the farmers' privilege to save seed for propagating the product of the harvest they obtained by planting a protected variety "on their own holdings," "within reasonable limits and subject to the safeguarding of the legitimate interests of the breeder." Earlier versions of UPOV permitted farmers to sell or exchange seeds with other farmers for propagating purposes.

The seed saving privilege and the permitted development of nonessentially derived new varieties from protected material were compromises built in to the legislation to take account of public policy concerns. It was appreciated that permitting individuals to privatize food varieties might compromise food security if breeding material was locked up and if farmers were prevented from saving seed for further harvests. However, from the perspective of plant breeders any derivation of new varieties from their protected varieties, whether essential or nonessential, was inconvenient for them and any seed saving by farmers deprived them of new sales. Consequently, they looked to patents law, which does not contain these exceptions, to protect their new varieties.

Plant varieties can be protected in the US under a system of plant patents, under a system of utility patents, or under the Plant Variety Protection Act (PVPA). The Plant Patent Act makes available patent protection to new varieties of asexually reproduced plants. Under this scheme a plant variety must be novel and distinct and the invention, discovery, or reproduction of the plant variety must not be obvious. One of the disadvantages of the scheme is that only one claim, covering the plant variety, is permitted in each application. The Federal Circuit Court of Appeals resolved any potential conflict between patent protection and protection under the Plant Variety Protection Act (PVPA) in its decision in *Pioneer Hi-Bred*

International Inc. v. J.E.M. Ag Supply Inc. (200 F.3d 1374 (Fed. Cir. 2000), *cert. granted,* 148 L. Ed. 2d 954 (2001)).

Pioneer's patents covered the manufacture, use, sale, and offer for sale of the company's inbred and hybrid corn seed products as well as certificates of protection under the Plant Variety Protection Act for the same seed-produced varieties of corn. The defendants argued that the enactment of the Plant Variety Protection Act had removed seed-produced plants from the realm of patentable subject matter under the Patents Act. The Federal Circuit rejected this argument noting that the Supreme Court held that "when two statutes are capable of co-existence, it is the duty of the courts . . . to regard each as effective."

This was illustrated by *Monsanto Co. v. McFarling* (302 F.3d 1291 (Fed. Cir. 2002)) which concerned Monsanto's patent for glyphosate-tolerant plants, the genetically modified seeds for such plants, the specific modified genes, and the method of producing the genetically modified plants. Monsanto required that sellers of the patented seeds obtained from purchasers a "Technology Agreement," in which they agreed that the seeds were to be used "for planting a commercial crop only in a single season," that the purchaser would not "save any crop produced from this seed for replanting, or supply saved seeds to anyone for replanting." Mr. McFarling, a farmer in Mississippi, purchased Roundup Ready soybean seed in 1997 and again in 1998; he signed the Technology Agreement. He saved 1,500 bushels of the patented soybeans from his harvest during one season, and instead of selling these soybeans as crop he planted them as seed in the next season. He repeated this activity in the following growing season. This saved seed retained the genetic modifications of the Roundup Ready seed. Mr. McFarling did not dispute that he violated the terms of the Technology Agreement but claimed that the contractual prohibition against using the patented seed to produce new seed for planting, when he produced only enough new seed for his own use the following season, violated the seed saving provision of the PVPA. The Court declined to limit the patent law by reference to the PVPA and Mr. McFarling was found to have infringed Monsanto's patent.

12.6 Patenting of Plant Breeding Methods

In addition to the patenting of the products of plant breeding, some patent laws allow for the patenting of plant breeding methods. For example, in the US a patent has been obtained for the "selective increase of the anticarcinogenic glucosinolates in brassica species" (US Patent 6,340,784, January 22, 2002) and an application published concerning a "method for breeding tomatoes having reduced water content" (US Patent Application 20100095393, April 15, 2010). This raises the possibility that methods of crop breeding to withstanding climate stress can be privatized in the US, which permits so-called methods patents.

Conversely, in Europe the exclusion in its patent legislation of "essentially biological processes for the production of plants or animals" defined in Article

2.2 of the EU Biotechnology Directive as consisting "entirely of natural phenomena such as crossing or selection," resulted in the denial of patent protection for the same methods for breeding brassica and tomatoes (Blakeney 2012). The Board of Appeals of the European Patent Organization (EBA) observed that with the creation of new plant varieties, for which a special property right was going to be introduced under the subsequent UPOV Convention in 1960, the legislative architects of the European Patent Convention were concerned with excluding from patentability the kind of plant breeding processes which were the conventional methods for the breeding of plant varieties of that time. These conventional methods included in particular those based on the sexual crossing of plants deemed suitable for the purpose pursued and on the subsequent selection of the plants having the desired trait(s). These processes were characterized by the fact that the traits of the plants resulting from the crossing were determined by the underlying natural phenomenon of meiosis. On the one hand processes for changing the genome of plants by technical means such as irradiation were cited by the EBA as examples of patentable technical processes. On the other hand it pointed out that the provision of a technical step, be it explicit or implicit, in a process which is based on the sexual crossing of plants and on subsequent selection does not cause the claimed invention to escape the exclusion from patentability if that technical step only serves to perform the process steps of the breeding process.

Thus, if a process of sexual crossing and selection includes within it an additional step of a technical nature, which step by itself introduces a trait into the genome or modifies a trait in the genome of the plant produced, so that the introduction or modification of that trait is not the result of the mixing of the genes of the plants chosen for sexual crossing, then that process leaves the realm of plant breeding and consequently, is not excluded from patentability in Europe. This principle applies only where the additional step is performed within the steps of sexual crossing and selection, independently from the number of repetitions, otherwise the exclusion of sexual crossing and selection processes from patentability could be circumvented simply by adding steps which do not properly pertain to the crossing and selection process, being either upstream steps dealing with the preparation of the plant(s) to be crossed or downstream steps dealing with the further treatment of the plant resulting from the crossing and selection process. The EBA noted that for the previous or subsequent steps per se patent protection was available. This will be the case for genetic engineering techniques applied to plants which differ from conventional breeding techniques as they work primarily through the deliberate insertion and/or modification of one or more genes in a plant.

12.7 Patenting of Genetic Resources

One of the problems with determining the legal protection of genetic resources through IPRs or any other kind of law is the fact that scientific constructs do not sometimes lend themselves to legal categorization. For example, the TRIPS

Agreement in Article 27.3(b) provides that WTO Members may also exclude from patentability: "plants and animals other than micro-organisms, and essentially biological processes for the production of plants or animals other than nonbiological and microbiological processes." Adcock and Llewelyn (2000) observe that the division between plants and animals on the one hand and microorganisms on the other is not as scientifically certain as the legal categories seem to suggest. Additionally, a number of international organizations, with varying levels of scientific competence, are now concerning themselves with IPRs and genetic and biological resources. At its 16th session, held from May 3 to May 7, 2010, WIPO's Intergovernmental Committee on Intellectual Property and Genetic Resources, Traditional Knowledge and Folklore (IGC) Member States identified the need for a glossary to clarify the meanings of key terms related to genetic resources to facilitate the negotiations of the Committee (WIPO 2010a, b). The Secretariat prepared a document drawing, as far as possible, from previous glossaries of the IGC and from existing United Nations and other international instruments, also taking into account definitions and glossaries which can be found in national and regional laws and draft laws, multilateral instruments, other organizations and processes and in dictionaries.

The term "genetic resources" is defined in the glossary by reference to Article 2 of the Convention on Biological Diversity (CBD) which defines the term as "genetic material of actual or potential value." Further, it defines the term "genetic material" as meaning "any material of plant, animal, microbial or other origin containing functional units of heredity." "Plant genetic resources" are defined in Article 2 of the FAO International Treaty on Plant Genetic Resources for Food and Agriculture as "any material of plant origin, including reproductive and vegetative propagating material, containing functional units of heredity." Article 2 of the FAO International Code of Conduct for Plant Germplasm Collecting and Transfer defines plant genetic resources as "the reproductive or vegetative propagating materials of plants."

Article 2.1 (a) of the FAO International Undertaking on Plant Genetic Resources (1983) defines plant genetic resources as "the reproductive or vegetative propagating material of the following categories of plants (1) cultivated varieties (cultivars) in current use and newly developed varieties; (2) obsolete cultivars; (3) primitive cultivars (land races); (4) wild and weed species, near relatives of cultivated varieties; and (5) special genetic stocks (including elite and current breeders' line and mutants)."

Other legal instruments on IPRs do not use the term genetic resources and refer to "biological material." For example, the EU Directive on the legal protection of biotechnological inventions defines it as "material containing genetic information and capable of reproducing itself or being reproduced in a biological system."

12.8 Farmer's Rights

The concept of Farmers' Rights was developed as "a counterbalance to intellectual property rights" (IWG 2011). This was a moral commitment by the industrialized commitment to reward "the past present and future contributions of farmers in conserving, improving and making available plant genetic resources particularly those in centres of origin/diversity. Farmers' rights were intended to promote a more equitable relation between the providers and users of germplasm by creating a basis for farmers to share in the benefits derived from the germplasm, which they had developed and conserved over time" (Glowka 1998). The first international enactment of Farmers' Rights occurred in the FAO International Treaty on Plant Genetic Resources for Food and Agriculture (PGRFA). The preamble to the Treaty acknowledges that "the conservation, exploration, collection, characterization, evaluation and documentation of plant genetic resources for food and agriculture are essential in meeting the goals of the Rome Declaration on World Food Security and the World Food Summit Plan of Action and for sustainable agricultural development for this and future generations." It also acknowledges that PGFRA "are the raw material indispensable for crop genetic improvement" and affirms "that the past, present and future contributions of farmers in all regions of the world, particularly those in centres of origin and diversity, in conserving, improving and making available these resources, is the basis of Farmers' Rights."

The Preamble outlines that "fundamental to the realization of Farmers' Rights, as well as the promotion of Farmers' Rights at national and international levels" are the rights "to save, use, exchange and sell farm-saved seed and other propagating material, and to participate in decision-making regarding, and in the fair and equitable sharing of the benefits arising from, the use of plant genetic resources for food and agriculture."

Under Art. 5.1(c) the Contracting Parties agree, subject to national legislation, to promote or support, as appropriate, farmers and local communities' efforts to manage and conserve on-farm their plant genetic resources for food and agriculture and in Art. 51(d) to promote in situ conservation of wild crop relatives and wild plants for food production, by supporting, *inter alia*, the efforts of indigenous and local communities.

In Art. 9(1) of the Treaty the Contracting Parties "recognize the enormous contribution that the local and indigenous communities and farmers of all regions of the world, particularly those in the centres of origin and crop diversity, have made and will continue to make for the conservation and development of plant genetic resources which constitute the basis of food and agriculture production throughout the world."

Article 9.2 of the WTO International Treaty on PGRFA envisages that "the responsibility for realizing Farmers' Rights, as they relate to Plant Genetic Resources for Food and Agriculture, rests with national governments" and that national legislation should include measures relating to:

(a) Protection of traditional knowledge (TK) relevant to plant genetic resources for food and agriculture
(b) The right to equitably participate in sharing benefits arising from the utilization of plant genetic resources for food and agriculture
(c) The right to participate in making decisions, at the national level, on matters related to the conservation and sustainable use of plant genetic resources for food and agriculture

Finally, Article 9.3 provides that the Article shall not be interpreted "to limit any rights that farmers have to save, use, exchange and sell farm-saved seed/propagating material."

An assumption of Art. 9 is that the landraces used by traditional farmers are a dynamic genetic reservoir for the development of new varieties and for the transmission of desirable genetic traits. The traditional knowledge of local and indigenous communities is similarly perceived. Farmers in subsistence systems have tended to utilize a diverse selection of crop species in order to assure their annual harvests and thus to guarantee a minimal level of production and to prevent food shortage. Seed production in many instances has been based on the collection of and domestication of locally known, wild varieties. Modern agricultural practices depend on crop species that promote productivity and resistance to disease that can only be maintained with the continuous input of new germplasm. The diversity of landraces and the associated information on their specific qualities contribute invaluable information to formal breeding processes. It has been noted that the loss of biological diversity is paralleled by the loss of traditional knowledge. Where a plant variety becomes extinct, then the entire body of knowledge about its properties is condemned to irrelevancy.

As a means of remunerating these groups for their past contributions to the development of plant genetic resources for food and agriculture production, there can be little argument, except about the quantum and distribution of this remuneration. Inevitably, any calculation of the equitable share, which traditional farmers and indigenous communities might enjoy under a Farmers' Rights or Traditional Knowledge regime, will be arbitrary. However, the intellectual property system is no stranger to arbitrary calculations, thus the 20 year length of a patent term is intended to provide an opportunity for the compensation of all inventors, whatever the area of technology. Similarly the 25 years exclusivity which the UPOV Convention provides for new varieties of trees and vines, takes no account of variations in R&D costs between the different varieties.

The principal ways in which plant genetic resources are translated into food and agriculture production is through plant breeding and plant patenting. Standing at the heart of a Farmers' Rights regime is the concept of the equitable benefit sharing of benefits with farmers for their contribution to innovations in plant breeding and plant patenting.

Article 9.2 obliges the Contracting Parties to the Plant Genetic Resources Treaty "to take measures," subject to their national legislation to protect and promote Farmers' Rights. The content of these rights is defined in the balance of that

provision and embraces the protection of traditional knowledge, equitable benefit sharing, and the right to participate in decision making. The Treaty leaves open the legal context within which Farmers' Rights are to be enacted.

To date the only measure which has been implemented to provide for Farmers Rights is the International Fund for Plant Genetic Resources, which was envisaged in the Undertaking which preceded the Treaty. This Fund was to operate as a means of capacity building in the field of agricultural biotechnology in developing countries rather than as a reward to individual farmers or farming communities for their contribution to the development or improvement of plant varieties. To date this fund has not been established because funds were not made available by donor countries.

At its Third Session in Tunis in 2009, the Governing Body of the ITPGRFA adopted a resolution on Farmers' Rights (Resolution 6/2009), in which it requested the Secretariat to convene regional workshops on Farmers' Rights, subject to the agreed priorities of the Program of Work and Budget and to the availability of financial resources. The aim of the workshops was to discuss national experiences on the implementation of Farmers' Rights as set out in Article 9 of the International Treaty, involving, as appropriate, farmers' organizations and other stakeholders.

The fourth session of the Governing Body of the ITPGRFA held from March 14 to March 18, 2011 in Bali, Indonesia adopted a resolution on Farmers' Rights that, *inter alia*:

- Requests the Secretariat to convene regional workshops on Farmers' Rights, subject to availability of funding
- Encourages parties to submit views, experiences, and best practices on the implementation of Farmers' Rights
- Invites parties to consider convening national and local consultations on Farmers' Rights with the participation of farmers and other Stakeholders
- Requests the Secretariat to collect and submit these views, as well as reports from regional workshops to GB 5
- Encourages parties to engage farmers' organizations and relevant stakeholders in matters related to the conservation and sustainable use of PGRFA, through awareness raising and capacity building

12.9 Traditional Agricultural Knowledge

The traditional knowledge of indigenous peoples throughout the world has played an important role in identifying biological resources worthy of commercial exploitation. For example, the search for new pharmaceuticals from naturally occurring biological material has been guided by ethnobiological data (Balick et al. 1996; Kate and Laird 2000). Examples of traditional knowledge with an agricultural application include: "mental inventories of local biological resources, animal breeds, and local plant, crop, and tree species" as well as plants, which are

indicators of soil salinity, seed treatment and storage methods and tools used for planting and harvesting (Hansen and van Fleet 2007). A significant contribution can be made to developing crops, which are resistant to climate change by the knowledge of indigenous peoples and traditional farmers.

The economic value of biological diversity conserved by traditional farmers for agriculture is difficult to quantify. Correa (2000) has suggested that "the value of farmers' varieties is not directly dependent on their current use in conventional breeding, since the gene flow from landraces to privately marketed cultivars of major crops is very modest" because "conventional breeding increasingly focuses on crosses among elite materials from the breeders own collections and advanced lines developed in public institutions." Conversely, those collections and advanced breeding lines are often derived from germplasm contributed by traditional groups. An increasingly significant economic value of biodiversity is the extent to which it provides a reservoir of species available for domestication, as well as genetic resources available for the enhancement of domestic species. The modern biotechnological revolution has enabled the engineering of desirable genetic traits from useful local species. McNeely (2001) estimates that about 6.5 % of all genetic research undertaken in agriculture is focused upon germplasm derived from wild species and landraces.

Traditional knowledge is particularly important in the development of farming systems adapted to the local conditions, and farming practices. This may enable the utilization of marginal lands, contributing to food security in enabling access to food in remote areas, and in contributing to the management of the environment by preventing erosion, maintaining soil fertility, and agrobiodiversity.

The first international consideration of the protection of TK occurred in a joint UNESCO/WIPO World Forum on the Protection of Folklore, which was convened in Phuket in April 1997. At that meeting the representatives of organizations of indigenous peoples called for the promulgation of an international convention to protect TK. In response, WIPO in its 1998–1999 biennium instituted a schedule of regional fact-finding missions "to identify and explore the intellectual property needs, rights and expectations of the holders of traditional knowledge and innovations, in order to promote the contribution of the intellectual property system to their social, cultural and economic development." Australia was chosen as the first port of call for this expert mission, which visited Darwin and Sydney from June 14 to June 18, 1998 and during 1998 and 1999 similar expert, fact-finding missions visited Peru, South Africa, Thailand, and Trinidad and Tobago and in November 1999 WIPO convened a World Forum on Traditional Knowledge.

Following the failure of the Seattle Ministerial in November 1999 WIPO became the focus of agitation for the inclusion of traditional knowledge within the international intellectual property regime. In a note, dated September 14, 2000, the Permanent Mission of the Dominican Republic to the United Nations in Geneva submitted two documents on behalf of the Group of Countries of Latin America and the Caribbean (GRULAC) as part of the debate in the WIPO General Assembly on "Matters Concerning Intellectual Property and Genetic Resources, Traditional

Knowledge and Folklore."[1] The central thrust of these documents was a request for the creation of a Standing Committee on access to the genetic resources and traditional knowledge of local and indigenous communities. "The work of that Standing Committee would have to be directed towards defining internationally recognized practical methods of securing adequate protection for the intellectual property rights in traditional knowledge."[2]

In order to clarify the future application of intellectual property to the use and exploitation of genetic resources and biodiversity and also traditional knowledge, it was suggested that the Committee could clarify (a) the notions of public domain and private domain; (b) the appropriateness and feasibility of recognizing rights in traditional works and knowledge currently in the public domain, and investigating machinery to limit and control certain kinds of unauthorized exploitation; (c) recognition of collective rights; (d) model provisions and model contracts with which to control the use and exploitation of genetic and biological resources, and machinery for the equitable distribution of profits in the event of a patentable product or process being developed from a given resource embodying the principles of prior informed consent and equitable distribution of profits in connection with the use, development, and commercial exploitation of the material transferred and the inventions and technology resulting from it; (e) the protection of undisclosed traditional knowledge.

At the WIPO General Assembly the Member States agreed to the establishment of an Intergovernmental Committee on Intellectual Property and Genetic Resources, Traditional Knowledge and Folklore. Three interrelated themes were identified to inform the deliberations of the Committee: intellectual property issues that arise in the context of (1) access to genetic resources and benefit sharing; (2) protection of traditional knowledge, whether or not associated with those resources; and (3) the protection of expressions of folklore. As it has turned out the work of the IGC has been very slow. During the first 10 years of its existence the IGC has concentrated on the formulation of "objectives" and "principles" which should animate the protection of TCEs and TK. The African group of countries at WIPO were in the forefront of agitation there to accelerate the international negotiations, but a true reflection of their appreciation of the realistic likelihood of action was the promulgation by a diplomatic conference on August 9–10, 2010 in Swakopmund, Namibia, organized by the African Regional Intellectual Property Organization (ARIPO) of a Protocol on the Protection of Traditional Knowledge and Expressions of Folklore. The Protocol is meant to "protect creations derived from the exploitation of traditional knowledge in ARIPO member states against misappropriation and illicit use through bio-piracy." The protocol is also intended to prevent the "grant of patents in respect of inventions based on pirated traditional knowledge . . . and to promote wider commercial use and recognition of that knowledge by the holders, while ensuring that collective custodianship and ownership are not

[1] WIPO Doc. WO/GA/26/9

[2] Ibid, Annex I, 10.

undermined by the introduction of new regimes of private intellectual property rights."

A particular contemporary impetus for the formulation of an international position on the protection of traditional knowledge has been the debate concerning the review of Art. 27.3(b) of the plant variety provision of the TRIPs Agreement. Review of this provision was mandated by the TRIPs Agreement itself, to be completed by the end of 1999. Developing country participants in the review process have suggested the importation into the TRIPs Agreement of the provisions in the Convention on Biological Diversity, which provide for equitable sharing with indigenous peoples of the benefits of the utilization of traditional medical knowledge. The African Group of countries proposed the inclusion of this issue in the Ministerial Conference to set the agenda for the Seattle Round of the WTO.[3] On July 25, 1999 a federation of Indigenous Peoples groups issued a statement for the purposes of the review, pleading for a legislative structure which "Builds upon the indigenous methods and customary laws protecting knowledge and heritage and biological resources" and which prevents the appropriation of traditional knowledge and integrates "the principle and practice of prior informed consent, of indigenous peoples' as communities or as collectivities". The Statement concluded with an affirmation of the commitment of Indigenous Peoples "to sustain our struggle to have our rights to our intellectual and cultural heritage and our lands and resources promoted and protected."

On October 4, 1999 Bolivia, Columbia, Ecuador, Nicaragua, and Peru specifically proposed that the Seattle Ministerial Conference establish within the framework of the Round a mandate:

(a) To carry out studies, in collaboration with other relevant international organizations in order to make recommendations on the most appropriate means of recognizing and protecting traditional knowledge as the subject matter of intellectual property rights.
(b) On the basis of the above-mentioned recommendations, initiate negotiations with a view to establishing a multilateral legal framework that will grant effective protection to the expressions and manifestations of traditional knowledge.
(c) To complete the legal framework envisaged in paragraph (b) above in time for it to be included as part of the results of this round of trade negotiations.[4]

A communication of August 6, 1999 from Venezuela proposed that the Seattle Ministerial should consider the establishment "on a mandatory basis within the TRIPS Agreement a system for the protection of intellectual property, with an ethical and economic content, applicable to the traditional knowledge of local and

[3] *Communication to the WTO from Kenya, on behalf of the African Group,* WT/GC/W/3026, August 1999.

[4] WT/GC/W/362 12 October 1999.

indigenous communities, together with recognition of the need to define the rights of collective holders."[5]

A practical proposal for the integration of traditional knowledge with intellectual property rights is India's suggestion that material transfer agreements be required where an inventor wishes to use biological material identified by traditional knowledge. That obligation would be incorporated through inclusion in Article 29 of the TRIPs Agreement, the requirement that the country of origin of source material be identified in patent applications.[6] Following the failure of the Seattle Ministerial this agitation for the inclusion of traditional knowledge within the international intellectual property regime, shifted to WIPO, until it was picked up again at the Doha Ministerial.

Article 19 of the November 2001 Doha Declaration, instructed the Council for TRIPS, in pursuing its work program concerning both its review of Article 27.3(b) and its general review of the implementation of the TRIPS Agreement under Article 71.1 "to examine, *inter ali*a, the relationship between the TRIPS Agreement and the Convention on Biological Diversity, the protection of traditional knowledge and folklore, and other relevant new developments raised by Members pursuant to Article 71.1."

Following the Doha approach, amendments have been proposed to the TRIPS Agreement (Art. 29*bis*), which would require WTO Members to oblige patent applicants to disclose the source of any TK and evidence of compliance with legal requirements in the source country of prior informed consent for access and fair and equitable benefit sharing arising from the utilization of the TK. The African Group of Countries has proposed that as part of the review of Art. 27.3(b) TK should be protected as a "category of intellectual property rights."[7] The scheme of protection which they proposed would include the grant of rights to local or traditional communities concerning (1) respect for those communities on the commercialization of TK; (2) prior informed consent to the use of that TK; (3) full remuneration; and (4) the prevention of unauthorized third parties from utilizing that TK and incorporating that TK into any article or product.

Debate is still continuing within the TRIPS Council as to the whether it has a mandate to amend TRIPS by the inclusion of an Art. 29*bis* or whether that discussion is to be confined to the implementation of the existing text.

An alternative approach to the protection of traditional knowledge as a category of intellectual property, is its recognition as part of "prior art." As prior art it would call into question the novelty and inventiveness of inventions which are the subject of patent applications. The practical difficulty which patent examiners have in identifying relevant traditional knowledge as prior art, arises from the fact that they do not have access to traditional knowledge information in classified nonpatent literature and because there are no effective search tools for the retrieval of such

[5] WT/GC/W/282.

[6] WT/GC/W/147.

[7] IP/C/W/404, 26 June 2003.

information. The WIPO Intergovernmental Committee on Intellectual Property and Genetic Resources, Traditional Knowledge and Folklore (IGC) has begun to address practical measures to establish linkages between IP Offices and traditional knowledge documentation initiatives.

The draft Substantive Patent Law Treaty, which was submitted to the fifth session of WIPO's Standing Committee on the Law of Patents (SCP), held in Geneva from May 14 to May 19, 2001, contained two alternatives for a draft article on the definition of prior art. The draft provisions on the definition of prior art provide that any information made available to the public, anywhere in the world, in any form, including in written form, by oral communication, by display and through use, shall constitute prior art, if it has been made available to the public before the filing date, or, where applicable, the priority date.

12.10 Disclosure of the Source of GRs, Access and Benefit Sharing: Recent International Developments

One of the foundational tasks of the WIPO IGC has been the formulation of guidelines on the IP aspects of access and benefit-sharing in relation to GRs. A draft set of guidelines was submitted to the seventh session of the IGC in November 2004 which sought to provide assistance in the negotiation of contracts for access to genetic resources and related information, including traditional knowledge, and for benefit-sharing arrangements (WIPO 2004). This document has been through a number of drafts, the most recent of which was prepared for the third Intersessional Working Group which met from February 28 to March 4, 2011 (WIPO 2011). This document, together with documents which have been prepared on the subjects of traditional knowledge and traditional cultural expressions, are to be taken into account in "text-based negotiations" by the IGC, ultimately with a view to formulating an international treaty.

At the 17th Session of the IGC which met in Geneva, December 6–10, 2010, the Secretariat identified the options, which were then under consideration. There were three categories of options (a) those concerning the defensive protection of genetic resources; (b) those in relation to disclosure requirements; and (c) those concerning the IP aspects of access and benefit-sharing.

In relation to defensive protection, one category of options was the compilation of an inventory of existing periodicals, databases and other information resources, which document disclosed genetic resources, with a view to discussing a possible recommendation that certain periodicals, databases and information resources may be considered by International Search Authorities for integration into the minimum documentation list under the Patent Co-operation Treaty. The second option in this regard concerned the extension of the Online Portal of Registries and Databases, established by the Committee at its third session, to include existing databases and information systems for access to information on disclosed genetic resources. A

third option was for the formulation of recommendations or guidelines for search and examination procedures for patent applications to ensure that they better take into account disclosed genetic resources.

Options on disclosure requirements, included: the development of a mandatory disclosure requirement. Alternatively, it was proposed that the IGC could consider whether there is a need to develop appropriate (model) provisions for national or regional patent or other laws which would facilitate consistency and synergy between access and benefit-sharing measures for genetic resources, on the one hand, and national and international intellectual property law and practice, on the other. Another disclosure option was the development of guidelines or recommendations concerning the interaction between patent disclosure and access and benefit-sharing frameworks for genetic resources.

On May 6, 2010, the delegations of Australia, Canada, New Zealand, Norway, and the US submitted a working document[8] on GR for the 17th session of the IGC held December 6–10, 2010. Comments on this document[9] were made by the Delegations of Chile, Colombia, and the Russian Federation and a number of accredited observers, which resulted in a revised document identifying five objectives with underlying principles[10]: On December 8, 2010, the Delegation of Angola submitted the proposals of the African Group.[11] This suggested the commencement of negotiations on a mandatory disclosure requirement and an appropriate way to ensure prior informed consent and fair and equitable benefit sharing, in line with the Nagoya Protocol. The African proposal suggested that negotiations be based upon two current proposals on a mandatory disclosure requirement, and the incorporation of the "internationally recognized certificate of compliance" as stipulated in the Nagoya Protocol, together with any other submission that may be tabled by member countries. In relation to the option for guidelines and recommendations on defensive protection, the African Group proposed consideration of the use of available databases on GR and/or associated TK.

The African Group proposed a number of amendments to the Submission made by Australia, Canada, New Zealand, Norway, and the US. The common position between all groups of countries is that the objectives of the mandatory disclosure requirement should be that (1) the use of GRs and associated TK should be on the basis of benefit sharing; (2) patents should not be granted for inventions that are not novel or inventive in light of genetic resources and/or associated traditional knowledge; (3) patent offices should have available the information needed to make proper decisions on patent grant; (4) the principles developed should be consistent with other international and regional instruments and processes; and (5) it should maintain a role in promoting creativity and innovation. At the Third Intersessional Working Group of the IGC, which met from February 28 to March 4, 2011, a

[8] WIPO/GRTKF/IC/16/7.

[9] WIPO/GRTKF/IC/17/INF/10.

[10] WIPO/GRTKF/IC/17/7.

[11] WIPO/GRTKF/IC/17/10.

Working Group was appointed to review and rationalize the various Objectives and Principles which had been received by the IGC with a view to clarifying the key and divergent policy positions and issues, on which the IGC would need to make informed decisions. This report[12] is to be transmitted to the IGC for its consideration at its 18th session (May 9–13, 2011).

12.11 Substantive Patent Law Treaty

In an endeavor to reach a consensus on substantive patent law issues a Committee of Experts and WIPO's Standing Committee on Patents (SCP) considered a draft Patent Law Treaty (PLT), which had been prepared by the International Bureau of WIPO. The Draft PLT dealt with various procedural aspects of patenting. At the third session of the SCP in September 6–14, 1999, the delegation of Colombia proposed the introduction into the PLT, as a means of achieving some global harmonization of patent registration procedures, an article which provided that:

1. All industrial protection shall guarantee the protection of the country's biological and genetic heritage. Consequently, the grant of patents or registrations that relate to elements of that heritage shall be subject to their having been acquired or made legally.
2. Every document shall specify the registration number of the contract affording access to genetic resources and a copy thereof whereby the products or processes for which protection is sought have been manufactured or developed from genetic resources, or products thereof, of which one of the member countries is the country of origin.

This proposal generated a heated debate about whether, in the first instance, it raised a matter of procedural or substantive patent law. Agreement was eventually reached to defer consideration of this proposal to the occasion of the discussion of a proposed Substantive Patent Law Treaty. The SCP requested the International Bureau to include the issue of protection of biological and genetic resources on the agenda of a Working Group on Biotechnological Inventions, to be convened at WIPO in November 1999. The Working Group, at its meeting, the following month, recommended the establishment of nine projects related to the protection of inventions in the field of biotechnology. The Working Group decided to establish a questionnaire for the purpose of gathering information about the protection of biotechnological inventions, including certain aspects regarding intellectual property and genetic resources, in the Member States of WIPO.

An alternative approach to the protection of traditional knowledge, is its recognition as part of "prior art." As prior art it would call into question the novelty and

[12] "Draft Objectives and Principles Relating to Intellectual Property and Genetic Resources Prepared at IWG 3", WIPO/GRTKF/IWG/3/17, March 16, 2011.

inventiveness of inventions which are the subject of patent applications. The practical difficulty which patent examiners have in identifying relevant traditional knowledge as prior art, arises from the fact that they do not have access to traditional knowledge information in classified nonpatent literature and because there are no effective search tools for the retrieval of such information. The WIPO IGC has begun to address practical measures to establish linkages between IP Offices and traditional knowledge documentation initiatives. A number of the characteristics of traditional knowledge present difficulties in identifying the prior art effect of technological information. These include:

(a) The transmission of traditional knowledge through oral communication. This requires the codification and fixation of traditional knowledge into what it is not.
(b) Traditional knowledge systems tend to dynamic evolution without necessarily being identified as "new."
(c) Traditional knowledge is expressed in local languages and its expression is contingent upon such languages.
(d) The transfer of knowledge from oral into written, printed, and electronic forms may involve a cultural, semantic and symbolic transformation of the knowledge, which may affect the value of databases as a tool for the conservation of culture and knowledge.
(e) As knowledge must be in the public domain to be considered as prior art, this may provide some difficulties in those communities where knowledge is to be kept confidential.

The draft Substantive Patent Law Treaty, which was submitted to the fifth session of the WIPO's Standing Committee on the Law of Patents (SCP), held in Geneva from May 14 to May 19, 2001, contained two alternatives for a draft article on the definition of prior art. The draft provisions on the definition of prior art provide that any information made available to the public, anywhere in the world, in any form, including in written form, by oral communication, by display and through use, shall constitute prior art, if it has been made available to the public before the filing date, or, where applicable, the priority date.

12.12 Convention on Biological Diversity

The Rio Declaration in Principle 22 stated that "Indigenous peoples and their communities ... have a vital role in environmental management and development because of their knowledge and traditional practices." Chapter 26 of Agenda 21 detailed the relationship which conference participants recognized between indigenous peoples and their lands. The Agenda, at para. 26.3(a), required governments:

to establish a process to empower indigenous peoples and their communities' through measures that include:

- Recognition of their values, traditional knowledge, and resource management practices with a view to promoting environmentally sound and sustainable development.
- Enhancement of capacity-building for indigenous communities based on the adaptation and exchange of traditional experience, knowledge, and resource-management practices, to ensure their sustainable development.
- Establishment, where appropriate, of arrangements to strengthen the active participation of indigenous peoples and their communities in the national formulation of policies, laws, and programs relating to resource management and other development processes that may affect them.

The Preamble to the CBD recognized the

. . .close and traditional dependence of many indigenous and local communities embodying traditional lifestyles on biological resources, and the desirability of sharing equitably benefits arising from the use of traditional knowledge, innovations and practices relevant to the conservation of biological diversity and sustainable use of its components.

Article 8(j) of the Convention required each signatory

. . .subject to its national legislation, respect, preserve and maintain knowledge, innovations and practices of indigenous and local communities embodying traditional lifestyles relevant for the conservation and sustainable use of biological diversity and promote their wider application with the approval and involvement of the holders of such knowledge, innovations and practices and encourage the equitable sharing of the benefits arising from the utilization of such knowledge, innovations and practices.

The provisions of Art. 8(j) require implementation through national legislation. It is expressed to be subject to national legislation, in order to preserve legislation on this subject which predates the CBD.

After 6 years of negotiations the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization was adopted in October 2010 as a global agreement to implement the access and benefit-sharing obligations of the CBD. The Protocol applies when GRs are accessed and "used" except where they are covered by specialized access and benefit-sharing agreements such as the International Treaty on Plant Genetic Resources for Food and Agriculture, or the framework for pandemic preparedness of the World Health Organization. Each Party to the Protocol must establish rules that GRs and associated TK used in their jurisdiction have been acquired legally. Signatories must also establish one or more "checkpoints" to gather information on the source of the GRs, the establishment of mutually agreed terms and the use of the GRs. Users have to demonstrate at Protocol checkpoints that GRs were legally acquired. A competent national authority authorized to make a decision on access must be publicly identified for each signatory country and they must provide a decision in writing in a cost-effective manner and within a reasonable time. Where access is granted, a permit must be issued to enable researchers to demonstrate their compliance with the access rules. When the permit is filed with the International Access and Benefit-Sharing Clearing House, set up under the Protocol, it becomes an internationally recognized certificate. The clearing house will facilitate due

diligence, and underpin legal certainty by providing evidence that GRs and associated TK were acquired with prior informed consent and on mutually agreed terms.

12.13 Conclusion

The application of IPRs to GRs has become a pronounced feature of agricultural innovation in the past decade. The FAO' Panel of Eminent Experts on Ethics in Food and Agriculture has observed that "while most innovation for food and agriculture does not depend on IPRs, the acquisition and exercise of IPRs in this field raise a variety of ethical concerns" (FAO 2005). These include the fact that "IPRs protection may just mean the lack of access to innovations for the poor" and the concerns raised by the "patenting of merely isolated genes, the basic building blocks of life," which "are not invented, but are part of nature." More practically, the ability of individuals and corporations to obtain proprietary rights over agricultural innovations has important implications for food security (Blakeney 2009), particularly as the expense and general transactional costs has tended to concentrate such IPRs in a few hands. In particular, IPRs on GRs may impede their use by third parties for further research and breeding during the term of protection, and thereby inhibit the development of new products and the capacity to address emerging problems, such as agricultural stresses caused by climate change.

The IPRs landscape, which confronts countries, is of course dominated by the TRIPS Agreement. However, that Agreement contains a number of flexibilities. First, it allows the exclusion from patent protection of plants and animals (whether genetically modified or not). Secondly, the criteria under which patents are granted, i.e., novelty, and inventive step and industrial applicability may exclude materials identified through the application of TK, or GRs which exist in nature (even if isolated) as well as microorganisms. Article 30 of the TRIPS Agreement, provides that "Members may provide limited exceptions to the exclusive rights conferred by a patent, provided that such exceptions do not unreasonably conflict with a normal exploitation of the patent and do not unreasonably prejudice the legitimate interests of the patent owner, taking account of the legitimate interests of third parties." Thus, patent laws may allow third parties to undertake research and breeding during the patent term and farmers may be granted the right to save and re-use seeds where plant varieties, or certain components thereof, are subject to patent protection, in a way similar to the "farmer's privilege" under PVP.

Over the last few years, there has been a significant amount of patenting in relation to genetic material which might be useful in permitting organisms to resist the stresses of climate change. This patenting mirrors the high market concentration levels, which has already been observed in the seed industry and the control of patent thickets by a small number of companies. It should be noted in this regard, both in relation to patent rights and PVP, national laws may provide for compulsory licenses in situations of national emergency. There is also the possibility for the

intervention of the competition authorities to remedy abuses in the exercise of patent rights.

The practical effects of the application of IPRs to GRs, is reflected in the actions which are brought for infringements of IPRs. To date, these actions have mainly been brought against farmers who have cultivated patented GM crops without the permission of the relevant rights holder, as well as actions against importers of products containing patented GM ingredients. Potential IPR liability lies against governments, research institutes (international and national), and seed breeders who supply or utilize proprietary technologies. The TRIPS Agreement establishes machinery to deal with the international trade in infringing goods and this machinery is currently being supplemented by the proposed Anti-counterfeiting Trade Agreement (ACTA), of which the final text was settled on December 3, 2010.

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Chapter 13
JOINING UP: The Social and Political Dimensions

Stephen G. Hughes, Paul Richards, John A. Bryant, and Xiaobai Shen

Abstract Although this book project focuses on crop development to meet the challenges posed by climate and climate change we need to recognise that climate is but one of several interlinked factors, which bear on crop productivity and food security. Population increase, demographic changes, resource depletion and loss of agricultural land all combine with climate change to produce a "perfect storm".

We note that just as exploitation of resources is not evenly spread over the globe, neither is vulnerability to climate change. It is in general the most resource-poor countries that are most at risk and are least able to ameliorate the effects. The situation is further complicated by the considerable agro-sociological differences in respect of seed systems and crop husbandry between the industrial-scale farming systems of the global north and the small and diverse systems, which support livelihoods in the global south. Both represent agro-sociological capital but in very different ways and any breeding of crops in response to climate change needs to take this into account.

The range of issues considered extends to environmental justice, power and economic imbalances, differing economic statuses, different demographic structures as well as regulatory provisions and institutional policy.

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We acknowledge the need to engage the local and traditional knowledge and practices of those who already face extreme climatic challenges and have acquired adaptive capacity.

In the context of top-down institutional drivers for adaptive capacity building and bottom-up adaptive capacity acquired through experiential learning we examine the social implications of research and technology. Which properties of plants should we be looking at, where should the research be done and by whom? We need to consider too the possibility of new pathogens in a changing climate and their possible impact on traditional or new crops. The social acceptability of biotechnology approaches is considered as are problems in knowledge sharing and transfer.

Finally, we examine socio-political aspects of plant breeding for crop improvement. Who is making the decisions and who benefits? Can formal and informal seed systems work side by side or even be integrated together. Other forms of collaboration and especially farmer participatory plant breeding are then discussed.

13.1 Introduction: Society and the Contexts of Change

13.1.1 Inexorable Change

> "This different perspective is firmly embedded in the knowledge of specific, identifiable changes occurring in the natural and social worlds around us. These changes are so vast, so pervasive, and so important that they require our immediate attention. Scientific knowledge is urgently needed to provide the understanding for individuals and institutions to make informed policy and management decisions and to provide the basis for new technologies"—Luchenko 1998 presidential address to AAAS

The quotation above is taken from an address, which envisages a new form of compact between science and society but serves also to promote a view of a changing world of reticulated challenges that demand both clarity and concerted action.

Climatic stress is just a part of the challenge of inexorable global change. As we shall see, demographic change, population growth and resource depletion are inseparable from it. Furthermore, from the perspective of this book project, climate change is set in a dynamic discourse of institutional global imperatives concerning food security and in particular the Millennium Development Goals of freedom from poverty and hunger. FAO (Food and Agriculture Organisation) considers climate change an additional challenge to hunger eradication—one that needs to be addressed as a crosscutting theme rather than a separate activity (FAO_adapt http://www.fao.org/climatechange/2759403ecd7bd225b93086e7dca3944de64307.pdf). Top-down global institutional calls for the development and diffusion of adaptive responses, of which crop breeding improvements are a part, are further complicated by a competing and proliferating set of climate change-related imperatives related to carbon mitigation. The latter institutional ambitions are linked to the reduction of the carbon release implicit in crop production and also to changes in land use, the enhancement of carbon sequestration through agronomic

practice, or the wholesale switch from food crops to renewable energy crops. Beyond these immediately agrarian and humane settings in which agriculture is configured coincidently as victim and solution to climate change, further institutional agents converge. The list includes powerful influences from the governance of global trade, the management of biological diversity, intellectual property and benefit-sharing regimes, NGOs concerned with rural livelihoods, to research and development promotional and funding organisations, to biosecurity and regulatory authorities.

The social context of change plays out in terms not only of this extended set of global institutional drivers but also in terms of diverse vulnerabilities to the effects of change and further of disparities in the distribution of exposure to concomitant risks among the advantaged and disadvantaged peoples and communities of the planet. Manifest disparities in the distribution of environmental risk exposure and vulnerability are dominated by geographic location, poverty and demography as well as the climate per se and readers are directed to an extensive contemporary analysis by Gordon Walker, Environmental Justice (2012), which deconstructs the various impacts of these conspiring issues. Also we should note that different elements of the disruption caused by climate change will affect different countries to different extents. The Global Adaptation Institute has carried out a very interesting study comparing vulnerability to disruption with preparedness to deal with the disruption, producing for each country a "Global Adaptation Index".[1] The most recent listing shows that the bottom five countries are all in Africa, namely Ethiopia, Chad, Burundi, Zimbabwe and at the very bottom, the Central African Republic. The top five are Denmark, Switzerland, Ireland, New Zealand and Australia (the USA and UK are 8th and 10th, respectively). This generalisation of course hides more specific detail. Different countries will experience different types of disruption, including drought, reduction in water supply, flooding, rise in sea level, reduction in food supply and so on. However, one obvious feature is that it is mostly the poorer countries that are suffering and will continue to suffer most from the effects of climate change.[2]

13.1.2 Climate Change

If we isolate climate change for a moment, its implications for crop production and rural livelihoods themselves map very unevenly across human populations. They tend to concentrate their impact where pre-existing vulnerabilities are the greatest—that is to say where resource depletion, demographic pressures and poverty are constantly worsening and the security of infrastructures declining.

[1] http://gain.globalai.org/.

[2] See Harmeling S (2011) Global climate risk index 2011. Germanwatch Briefing Paper http://www.germanwatch.org/cri.

With severe vulnerabilities across regions and communities comes the further risk of self reinforcement and a spiralling threat to infrastructures and to sustainable livelihoods and even to governance and institutions.

To explore the social inequality, which we have elaborated above, and to construct a frame for the mapping of ecosocial diversity onto challenges to plant breeding, it serves to dissect the challenge of climate change into its three direct manifestations:

- The gradual and progressive changes forecast by global statistical projections and broadly attributed to atmospheric shifts in greenhouse gas emissions of fossil fuel combustion. These are generally expressed as increases in mean temperatures and sea level rises but themselves are within the bounds of changes to which life on the planet has adapted in the past.
- Embedded within these overall trends are the periodic abrupt changes typical of phenomena like El Nino La Nina and other oscillations deriving from interactions of ocean currents and the land. These can give rise to alternating bouts of drought or flood. The classical prediction of the climate change savants is that such fluctuations will become more erratic and more widespread in their impact on the vulnerable (IPCC 2007).
- Thirdly, we have to take account of a predicted increasing frequency of extreme events of the kind recognised as environmental disasters. These include floods, protracted droughts and freezes, and cyclonic storms, which often leave a wake of lasting damage (IPCC 2012).

Since society in its broadest context functions as a collective, continuing endeavour to alleviate individual vulnerabilities, we prefer to elaborate our analysis of social factors in a positive frame i.e., as elements, sometimes grouped as social capital, which limit vulnerabilities.

We recognise three concepts, which constitute the counterpart of vulnerability. These are resistance, robustness and resilience. Although we shall argue for the distinctness of these categories of systems–properties in relation to confronting the challenges of climate change to food production [see Environmental Justice (Walker 2012)] and to agricultural systems and livelihoods, they do have features in common in some contexts (e.g., all three entail social capital as a marshalling principle for responsive capacity) and are used interchangeably by some analysts (Conway 2008; Fellmann 2012).

We use resistance to denote pre-existing capacity for adaptation to the range of environmental challenges (stresses) encountered within an agronomic system. These adaptations may be embodied in physiological plasticity (strain) or in life-cycle plasticity (timely avoidance) and can apply to all tiers of agronomic systems from growing crop stands to farming communities. In this sense resistance implies refractoriness to exposure. But, of course the role of the plant breeder or seed system is to ensure that adaptive potential is progressively enhanced against the expected trajectory of stresses resulting from climate change. While natural selection during breeding is a powerful ally of this endeavour during gradual environmental change, which should ensure success, other features of plant–environment

interaction, in particular biotic stress, complicate this proposition. Shifts in climatic zones coupled to the international trade in plants and plant products may lead to a redistribution of disease vectors and the emergence of sudden and unexpected pathogen pressure and even to novel pathotypes (Brasier 2000). There is evidence on which we shall enlarge later, that such outbreaks are already happening in the world of silviculture Although this may be seen as largely an issue of phytosanitary controls it does raise issues for anticipatory identification and diffusion and introgression of disease resistance loci beyond the scope of the adaptive pace of natural selection. This constitutes the first of our examples of challenges to the plant breeder stemming from patterns of governance and human behaviour in concert with climate change.

Robustness is used to denote the ability to cope with damage or set-backs and relies on a degree of systemic redundancy or a diversity of alternative developmental pathways, which a system may draw upon. Robustness is particularly relevant to the management of environmental challenges stemming from instability or abrupt fluctuations such as alternating cycles of flood (water-logging) and drought beyond the innate adaptive potential of resistance. A major feature of such scenarios is working with uncertainty and choosing the best hedging strategies. The role of the plant breeder or seed system here is to develop, maintain and provide access to a diversity of varieties and crop types from which farmers can draw on the basis of their experiential knowledge of the dynamics of the local challenges to agronomy that they face. Breeding strategies, which might be appropriate to the challenge, are: the development of mixtures (Wolfe 1985),[3] synthetics and hybrids, all of which constitute broadly adaptive assemblies of diversity, or the development of a set of derivatives of elite varieties with significant "clip on" traits designed to enhance adaptive potential to one or another of the climatic extremes.

The social capital enjoyed by farmer communities in the sharing and conservation of seed diversity, and in the exchange of acquired knowledge (social and individual learning) are a strong feature of robustness. We shall examine this proposition and its significance to breeding practices in the context of climatic instabilities in West Africa in Sect. 13.3 informed by the extensive studies of Edwin Nuijten (2005) in the Gambia and more broadly by studies of the rice cultures of the coastal states (Offei et al. 2009). Also, from Harwood's study of the Rockefeller Mexican Agricultural Program we see how sharing of varieties between linked communities and the construction of synthetics from them can add a significant and robust dimension to yield improvement (Harwood 2012).

Resilience denotes the set of systems properties favourable to maintenance of a developmental trajectory attributable to recovery from incidences of serious damage or disruption such as might accrue during extreme climatic events (Lambin 2005). The frequency of such events is expected to increase according to current framings of climate change (Cruz et al. 2007) articulated by IPCC. The associated narrative is that during serious environmental disasters such as regional devastating

[3] http://www.apsnet.org/edcenter/advanced/topics/cultivarmixes/Pages/default.aspx.

floods, prolonged unseasonal episodes of freezing or frying temperatures, or sea water inundations during cyclones, resistance and robustness thresholds are overcome and crops destroyed, and agronomic affordances and supporting infrastructures temporarily disabled. Under such circumstances resilience may initially be dependent upon material reserves, but, subsequently recovery may be dependent upon external institutional interventions designed to assist the re-establishment of livelihoods and agriculture as the agro-ecosystem re-equilibrates. Re-equilibration may infer an altered set of conditions if, for instance, topsoil has been eroded or a legacy of salinity left behind, in which case access to new plant varieties and plant types specifically adapted to the new and challenging circumstances will be required. This raises a distinct set of challenges to the plant breeding systems. Types adapted to perform in the extreme conditions of damaged agro-ecosystems will have a limited and transient market and may not be attractive propositions for investment by commercial breeders.

As we observed for robustness, resilience may be configured as a system of learning though it is worth remarking that because of the regional nature of environmental disasters social learning is likely (instructional learning coupled to imitation) to be of greater prominence than individual or empirical learning.[4]

Analysis by agents of IPCC (Cruz et al. 2007) of the causes and consequences of the recent flood disaster in the Indus Valley of Pakistan for instance demonstrates that vulnerability was exacerbated by demographic change linked to the urbanisation of the flood plain.

Similar considerations of robustness and resilience apply to disruption and loss of infrastructures and agricultural resources during civil conflict, from which we may also learn. A particular example is the loss of indigenous rice varieties and breeding materials during the civil war in Sierra Leone. The studies reported in Richards et al. (2009), Richards and Ruivenkamp (1997) and Longley (1997) showed that the farmers were quickly able to assess the value of new varieties they were offered and, recognising the new circumstances in which they found themselves, did not always go back to the types they were growing previously. This illustrates that individual experiential learning can have a part to play even in resilience.

[4] See Oxley (2011): For many poor people dependent on agricultural-based livelihoods the recovery will be a long and difficult process; it is likely they have lost at least one year's crop production together with livelihoods and households assets. Food production levels may be significantly lowered for the next few years because of the combined effect of soil erosion, destroyed infrastructure and contaminated soils.

Effective recovery will depend significantly on how quickly livelihoods and supporting infrastructure are restored. Fortunately the Pakistan authorities have considerable experience of organizing and mobilising large-scale social protection and food security schemes through cash or food-for-work program. Such people-intensive approaches could be well suited to major infrastructure and environmental restoration work. For example, poor people whose livelihoods are dependent on natural resources have substantial local environmental knowledge and are well positioned to support national efforts to restore and enhance the functioning of the river basin ecosystems.

The forgoing discourse has been elaborated in order to introduce some of the challenges for plant breeding in a changing world and adaptive measures introduced to reduce vulnerability have features in common whether viewed across the technical exigencies of plant breeding or the ecosocial dimensions of sustainable communities of agricultural production.

13.1.3 Technological Change

We now move on briefly to consider the third element of change within which plant breeding challenges are elaborated, that of technology and opportunism. This will lead us at a later stage to examine more closely how the socio-technical settings of breeding map onto the ecosocial dimensions of robustness and resilience. As the many technical articles in the two volumes attest the scope of application of new techniques for mobilising and introgressing valuable alleles from adapted ecotypes into highly tuned idiotypes is continuously advancing. This raises issues relating to the ownership of traits as well as the means by which they are accessed as material resources of breeding in its many contexts. It also raises questions, though patchily distributed and often voiced by external advocates of particular agronomic persuasions, of the acceptability of some technical approaches in particular the transgenic assembly of novel traits (aka GM). Of course there is an institutional dimension to this issue manifest in the Cartegena Protocol, a spinoff of the Convention on Biodiversity (CBD), which sets conditions for the management and transboundary movement of modified organisms and the distribution of liabilities.

The final dimension of the advance of technology in crop plant improvement to which we will draw attention in this introduction lies in the distribution and support of capacities and competencies. Here we include the set of capacities, from fundamental research on traits and adaptation, to knowledge of advanced germplasm, to plant breeding skills and training, to agronomy and the matching of varieties to agronomic and cultural circumstances. Commercial breeding companies are at liberty to locate their investment in these capacities as best suits their perceptions of the eventual market opportunities. The support for these capacities in the public sector of the formal seed system is dependent on many distributed institutional decisions concerning the allocation of resources and the prioritisation and recognition of the need for each element. It has been noted that public sector breeding in general has latterly been in decline (Murphy 2007) linked to a lack of trained capacity (human resource) for distributed plant breeding. A recent report by the Royal Society of London (Reaping the Benefits 2009), while emphasising the potential of current public investment in molecular plant science also recognised a systemic weakness in capacity for crop physiology, phenotypic evaluation and the translation of advanced knowledge into benefits at the field level. As we shall see in the next section these lacunae are recognised as significant to institutional goals for adaptive capacity relative to resilience and robustness, and should not be taken merely as consequences of misguided policy decisions driven by the hegemony of

elite science, however compelling that may seem. External pressures from economic limitations, educational opportunities and demographic shifts have had a part to play.

13.1.4 Sociological Groundings

As a coda to this introductory sector in which we have tried to position social considerations in the discourse on climate change and adaptation via plant breeding, we are bound to observe that climate change and its social issues has attracted little attention from practicing social scientists (Langenhove 2012). This is so despite the critical importance of this subject to human welfare and social continuity. Keywords associated with climate change are found in just 1.6 % of the 10,000 social science papers published.

However, we are fortunate that a small group of agrarian sociologists and anthropologists have focused their gaze on breeding technologies and seed systems. Their studies extend across a diversity of social settings where the impacts of climate change are of pressing significance or where farmers, for one reason or another (poverty, lack of access to inputs, or the adoption of the organic persuasion) are unable to intervene in order to moderate their agro-ecosystem. This group of analysts have contributed both to the conventional literature but also to reviews and policy investigations on behalf of global institutions such as FAO. We have drawn heavily on the work of this community especially in Sect. 13.3. The other group of sociologists we have drawn upon are those who work with theories of resilience and robustness, for example Fellmann (2012) and Lambin (2005).

13.1.5 The Institutional Challenge

Against the broadly shared imperative of enhancing resistance robustness and resilience of communities and agricultural systems we are now ready to ask how decisions on priorities for crop improvement through breeding are made including how, where and by whom and by what criteria?

We anticipate at this stage that where considerations entailing technological affordances are salient then top-down policies and decisions made by institutions (including commercial and public sector breeding undertakings and those international agencies whose mandate includes agricultural development, food security, the sustainability of ecosystems, or trade and infrastructure) will prevail. Correspondingly, we assume that bottom-up choices made by farmers and farmer communities actively or passively (through practice) based on observation and experience will prevail under local circumstances where livelihoods are at stake.

Within this dichotomy our attention is drawn to an additional underlying tension between the broad imperatives of systems sustainability versus the ambient

pressures on productivity in relation to market supply and demand, as well as the immediate motive of sustaining livelihoods.

Institutional top-down imperatives intended to translate into schemes to support the enhancement of robustness and resilience are generally reckoned to be configured through the elaboration of enablements (adaptive capacity), motivational incentives, and information (Lambin 2005). The expectation is that these will feed in to the diversity of agricultural settings and act as transformative principles where adaptive systems are likely to push close to or beyond their tipping points, either by the environmental stresses of climate change acting alone or in concert with social demographic or economic factors.

Against this expectation we will next examine the positions and modalities adopted by major global institutions in promoting and distributing adaptive strategies, supporting responsive capacities while recognising relevant knowledge systems and social practices.

13.2 Institutions, Demography and Trade

13.2.1 Institutions and Adaptation

Beyond the Intergovernmental Panel on Climate Change an array of powerful national and international institutions have actively pursued the imperative of their mandates to monitor the threats to global food security, local food sufficiency and effective, sustainable use of agro-ecological resources, posed by climate change. In numerous published reports and exhortations *adaptation and mitigation* are the clarion calls. Let us take as a first example the Food and Agriculture Organisation (FAO) of the UN. The manifesto for its framework program, appropriately entitled FAO-Adapt, recognises the diversity of circumstances under which the impacts of climate change will play out and looks to building robustness and resilience through participatory development of local capacity and through institutional agility (http://www.fao.org/climatechange/27594-03ecd7bd225b93086e7dca3944de64307.pdf). This ambition carries an intrinsic, socially oriented recognition of human rights and justice as well as of disparities of exposure to environmental risks. It also carries the expectation of participative development of adaptive capacity:

> Through its long experience in people-centred work on agriculture, rural development and climate change, FAO recognises that adaptation work also calls for demand-driven, location-specific approaches and requires participatory modalities that consider gender-specific vulnerabilities, needs and capabilities as well as the priorities of indigenous people and vulnerable communities.

FAO-Adapt promotes an ecosystem approach alongside the above "people centred-activity-distributed", social systems/institutionally oriented strategy, with

attention paid to resource management and balancing food production demands against ecosystem capacity.

Among its many active adaptive themes focussed on agricultural productivity is the declared ambition to:

> Promote the breeding and conservation of crops, trees, livestock and fish adapted to changed climate conditions. Support the development and dissemination of technologies and practices and enhance local knowledge to improve the adaptive capacity of production and management systems and value chains in agriculture, forestry and fisheries.

Against this tier of ambition, GIPB, the Global Partnership for Plant Breeding and Capacity Building, which is facilitated by FAO, takes account of breeding priorities and draws attention to the need for better use of plant genetic resources (not surprisingly given the FAO undertaking under ITPGRFA). The link between breeding and germplasm is expressed by a further body under the auspices of the FAO. The Intergovernmental Technical Working Group on Plant Genetic Resources for Food and Agriculture (ITWGPRFA) in its report Strengthening Plant Breeding Capacities (http://typo3.fao.org/fileadmin/templates/agphome/documents/PGR/ITWG/ITWG5/ITWG5_INF4FINALUpton.pdf) commented for instance:

> The Working Group also noted with satisfaction the achievements of the FAO-led Global Partnership Initiative on Plant Breeding Capacity Building, highlighting the importance of plant breeding to address climate change and the need for capacity development and long-term national strategies for strengthening linkages between the conservation of plant germplasm, crop improvement, and the dissemination of quality seeds and planting materials.

Significantly in Part VII of this report subtitled Charting the Course for Re-invigorating Plant Breeding, the intergovernmental panel assert and this is a *key* assertion, since the role of social linkages and of client farmers is clearly acknowledged:

> The development of successful new crop varieties fundamentally depends on well-defined partnerships among multiple institutions and the client farmers, who in turn are attuned to consumer interests . . . (and) . . . sustainability of the system. When all these elements are in place, the payoff is very high in terms of income generation for smallholder farmers, benefits for the environment, and food security for society as a whole. The time is opportune to develop "best practices" to anchor policy recommendations for the establishment of results-oriented breeding programmes.

This acknowledgement is all the more poignant as it makes the link between policy and best practice and introduces people in communities, societies and cultures as the interface of translation between policy and practice and outcomes. We shall return to these operational linkages and farmer engagement in Sect. 13.3 in the context of building adaptive capacity through plant breeding. But in the meantime we should take note of the absolute shortfall in breeding capacity as well as the need for reorientation as a general bottleneck in the translation of policy to outcomes.

The erosion of plant breeding capacity and cognate skills has been remarked upon by other institutions too, notably, because of its frontline role in training, the Wageningen Plant Breeding Business School, pointing to the low status of agriculture relative to the expectations of educated cadres who might be expected to train as specialists in the profession:

> This is due to demographic development, broadened education possibilities, but also to the poor image of agriculture in general.[5]

The shortfall is also recognised in reports authored by expert analysts from major Policy Oriented Donor organisations like the World Bank, which articulate the need for training initiatives across the world, initiatives which include tuition in the full portfolio of field, laboratory and commercial (including IP) skills (Morris et al. 2006). However, the report makes little mention of the skills needed to link and converge with farmers and to draw on knowledge derived from experiential learning in informal settings. This global shortfall in distributed breeding capacity implies a continuing dependence on a narrow portfolio of varieties broadly adapted to high input systems and the international seed market.

Plant breeding is nested within seed systems, which will be explored in detail in the next section, but it is worth introducing them here in the context of institutional policy and capacity. Formal seed systems involve trained and resourced plant breeders operating in centralised private or public sector institutions and providing broadly adapted seed for distribution to remote locations. The informal system involves farmers embedded in their society often as coincidental but none the less effective breeders who produce locally adapted unregulated seed for themselves and their communities. Despite their recognition of the importance of social and farmer linkages as discussed above the ITWGPGRFA in their report on Strengthening Seed Systems (Gap Analysis of the Seed Sector 2011) adopts the position that, in areas where the informal sector is predominant it should be strengthened by acquiring the capacities of the formal and that the public sector be enabled in the direction of the private system. The report recommends, for instance the modification of seed legislation to accommodate the informal. It also recommends strengthening farmer's capacities in seed multiplication in order to improve the quality of seed produced in the informal sector, as well as support for the emergence of local private sector enterprises. Clearly the institutionalised regulated market-oriented formal model is what is aspired to, and perhaps not surprisingly so since the formal sector is rather better placed to make use of the genetic resources in the ex situ collections of the FAO undertaking. But we should not forget that ITWGPRFA also recommends an increase of farmers' participation in crop improvement activities in order to ensure that new varieties are appropriate to farmer practices and experiences. Perhaps the intention then is not to

[5] http://www.wbs.wur.nl/UK/Partnerships/Plant+Breeding+Business+School/Plant+Breeding+Business+School+detail/default.htm.

disenfranchise the informal sector completely. The significance of this cooperation between formal and informal sectors will be explored in the next part of this account.

Erosion of breeding capacity is also evident within the networks of public sector breeding establishments embodied in national and international agricultural research centres (Harwood 2012; Murphy 2007). This corresponds to a gradual withdrawal of funding from the sector and it remains to be seen whether the encouragement of the emergence of a new and less centralised private sector can fill the gap.

The World Bank has recently sponsored, together with other institutions including FAO, UNDP and UNEP, an extensive assessment involving inputs from 400 experts, of the challenges to agricultural development that lie ahead. The following quote from the International Assessment of Agricultural Knowledge, Science and Technology for Development (IAASTD) report of 2008 sets the tone:

> The way the world grows its food will have to change radically to better serve the poor and hungry if the world is to cope with growing population and climate change while avoiding social breakdown.

The IAASTD report has attracted some notoriety for its prioritisation of integrative approaches drawing upon traditional technologies, over advanced technologies oriented toward production and yield.[6] In fact, industrial contributors withdrew from participation when this stance started to emerge. The tension between the imperatives of production, aligned with supply and demand and commodity markets on the one hand, and of integration, which align with sustainability, livelihoods and the socio-ecological factors that surround farmers and farming practice on the other, forms an undercurrent to our thesis. So in the elaboration of our arguments it is encouraging that at least one group of experts have articulated the need for a more broadly negotiated approach to development, which takes account of the inequities and institutional barriers to participation.

In a further paper prepared for the FAO/OECD workshop on Building Resilience for Adaptation to Climate Change in the Agricultural Sector, Fellmann (2012) provides a discussion of frameworks for assessing vulnerability to climate change. He represents vulnerability in terms of three components, exposure, sensitivity and adaptive capacity. Exposure, as Walker (2012) explains in his book Environmental Justice, is influenced by socio-demographic accidents of time and location linked to disparities of wealth/poverty, age and gender. Sensitivity is the counter part of our concept of resistance and relates to the ability of varieties or systems, thanks to their innate or acquired qualities, to exhibit refractoriness in the face of environmental perturbations and challenges. Adaptive capacity represents the two components, robustness and resilience (recovery, resurgence) of the framework we have elected to work with. Fellmann (2012) goes on to distinguish between outcome

[6] http://web.worldbank.org/WBSITE/EXTERNAL/EXTOED/EXTGLOREGPARPROG/0,,contentMDK:21106947~menuPK:4426475~pagePK:64829573~piPK:64829550~theSitePK:4426313,00.html.

vulnerability and contextual vulnerability. He positions the former as an endpoint (future) interpretation and the latter as a starting point or current interpretation. The former is based on physical predictions and focused on technological solutions to adaptation and mitigation, while the latter is based on the dissection of susceptibilities related to socio-economic factors and focuses on building adaptive capacity through the exploration of alternative pathways of development. He is then able to deconstruct the contextual and the endpoint interpretations against the functioning of agricultural systems as a means of exploring the nature and location of power and inequity issues which constrain robustness and resilience. Plant varieties can then constitute a bridge between the end point and contextual interpretations. It will be interesting to see if this analytical framework is taken up by the lead institutions in focusing their policies more sharply on the social issues governing adaptive capacity.

13.2.2 Institutions and Research

Although The Coordinating Group for International Agricultural Research (CGIAR) and its constituent Centres represent only a small fraction, and a declining one (Harwood 2012) of the overall global agricultural research on climate change, their high status coupled to their proximity to major donors and policy makers coupled to their ability to link to the cutting edge of science renders them very influential.

The CGIAR together with the Platform for Rural development has supported the Commission on Sustainable Agriculture and Climate Change (CSACC) in constructing a report for policy makers (including UNFCCC) (Beddington et al. 2012), which includes the following set of recommendations for action:

- "Integrate food security and sustainable agriculture into global and national policies
- Significantly raise the level of global investment in sustainable agriculture and food systems in the next decade
- Sustainably intensify agricultural production while reducing GHG emissions and other negative environmental impacts of agriculture
- Develop specific programmes and policies to assist populations and sectors that are most vulnerable to climate changes and food insecurity
- Reshape food access and consumption patterns to ensure basic nutritional needs are met and to foster healthy and sustainable eating patterns worldwide
- Reduce loss and waste in food systems, targeting infrastructure, farming practices, processing, distribution and household habits
- Create comprehensive, shared, integrated information systems that encompass human and ecological dimensions"

While the list contains few novel insights, it is significant in linking contexts with objectives in a clear and concise way and as such it provides a helpful checklist

for mapping proposed solutions onto situated outcomes. Interestingly in relation to the convergence of food systems and plant breeding we can perhaps abstract an indicator that post-harvest losses and the nutritional content of staples are valid targets for improvement alongside the obvious environmental stress-linked productivity goals, in the response to climate change.

Commensurate with the above set of broad principles, CGIAR has promoted a dedicated 10-year consortium research program entitled Climate Change Agriculture and Food Security (CCAFS), the goal of which is to identify appropriate policy and technical interventions to improve food security. It employs a food systems approach (Gregory et al. 2005), which supports integrative considerations beyond the simply commodity production oriented to the support of livelihoods and environmental goals in vulnerable settings. It has set up 36 benchmark sites across the world in which to collect integrated data concerning impacts and responses to climate change and its research is organised around:

> "Adaptation to Progressive Climate Change; Adaptation through Managing Climate Risk; Pro-poor Climate Change Mitigation and Integration for Decision-Making" (http://ccafs.cgiar.org/our-work/research-themes/pro-poor-mitigation)

This alignment of a food systems approach with recognition of regional diversity appears to us to have considerable merit in bringing together scientific and local knowledge and could provide a touchstone for the mapping of adaptive solutions onto local issues.

In the forgoing analysis we have surveyed and portrayed the changing institutional environment drawing upon policy positions, commentaries and programs articulated by some of the major global agencies. The institutions overlap considerably in their coverage and present varying degrees of recognition of the diversity of socio-economic factors, which influence the development of adaptive capacity, as well as some of the bottlenecks, like the absolute shortage of plant breeders.

Overall, we note a continuing alignment with production-oriented solutions but with contextual and integrative approaches starting to gain traction as we have witnessed with the CGIAR CAFFS program.

13.2.3 Institutions and Mitigation

Before moving on, in the context of technology and the opportunities for its deployment in support of breeding for climate resilience, we should devote some attention to the other element of the clarion call, that of mitigation of the carbon footprint of agriculture. In general the call for mitigative practices points us towards plant breeding targets related to input use efficiencies. One might cite, for instance, water (irrigation) use efficiency, NPK fertiliser use efficiency and reduced pesticide deployment as valuable features of plant ideotypes conceived with carbon input costs and environmental impact in mind.

Beyond this an example of the way in which FAO addresses mitigation relates to grasslands management and carbon sequestration.[7] Grazed grasslands constitute the majority (70 %) of agricultural lands and 26 % of the land area of the planet and are especially at risk of degradation through mismanagement or failure to adapt to climate change. Pastures lie under a variety of land tenure provisions and management regimes and this is all-important to the adaptive capacity of communities also, especially when we consider instances where degradation and overgrazing present classical examples of the tragedy of the commons (Hardin 1968).

The attempt to promote synergies between adaptive and mitigative strategies exists against a tension between initiatives for intensive management and replanting of rangelands, cerrado, etc. with genetically improved varieties, and initiatives for the reconstruction of sustainable evolved ecosystems containing diverse native species. Besides this, uncertainty about land tenure among smallholders and weak institutions are key issues that discourage potential participants from adopting carbon-sequestering practices (Grieg-Gran et al. 2005).

Nevertheless, in a supportive document prepared for the FAO by Abberton[8] it is argued that in general, breeding approaches to increasing the efficiency of grassland agriculture can be characterised as:

1. Accessible: through seed without other inputs
2. Providing lasting and cumulative impacts
3. Bringing other benefits, for example, varieties contributing to improved animal performance
4. Easy to use and relatively inexpensive to the farmer
5. Appropriate in the long term, representing sustainable "genetic" rather than "input" based solutions. Otherwise mitigation will not present an incentive for investment especially where social factors such as land tenure and poverty conspire with climate change.

The major challenges of tropical improved grasslands—uptake of improved germplasm, encouragement of on-farm diversity, and development of more sustainable production—mean that at the moment adoption of breeding programs aimed specifically at climate change mitigation is not a priority.

Plant properties such as digestibility and nutritional content favourable to the reduction of ruminant methane production, making nitrogen and phosphorous utilisation both in plants and animals more efficient, improving carbon sequestration through development of deep rooting systems are however, valid goals.

As in temperate systems the depth of deposition of C is important: the deeper the deposit, the longer the turn over time. In this context it is interesting that the forage

[7] http://www.fao.org/docrep/012/i1399e/i1399e00.htm and http://www.fao.org/fileadmin/templates/agphome/documents/climate/AGPC_grassland_webversion_19.pdf.

[8] http://www.fao.org/ag/AGP/agpc/doc/climatechange/papers/abberton_%20geneticimprovement.pdf.

grass *Brachiaria*, which we will consider further in Sect. 13.3 deposits C to a depth of 1 m due to its deep roots (Mannetje 2007).

Pasturelands will be a territory in which we shall examine the potential of a novel hybrid plant breeding technology to contribute to robustness in the face of flux and diversity in the ecosocial system in Sect. 13.3.

The issues raised above concerning land management and allocation are significant in relation to other aspects of mitigation in particular the issues of biofuel production. Breeding questions relating to the improvement of energy crops are significant in relation to the socially oriented issues of where and by whom should such crops be grown. The recent Nuffield Council on Bioethics report (2011) laid great emphasis on the rights of farmers not to have their livelihoods threatened by the sequestration of land and resources for subsistence under the mandates for bio-ethanol and biodiesel incorporation into transport fuels.

13.2.4 Agencies and Technologies

The FAO/International Atomic Energy Agency Joint Program supports projects on mutation-based breeding across Africa, Asia and South America, as a manifestation of the peaceful and nonthreatening use of nuclear technologies, which was established after the Second World War. It has now adopted climate change under its banner Enhancing Crop Varieties for Increased Adaptability to Climate Change Conditions as its driving imperative for the promotion of breeding goals, for which mutation breeding is seen as appropriate. As an example: the technical cooperation project, "Improving Food Crops in Latin America Through Induced Mutation", supports the increase of food production in drought-affected areas through the development and dissemination of drought-tolerant mutant lines of food crops (e.g., legumes, cereals, pseudo-cereals, and fruit trees) that have been traditionally cultivated in marginal and semi-arid areas.

The program also expresses concern, as we see below, over the emergence of new pathogens and the need to generate novel variation to provide resistance and, of course prioritises programs of mutation as a means of acquiring it.

This very interesting and well-established motivational initiative represents an example of the provision of resources, including a dedicated laboratory and information support system and project funding by UN Agencies in support of a particular technical solution. It is all the more interesting for its ideological foundations as a counter movement to weaponry.

13.2.5 Demographic Change

Demography, which for our purposes we regard as the structural account of populations and of people as agents in locations, has to be included in the discourse

on adaptation and mitigation. As remarked earlier, people in situ in agricultural settings are the only means of integrating policies, adaptive materials and sustainable practices into livelihoods and productivity. Put more simply: technical solutions to adaptation and mitigation have people inside them. This is seen as a Durkheimian (Durkheim 1912) view of a secular belief in a set of ethical principles based on human action and interaction, which appeals to a set of human rights to safety, sustenance and livelihoods on which the Millennium Development Goals (MDGs) are founded. In this context it is worth noting that the World Bank in aligning with the Millennium Development Goals of:

> the reduction of hunger and poverty, the improvement of rural livelihoods and human health, and facilitating equitable, socially, environmentally and economically sustainable development.

States that:

> Meeting these goals has to be placed in the context of a rapidly changing world of urbanisation, growing inequities, human migration, globalisation, changing dietary preferences, climate change, environmental degradation, . . .

The latter set of factors is linked in a complex multitude of ways well beyond our capacity for simultaneous commentary. However, urbanisation, inequities especially in relation to exposure to the risks of climate change, migration, and changing dietary preferences, can be seen as exerting powerful influences over our concepts of robustness and resilient trajectories in agriculture and are significant concomitants of demographic change. As an example, we can link migration and urbanisation in the context of the movement of young people in general and males in particular from rural agrarian settings into cities. Besides the general dilution of rural social capital, which this accords, the new urban environment will elicit many changes and shifts in behaviour. A notable one is dietary consumption. We are witnessing an upward shift in the demand for meat and animal products in Asia, which in turn puts pressure on production and thence onto pastures and their carrying capacity. Meanwhile back in the rural setting the corresponding shift is towards an aging population of farmers and household managers who are having to adapt their practices and take on extended roles as the holders of local knowledge.

As a further instance we saw in Sect. 13.1 that the infrastructural proliferations associated with accommodation of urban populations on the flood plains of agronomic systems have exaggerated the severity of flood events and their challenge to resilience.

Migration from land to cities is a trend, which started long ago but was accelerated by industrialisation. It has continued in turn to provoke and be precipitated by changes in agricultural technology and land tenure. However, nowadays the trend links to a change in the age profile of rural communities and farmers the significance of which is that the tacit knowledge, which supports robust practice and the deployment and custodianship of seed systems, is invested in an age and gender-biased subpopulation. This lends urgency to the proposition, which we shall explore in Sect. 13.3, that experience and materials of agriculture in climatically marginal zones embody the adaptive capacity and robustness which

has accumulated in agrarian communities and which are important to informing the trajectory of plant breeding.

While discussing people and locations, age and gender it is critical to remember that demographic pressures have the tendency to push those with the least financial assets into the zones of self-sustaining exposure to environmental risk at the periphery. This reflects their lack of a voice and, even in democratic systems their lack of negotiating power. As we have emphasised from the start poverty and exposure form a self-sustaining trap, which compounds with voicelessness: Those who are poorly resourced are least able to achieve rights of recognition; those who are not recognised are discriminated against in the allocation of and access to resources (Walker 2012).

We have noted the loss of a cadre of young people with the agricultural experience, which might make them into good breeders that has been attributed to demographic change. This is mirrored by a growth from the urban elite of a cadre of decision-makers with little knowledge of rural realities (Lambin 2005).

13.2.6 Trade and Exchange

The dominant and persistent economic model of growth and consumption into which we are locked tends to favour considerations of production, intensification, markets and distribution over sustainable livelihoods and food systems. A clear illustration of this is the effects which remote decisions on economic priorities can have on investment cycles and commodity prices. For instance, mandates placed on biofuel incorporation and therefore on production and trading have been seen to push agricultural food commodity prices up (Gallagher Review 2008) due to an anticipated unmet demand. Competing markets will inevitably lead to exaggerated cycles of supply and demand which are likely to create a lose–lose situation for smallholders. These fluctuations will likely be exaggerated further by the oscillations of climate change and other oscillations in investment cycles as investors shift their capital.

One of the positive consequences of the discourse over climate change mitigation and carbon trading is that it may have helped the world as we saw in the IAASTD evaluation, to start to take notice of integrative imperatives rather than the production-oriented ones that have driven us this far.

Nevertheless, trade across a heterogeneous planet is an essential function of feeding people. But while trade separates production and consumption as processes and communities of action, it links them materially. Material exchange and relocation of agricultural products raises very diverse sets of issues of which we shall highlight three in relation to our interests in building resistance, robustness and resilience in the face of climate change. One relates to the knowledge embodied in the material, another to disease agents or pests inadvertently co-exported with objects traded across agro-ecological zones and the third to the trading of genetically modified materials.

13.2.6.1 Exchange of Material and Knowledge

The institutional arrangements whereby global trade is conducted, (and here WTO is very much in mind), accord very different status to formalised knowledge i.e., that which can be commodified as intellectual property, and other informal kinds collectively referred to as traditional and by inference of less value. The enforcement of intellectual property rights linked to breeding tools and valuable adaptive traits can restrict international trade in those agricultural products which embody the corresponding genes and enabling technologies (Hughes 2002). In contrast, the knowledge embodied in locally adapted seeds of farmers and sharing communities in the informal system is respected only in relation to the more diffuse concept of biodiversity.

This asymmetry is the subject of criticism in relation to the provision of TRIPS (Trade Related Intellectual Property System), whereby (trade) participating countries are required to subscribe to appropriate systems of IP protection including patents and plant variety rights.[9] These formal legal provisions align with the competitive market-oriented practices of the developed west. This coercive mechanism with its asymmetric prioritisation of knowledge raises issues for plant breeding and material exchanges (Louwaars 2007) and has supported the emergence of the counter-concept of farmer's rights and links to the concept of biopiracy developed within the framework of biodiversity and its regional sovereignty as assigned under the Convention on Biodiversity (CBD) (IPGRI 1999). Formalised systems of exchange have been developed according to the principles of CBD, which provide for informed consent on the part of the donor of in situ material and material transfer agreements (MTOs) governing material from ex situ collections under the ITPGRFA and the FAO undertaking. A principle of benefit sharing is attached to these arrangements. As De Jonge (2009) points out, this is founded on a pragmatic ethic rather than an ethic of distributive justice (fare shares for all), which is to say that it is intended to induce participation with a promise of a return rather than to match the rights assigned to those of the formal system who might exploit the material. Hence, we see a clear disparity between breeder's rights and the notion of farmer's rights. De Jonge in his in depth inquiry into the principles of fair and equitable benefit sharing Plants Genes and Justice (2009) highlights the difficulty of administering any system of benefit sharing which involves a material return given the very diverse nature of the materials and embodied knowledge that might be transferred, as well as the diverse and potentially indirect benefits that might accrue. An alternative suggestion is that benefit sharing could be grounded rather in the ethic of participative justice by which the voices of the custodians of in situ material and wherever possible the originators of ex situ material are heard during the process of translating their embodied knowledge into distributed benefit. This

[9] The technical details of this requirement have been reviewed by IPGRI (1999) http://www.bioversityinternational.org/fileadmin/bioversity/publications/pdfs/41_Key_questions_for_decision-makers.pdf?cache=1335669203.

suggestion has some merit as a pragmatic principle also. As Hughes and Deibel (2007) asserted, the formal and informal systems and their associated communities can all gain by cross-participation and the mutual sharing of breeding materials, a solution, which we shall explore in the form of participatory plant breeding in Sect. 13.3.

Nevertheless, the disparity of rights (Louwaars 2007) continues to raise issues both in relation to access to valuable genetic variation (varieties and traits) as inputs, as well as to the identity and status of the products of cooperation between formal and informal systems. The disparity is particularly manifest when we compare the material natures of varieties in the formal and informal systems. Under the formal system the identity of a variety is established within a legal framework based on its distinctness, uniformity and stability (DUS) as demonstrated under precise growing conditions. Strict application of DUS in variety registration and the assignment of proprietary rights is a feature of seed administration in those countries which subscribe to the UPOV convention. Adherence to the DUS principle is understandable given the competitive nature of private sector breeding and even for the public sector where breeders are expected to mitigate their costs via the recovery of royalties. In contrast informal varieties are likely to contain a degree of heterogeneity due to spontaneous gene flow (Nuijtens 2005) as well as adaptive phenotypic plasticity representing the marshalling of on-going adaptive capacity on the part of the breeder. It is also possible that irregularities of naming further complicate the picture as does the decentralised ownership of systems of sharing. The identity of ecotypes relates more to the reality of situated performance and farmer knowledge than to administrative constructions the like of DUS.

So breeder's rights assigned under IP regimes and farmer's rights conceptualised under CBD connote at the moment a barrier to cooperation between formal and informal systems and further offer little incentive to informal systems to commit their working material to ex situ collections. Louwaars (2007) reports that public sector research centres in the developing world have drawn more extensively on the accessions than has the private sector, questioning the proposition that ex situ collections constitute more of a resource for speculative trait browsing than for adaptive diversification. At the same time it seems that acquisitions of material from the ex situ collections has latterly shown a downward trend consistent with the expected burdens of negotiating material transfer agreements and a perceived general disincentive to invest in breeding within the ambient rights regimes (Kingston 2007).

Perhaps we can look to those Civil Society organisations (NGOs) more concerned with negotiating integrative adaptive outcomes than with manicuring their own advocacy, to establish local safeguards within cooperative programs to provide the missing reassurance and to ease the log-jamb of rights. The Community Biodiversity Development and Conservation Network (CBDC)[10] for instance seem

[10] http://find-your-feet.org/blog/tag/seed-conservation/.

well placed to do this and through past programs has firsthand experience of the tensions involved (see Manicad 1996).

13.2.6.2 Emergent Pathologies

We will next examine the implications of the international trade in plant materials in the light of patterns of agronomic practice, social behaviours and biosecurity provisions in relation to emergent pathogens and their distribution. International trade and traffic in plant material has been going on for some time and it might be expected that phytosanitary oversight should take care of it these days. But this ignores the subtleties of emergent pathogens, which exist in equilibrium as saprophytes with their hosts in one set of climatic and agronomic conditions and then emerge as new threats when transposed to new cultures, cultural systems and emergent (changing) environments. The material trade may not even involve the vulnerable crop itself but other live-traded carrier/co-host species imported for local cultural purposes such as ornamental gardening (the cultural pursuit of leisure and the exotic). New pathogens may emerge from hybridisation between indigenous and casually introduced lines once geographic and climatic isolations have been compromised (Brasier 2000).

There are some salient lessons to be drawn from silviculture and its emergent pathogens of which there has been a recent upsurge paralleling the burgeoning international trade in plants (Ingram 2005; Brasier 2008). The names, sudden oak death and Dutch elm disease resonate with the issue. The threat is to indigenous species and woodland as much as to exotic ornamentals or timber plantations and it appears that the provisions of the Sanitary and Phytosanitary Agreement of the WTO are inadequate and may even be contributory to the problem. This gives space for the re-conceptualisation of plant disease as a phenomenon of disruptive human practices (monocultures, prospecting, vegetative propagation, promotional trade arrangements, spiritual high-ground of the well-maintained garden with a woody landscape) rather more than one of microbial mal-adaption and virulence (Döring et al. 2012). The classical approach to restricting exposure to disease risk has been the deployment of resistance genes derived from wild relatives (aliens) (Smilde et al. 2005) or resistant cultivars. Climate changes and international plant trade conspire to bring a new challenge to this approach especially for crops with long breeding cycles and where the perennial timber crop stands for the next 50 years are already planted. There is a case for reconsideration of the common binary host–parasite model of crop disease along with its associated gene for gene resistance breeding strategies and to give prominence to the other agents, which are implicated. We include here cultural and entrained agricultural practices, vectors and other agencies of transmission as well as climate, geography and the dynamics of adopted crop types. This paints a fuzzy picture of culture/climate conspiracy but we are confident that genomics will take its place alongside the supportive tools of crop science agronomy and social analysis in unravelling the complex interactions and in rationalising collaborative design of integrative strategies minimising

emergent diseases and their impacts. Plant breeders, private or public and resistance (R) genes cannot do it on their own.

Whatever else we learn from experiences of silviculture certainly it is clear that trade-related biosecurity will need an upgrade of provisions under the SPA regardless of how much this may be contested by competing interests in maintaining the status quo. The promotion of agroforestry as a means of stabilising vulnerable production systems reinforces this imperative.[11]

In the context of institutional drivers and recognition of the issue of emergent pathogens, it is worth noting the IAEA funded a project, responding to the Transboundary Threat of Wheat Black Stem Rust (Ug99).[12] This aims to promote the use of technology packages integrating mutation induction and efficiency enhancing bio- and molecular technologies for developing wheat varieties that are resistant to Ug99, a virulent race of the fungus causing the trans-boundary spread wheat black stem rust disease. According to surveys, this disease is destroying up to 80 % of affected crops, in some areas almost all. Some 90 % of commercial varieties fail to resist this new virulent race. Global warming may send stem rust into parts of the world where it was never seen before.[13]

This apocalyptic warning is coupled by the FAO/IAEA joint program to a set of centralised transformative technologies but carries no enquiry as to causality beyond the binary host parasite model. Our suggestion, based on the arguments above would be that as a principle the broader context of causality be considered alongside the deployment of such resistance mechanisms generated through novel genetic variation.

In relation to the general proposition introduced above the prospect of mutation coupled to TILLING should the golden promise be fulfilled, has been articulated as an extreme adaptation to salinity for rice for planting and recovery resilience following the damaging Japanese Tsunami (Abe et al. 2012). This emphasises the role of the formal public sector in relation to the response to disasters.

13.2.6.3 Genetically Modified Crops and Crop Products

It has been a part of our general argument that people and their local social groupings tend to be side-lined in the discussion of solutions to the challenges of climate change, relative to the imperatives of technical momentum. People and their patterns of consumption and pressure on global equilibriums are recognised as a part of the cause of climate change and also as a part of the solution, if we assume that those damaging behaviours can be reversed. But people and their interactions

[11] http://www.un.org/climatechange/projectsearch/print.asp?projID=285&ck=bzIQk94xeXu1IGT.

[12] http://www.globalrust.org/db/attachments/resources/863/4/Lagoda_BGRI2oo9.pdf.

[13] http://www.un.org/climatechange/projectsearch/print.asp?projID=285&ck=bzIQk94xeXu1IGT.

with technology tend to be an afterthought when new and potentially transformative technologies are being introduced as solutions to socially situated challenges (Feenberg 2005). Smith and Stirling (2008) have argued for new approaches in this regard, which recognise the negotiability of the links between transformations acting at the techno-social level and their implications for socio-environmental practices. This is particularly relevant to securing public buy-in to the products of adaptively capacitated agriculture as much as to mitigation via behaviour change.

In the case of crop-focused GM technologies, however, what is popularly represented as the public voice, has acquired significant agency. Peoples' misgivings concerning the application of genetic manipulation technologies to the production and to the nature of their food have been the subject of much high profile commentary (e.g., Nuffield Council on Bioethics 1999; Hughes and Bryant 2002) and numerous attitudinal surveys (e.g., AEBC GM Nation 2003; Getliffe 2012) and consultations. They have further been translated into oppositional movements and calls for moratoria on the further introduction of plant varieties carrying novel traits into agricultural practice. This has been the case particularly in the states of the European Union. The institutional response has been to impose regulatory oversight and to appoint national competent authorities to manage the perceived risks of environmental release and consumer concerns over food safety. These provisions extend to the international dimension under the CBD where the terms of the Cartegena Protocol govern the transfer of live GMOs between states and accords liability for adverse consequences.

The regulatory environment poses an on-going significant hurdle to the development of novel traits and adaptive capacity by the plant breeding community (especially in Europe and Australasia) and also to international trade in seed. Interestingly, in relation to the intellectual property constraints linked to the deployment of transgenic traits, it is anticipated that the proprietary IP embodied in regulatory dossiers will, as the corresponding patents expire, pose the most enduring barrier to distributed decentralised access to GM technologies.

Nevertheless, GM-bred varieties of crops have been widely adopted across the globe (and in fact, in one EU country, Spain, where BT-maize has gained significant market penetrance). Most recent figures indicate that crops bred by GM technology are grown on about 160 million hectares of land spread across 29 countries (ISAAA 2012).[14] It is also claimed that 90 % (15 million) of the farmers growing these crops are resource-poor, husbanding small parcels of land in less-developed countries (ISAAA 2012). However, we need to say that the range of GM-bred traits currently in use in commercial farming and horticulture is very limited and none are aimed specifically at adapting crops to the effects of climate change. Nevertheless, the recent creation of salt-tolerant durum wheat in Australia (Munns et al. 2011) and of

[14] ISAAA (2012) *Global Status of Biotech/GM Crops: 2011*; available at http://www.isaaa.org/.

drought-tolerant soybean in Argentina (Samuel 2012),[15] plus several other drought-tolerant crops in the R and D pipeline all point the way to traits that facilitate greater crop adaptation.

The example of salt-tolerant durum wheat in Australia is very pertinent here and thus merits further discussion. The scientists who undertook this work decided, because of the complex regulatory framework around and sensitivities about GM technology, to use non-GM techniques. By use of complex, and we need to say, highly un-natural methods, they forced a hybridisation between a salt-tolerant einkorn wheat (*Triticum monococcum*) and *Triticum durum* and then carried out several generations of backcrossing to *T. durum*. Overall, the project took 15 years but the irony is that the gene that confers salt-tolerance in *T. monococcum* has been identified and could have more easily been introgressed into *T. durum* by GM methodology. As the authors say in a commentary on their work, if we are to breed crops for a changing climate, we do not have the time to wait a further 15 years for the next advance. As an aside, we note that this case exemplifies the self-contradictory position that governments have got themselves into because of over-zealous responses to those campaigning against GM crops. "Conventionally" bred salt-tolerant wheat, once it is registered as a new variety, can be put straight onto the track to commercialisation. GM salt-tolerant wheat, carrying the same gene, would be beset by a mass of essentially inhibitory regulations.

It is indeed possible that as the pressures for greater crop adaptation increase and benefits accrue to those states and agricultural systems, which have embraced GM, opinion leaders and activists in Europe and Australasia will shift their position and reassure the public. We are already starting to witness some softening of and even backing away from extreme positions (e.g., Lynas 2011).[16] However, until such changes of mind become more widespread, the international seed market will remain tricky territory for navigation by plant breeders working on adaptive traits.

13.3 Breeding and Seed Systems

13.3.1 The Social Context of Plant Breeding: Who Breeds for Whom and Who Chooses for Whom?

The world of breeding constituencies is commonly split along the lines of the binary categories of formal and informal seed systems as contrasting representations of how breeding practices are determined and conducted and how the resulting seed and its value distributed and integrated into social practice. This distinction extends also to the contrasting ways in which traits and germplasm are evaluated, accessed

[15] Research by Raquel Chan and her team, reported by Samuel L (2012) Drought-resistant Argentine soy raises hopes, concerns. http://phys.org/news/2012-04-drought-resistant-argentine-soy.html.

[16] Lynas M (2011) *The God Species*. Fourth Estate, London.

and deployed. It can also be associated with the ways in which adaptation and the risks associated with climate change are perceived and experienced. Of course, as with all binary distinctions there is a risk of oversimplification and of allowing categories to determine rather than to reflect or account for differences, but in practice the formal/informal dichotomy has provided a reliable framing for the discourse on who breeds for whom and how choices are made. It also provides a convenient means of disaggregating and explaining the complex of influences inherent in global change discussed above. Furthermore, as we expect to demonstrate with examples from different agricultural scenarios, the convergence of the knowledge systems and practices and materials intrinsic to the formal and informal systems offers a reasonable way forward towards crop adaptation linking the capacities of the two kinds of communities (Louwaars 2007; Richards et al. 2009; Offei et al. 2009). In order to pursue the question of how the formal and informal seed systems, or at least representative examples of them, might cooperate in developing adaptive capacity as pillars to support robustness and resilience in socio/agricultural systems, it is first necessary to explore their distinguishing features in terms of both their practices but also their motivations and knowledge flows. To extend this investigation we shall highlight some examples of Public Private Partnerships, Participatory Plant Breeding and Participatory Varietal Selection as an interface of practice between the formal and informal systems. Differentiation of the formal and informal systems is based on the writings of Louwaars (2007).

13.3.1.1 Formal Seed Systems

Formal seed systems are characterised by centralised breeding operations often embedded within national or transnational companies or institutions (e.g., Monsanto, Pioneer, CGIAR centres), which face outward to global competitive markets and function within institutionalised seed certification and variety registration provisions. The most well recognised players tend to focus on the high tonnage crops of international trade and economies and classically represent a few-to-many configuration with a few varieties pushed into many production niches with an emphasis on broad adaptability and intensively managed agronomic inputs for the realisation of high yield potential.

Formal systems tend to be oriented around pedigree selection and F_1 hybrid breeding systems, exploiting existing elite germplasm coupled to the systematic introgression of adaptive performance traits and product quality traits and supported by best technologies of marker-assisted selection and trait identification. This restricts the genetic base (narrow variation) of the set of varieties, which reach the field and produce revenues, attract elite status and act as inputs to subsequent rounds of breeding. It has been estimated for example by Powell[17] that just ten wheat varieties represent and contain all of the genetic diversity present in the entire

[17] http://www.genomicsnetwork.ac.uk/media/IP_Workshop_Report_July08_Final.pdf.

UK wheat crop. The large investment implicit in these established approaches is redeemed by large sales' volumes and seed price margins, which reflect to some degree, the value added through potential advances in performance. A considerable portion of this investment is devoted to the process of capturing that value within the governance systems of seed marketing, distribution and accreditation. We have already looked in Sect. 13.2.6.1 at the national and international regimes such as the UPOV Convention, which trap the added value of crop plant varieties as a form of intellectual property, in terms of the influence this has on the exchange of traits and knowledge. At this stage we shall point to the path dependence which compliance with national laws and requirements for distinctness uniformity and stability[18] places on breeding practice and the concept of what constitutes a plant variety i.e., a fixed entity, which will behave predictably under precisely defined trial conditions. It is reasonable to ask how within these confines considerations of robustness and resilience of an agro-ecological system map within such a system where, for instance, functionally adaptive morphological plasticity would disqualify a variety from recognition.

The institutions themselves are hierarchical and their strategy driven in a top-down way in particular by the marketing arm as much as by what the breeders themselves think they can achieve technically. The visionary ideotype towards which traits are introgressed and selections are made resides at the core of breeding strategy. It depends upon the assumptions of genetic essentialism i.e., that alleles at loci determine traits and then manifest in the same way or procure the expected phenotype when transferred from one genetic background to another. Fortunately this works some of the time, sufficient to let us feel as if we have a capacity for design and prediction.

However, the recently elaborated principle of transgressive variation (Maas et al. 2010) suggests that adaptive traits managed within and between subpopulations can behave unexpectedly. This deepens the game. Arguably it calls for a closer consideration of individual trait interactions with background germplasm and probably more basic research on adaptive trait associated loci and their epistatic relationships.

The idealised business model elaborated above exists in association with a collection of smaller agents representing what is normally known as the ag-biotech sector. Here the focus is on identifying and modularising and mobilising traits as tradable knowledge flows.

They are often founded as spin offs from academic research centres. If really successful they are likely to be absorbed in to the larger breeding institutions or to sell or licence traits and technology packages. Few have the resources or experience to participate directly in the formal seed system. These relationships are based on commercial exchange, which is founded in the technology transfer paradigm and the formalities of intellectual property rights. A representative instance is Performance Plants (http://www.performanceplants.com/), a company which practices

[18] The DUS is a pragmatic requirement constructed to suit the needs of a formalised certificatory system rather than adaptive capacity.

trait development with a focus on temperature, drought and water use efficiency adaptation.

Links to agronomy can be eroded though the centralisation of formalised plant breeding, though the nesting of the large scale breeding companies in the agro-chemical sector is a strong feature of the formal system. This may help to explain the trait and gene locus orientation of the formal seed system. It is customary for the ag-industry to view single bio-agents as providing solutions. This fits very well also with the assumptions of the single additive transgene approach to modulating adaptation.

However, the ongoing disconnect between formal breeding and the experiences of practical agronomy at the farmer level has been widely noted. First the Seed, Kloppenburg's (Kloppenburg 1988) particularly insightful account of the commercialisation of F_1 hybrid maize breeding and seed management practices. He posits a division of labour as a turning point at which the cross-informing of farmers and breeders morphed into the one-way process of promoting hybrids marketing.

Knowledge among professional breeders is for the most part formalised and its transfer and deployment is instructional, which is to say that it is rules-based, prescriptive and supported by expertise which is taught, formulaic and professionally legitimised. This is augmented by entrenched and convergent evaluative norms and regulations, which as we saw above add to costs of maintaining competitive viability of the system. At the same time we have to appreciate that even in the formal seed system, and this is very much asserted by practicing breeders, tacit skills and knowledge acquired over years of close personal and physical engagement are important just as we shall see they are in the informal seed systems. However, both the fields of knowledge application and the modes of acquisition of tacit knowledge are very different across the formal and informal sectors.

This account of the formal seed sector, by concentrating on concepts and approaches and prescriptive governance suggests a rather more uniform collection of agents than actually exists in the world. There is in fact a wide diversity of breeding operations within the formal sector, differentiated by scale and locality, the focus on crop types as indicated by the contents of this book project (major grains versus specialist or orphan crops), narrow or broad portfolio but generically split into the categories of public and private sector. Although it may seem that the private sector has the power to make its own decisions on its breeding goals, because of the focus on ideotypes and traits it is restricted by the access to the knowledge and access to the genetic correlates of appropriate traits. We were reminded above that traits knowledge is elaborated in research institutions and specialist biotechnology ventures. Arguably then the funding agencies, which support research, as well as the investors who maintain the small specialist companies play a significant though potentially detached role in determining what goals breeders of the formal sector are able to include in the design of their ideotypes. This implies a distributed, delocated and pragmatically driven decision-making system; though of course we can locate many exceptions.

A very interesting exception is the private breeding centre called the Land Institute,[19] which is driven by a mission. That 30-year mission is to develop a set of perennial varieties of the major grain crops. In relation to the agro-ecological challenges of climate change the benefit of the perennial habit may lie agro-ecologically in the ability to stabilise the crop, ground cover and terrain during floods and droughts plus, from the social perspective, in the avoidance of having to seed and establish a rooted crop each year under uncertain conditions. Although their approach may seem to fly in the face of the adaptive capacity of varietal diversity, it is possible that interplanting of differently adapted perennials may provide a degree of compensatory plasticity to a crop stand. No perennial varieties have yet been released from the institute but it will be interesting to follow their performance as a response to climate change.

As we deliberated in Sect. 13.2, there is a general orientation of institutions around the assumption that the solution to adaptive capacity building lies in the proliferation of formal seed systems of the public or private persuasion in those regions which lack them and that national policies should be turned towards establishing appropriate governance and to promoting this ambition. This was well articulated by the ITWGPGRFA in their report on Strengthening Seed Systems (Gap Analysis of the Seed Sector 2011). Also perhaps the successes attributed to the green revolution varieties can be quoted in support of this proposition. We would like to suggest that judgement be suspended while we give some air first to Harwood's take on the costs of ignoring smallholders in the development of breeding policy and also have a chance to examine informal seed systems and the continuing contribution they can make (Harwood 2012).

13.3.1.2 Informal Seed Systems

In contrast to the centralisation of formal systems, informal seed systems are characterised by distributed nodes in a network of breeding activities, which are inward facing in so much as they embody the sharing of seed and knowledge between the members of the network, who are practicing farmers dispersed in communities.

They are further characterised by experimentation and non-instructive learning (Richards et al. 2009). Breeding and selection takes place across the set of ambient environments in which the seed will ultimately be cultivated which is to say that there is a close integration of the production, consumption and experiential learning functions of networks.

There is a tendency to regard the informal system disparagingly because of its low technology status and want of formalised process, empowered voices and systematisation. However, sociological studies warn us against falling into this trap. It is clear that farmer/breeders experiment effectively in evaluating the

[19] http://www.landinstitute.org/.

suitability of new seeds to their agro-ecological circumstances (Richards et al. 2009; Richards 2010; Mokuwa et al. 2012). They are clearly also enabled by their experience to recognise novel types produced by out crossing which appear in their cultivated material and to advance the selection of segregating progeny to develop varieties better viewed as ecotypes, which is to say they are adapted to local ecological but also cultural circumstances (Nuijten et al. 2009).

There is a further tendency to regard seed material in informal systems just as a reservoir of ecotypic variation and only as of value for the downloading of adaptive traits for introgression in ideotypes of the formal system.

Against this a strength of the informal system resides in its many-to-many configuration. Which is to say that many different seed varieties (agro-biodiversity) are available to be deployed in different locations dependent on socio-ecological circumstances in space and time. The many-to-many configuration portrays unfathomable complexity but in this case one which is kept within bounds by the scale of the networks and by the tacit knowledge of its members. Almekinders (2001) in her account of the management of crop genetic diversity at the community level makes the point that this position is under pressure worldwide and the loss of crop diversity is of great concern. Support for the maintenance of in situ diversity is founded on the premise that improving farmers' use of diversity contributes to their sustainable livelihood and thus to their ability to contribute to its maintenance. Almekinders (2001) provides an extensive analysis of the community processes, which are not constrained by the requirements of seed registration and the strictures of DUS, which means that there is scope for internal diversity (redundancy) and plasticity.

Choices made by farmers are intimately immersed in the agro-ecology of their terrain. They are also embedded in and adapted to cultural norms and needs of the local food system, especially when under the stewardship of women who at once breed, grow, harvest, store, cook and feed their families.

Formal and informal seed systems coexist all over the world but informal seed systems actually predominate over formal provisions in certain parts of central Europe, in South America and also in Sub-Saharan West Africa where they account for more than 70 % of the seed planted. Edwin Nuijten (2005) has made a systematic study of the social and agricultural practices as well as the dynamics of seed selection and adaptive diversity within The Gambia. This coastal region is notable for its climatic instabilities, floods and droughts coupled to the North Atlantic Oscillation, against which farmers exist under a severe challenge both to their own adaptive capacity and that of their chosen crops. The study is particularly poignant since it represents the technical and social context in which farmers are already facing the forms of climate instability, which are predicted to proliferate under the commonly adopted models of climate change.

Skilled both as a plant breeder and social anthropologist, Nuijtens is able to assess how the dynamics and sources of genetic variation (gene flow) map across the agrarian communities and contribute to robustness in terms of the adaptive choices farmers were able to make. He concludes that recurrent mass-selection by farmers (to farm is to select he asserts succinctly) plays a smaller than expected role in sustaining gene flow and varietal diversity. But against this seed exchange

between farmers coupled to the ability to recognise and propagate novel variation (off types), as it crops up though cross-pollination, is significant and arguably represents an informal verisimilitude of what formal breeders do, but in this case, intimately linked to social conservation of varietal diversity (Mokuwa et al. 2012). Observations of the choices farmers make to switch varieties and move to alternate types during climatic fluctuations reinforce the value of the corresponding reservoir of shared materials and the knowledge, which goes with them, and the way this can support robustness both at the level of plant and socio-ecological adaptation.

A significant observation of farmer selection of off-types in action has been illuminated by molecular genomic analysis of rice varieties in the extended region of West Africa (Nuijten et al. 2009) around The Gambia. The possibility of past hybridisation between indigenous varieties (*O. glaberrima*) and introduced varieties of *O. sativa* var. *japonica* or *indica* was first observed by Jusu (1999) as intermediate types in farmers' material in Sierra Leone and was all the more likely to be the case given social practices of seed exchanges and farming practices of interplanting, despite the botanical assumption that the species were incompatible. Some 80 amplified fragment length polymorphism (AFLP) markers were used to construct a relational map of the distribution of DNA polymorphisms across a collection of local farmer varieties. Within the collection there was a significant subset containing an intermediate distribution between *japonica* and *glaberrima*-specific polymorphisms, clearly indicating a past hybridisation event in the derivation of these varieties. Whatever construction we place on this example of the management of variation, fortunate accident, or playful experiment or acute observation linked to an understanding of what might constitute a valuable option for the future, it is clear that farmers' practice within the informal seed sector needs to be taken seriously when we look forward to mobilising the best of breeding practices to secure robustness and resilience in the face of climate change.

In this regard it is notable that the formal seed system, in this case the public sector represented by WARDA (West African Rice Development Association now the West African Rice Centre) has recently (recent in terms of varietal time lines) produced a set of varieties based on laboratory-based hybridisation between African and Asian rice (WARDA 2001) as a means of combining high yield potential with adaptation. New Rice for Africa (NERICA) varieties are now being distributed in the region and according to some evaluative reports, under appropriate managements are beginning to gain traction with farmers and to deliver yield and income benefits (Diagne et al. 2010). However, according to a critical study by GRAIN[20] uptake of NERICA by farmers in the absence of external inducements from aid agencies and pressures from private sector initiatives, has been slight despite the significant investment placed behind their promotion, multiplication, and distribution by donors (BMG, World Bank Rockefeller Japan UNDP). The problem does not lie with hybridity, as we have seen above, it is common in informal varieties. Perhaps the problem lies with the commodity-based approach

[20] http://www.grain.org/article/entries/111-nerica-another-trap-for-small-farmers-in-africa.

adopted by WARDA. WARDA acting as a formal agent set its own breeding targets based on its perception of yield components for upland rice with the ambition, according to the project leader, not of replacing informal varieties but of persuading farmers to integrate Nericas into their variety portfolios. The chosen traits included drought tolerance, heat tolerance, short straw and early flowering, but did not draw upon the voices or experience of farmers. Farmers and the informal seed systems were only brought into the equation when invited to view local demonstration plots in what was termed "participatory variety selection" (PVS). Small surprise then that there was no strong fit with robust farmer practice. A seemingly small example highlighted in the GRAIN critique is highly illustrative. The above-mentioned early flowering trait, though buffered from bird predation in large crop stands renders a variety as selectively prone to predation when interplanted in mixed stands of small-holder's plots. However, despite these subtleties the top-down campaign to install Nericas as Africa's green revolution continues while some might feel inclined to join Harwood (2012) in asking what the formal seed system has learned from the previous ones.

13.3.1.3 Convergence of Formal and Informal Systems

Nuijten (2005), from his studies in the Gambia and more broadly in West Africa, makes the conclusion that the formal seed system does in fact have much to learn from the social practices of seed sharing and the learning processes reflected in farmer selection and maintenance of robust adaptability. At the same time he recognises the capacity of the formal sector to access wider variation and to organise more extended and targeted seed dissemination in support of socio-ecological resilience during extreme events and environmental disasters. He envisions a coexistence of the formal and informal systems reinforced by cooperative practice and knowledge sharing.

Though more concerned with the way in which culture, economics and politics shape technologies Puente-Rodriguez (2008, 2010) makes similar types of observations from his ethnographic studies of potato cultures and seed distribution in marginal agro-ecosystems of the Bolivian Andes.

The region embodies an extended diversity of varieties with some communities maintaining and cultivating 60 varieties and more of the 5 cultivated species of the region. The varieties add not only agronomic diversity and robustness but also to dietary and cultural richness in terms of flavours, appearance and occasions of use, to the extent that communities regard them as cultural objects rather than material resources. At the same time communities share seed and there is a traditional periodic traffic from high to lower elevations for the provision of fresh virus-free seed. Demographic, institutional and economic changes offer to transform this scenario. There is significant migration away from agricultural communities and there is pressure to grow and send to market particular varieties of the formal seed system. These pressures, claims Puente-Rodriguez, are supportive of the generalisable correlates that informal systems persist within low input agronomies

and that the injection of varieties from the formal system erodes the broad varietal base of the informal. Furthermore, he notes that the formal seed sector jeopardises an opportunity to engage peasants and their material.

In trying to assess the potential of genomics to sustain the robustness of peasant varieties he develops the notion of territoriality, a concept ascribed to Deleuze and Guatari (2005), which describes the de-location of power and decision-making capacity from practitioners (deterritorialisation) as centralised technologies make their mark.

As his example he takes a genomic initiative called Whipala Genomics, which by using genomic molecular marker technology seeks to support identities for the diversity of peasant varieties in order to develop market recognition for them. But the key point is that the initiative is territorialised within the peasant communities. The potential of molecular marker technologies to untangle the complexities of varietal naming and distinctness in communities of sharing where varieties with the same name may be different and vice versa, for reasons of language and cultural history across sharing communities has been remarked upon by others (Nuijtens 2005; Richards et al. 2009). This represents an interesting departure for the interaction of the advanced technologies of the formal seed system with the informal system in that it places (reterritorialises) the driving imperative with the agricultural community and the context of its needs to stabilise its material (on-farm conservation) and cultural objects in sustenance of its robust adaptive capacity.

13.3.1.4 Participatory Plant Breeding

In further exploration of the location of decision making over seed management and breeding trajectories we will next examine an example of closely integrated cooperation between the formal and informal systems in southern China. Participatory Plant Breeding (PPB) emerged as a concept back in the 1980s arguably as a response to the perceived failure of the previous green revolution to engage with small holders, low-input systems, and marginal environments (Bentley 1994). It was recognised as a part of a general movement in farmer participatory research. Since then numerous examples of models for PPB involving varying degrees of participation have been elaborated. Participatory variety evaluation as we saw above involves a minimal participation at the output end of the operation in which farmers' voices are unheard except as a distant feed-back that may go unheard if the momentum of donor-backed policy is sufficient. At the other extreme fully participatory plant breeding engages farmers at all stages, extending to the inclusion of informal seed as a germplasm resource in crosses as well as in selections, seed distribution, and multiplication. It has attracted its detractors (a temporary fad which ignores the complexities of negotiating across the breeder farmer divide) and its promotors (Witcombe et al. 1996; Weltzien et al. 2000)[21]

[21] http://pdf.usaid.gov/pdf_docs/Pnack761.pdf.

some of who point to the potential of fully integrated PPB to support farmers' rights to a share in the benefits of the deployment of their adaptive traits (Halewood et al. 2006).[22] The FAO have sponsored a comprehensive volume (Plant Breeding and Farmer Participation 2009) which systematically specifies and rationalises best practices in PPB. Of particular relevance to the argument concerning the location of decision making regarding the selection of targets for plant improvement Chap. 4 by Weltzien and Christinck (2009) is dedicated to Methodologies for Priority Setting. The chapter sets out the issues to be taken into account in priority setting as well as listing the diverse stakeholders both institutional and social who need to be brought into participation. The authors also engage the discourse concerning the relative merits and significance of focusing on the goals of broad adaptation versus local adaptation. We infer from the above FAO initiative a growing enthusiasm for constructive convergence of the formal and informal seed systems at institutional level, which we regard as critical to capacity building for robustness and resilience from crops to communities of practice.

In the aforementioned review Halewood and colleagues provide a helpfully concise and accessible account of the practices of PPB pointing to successful examples from around the world and notably to the burgeoning engagement of the CGIAR-mandated breeding centres as anchors of the formal end of the practice and often women farmers as anchors of the informal. They highlight the case of a study of maize varieties in the southern provinces of China in particular Guangxi, which provides an encouraging example of farmers and formal breeders working together and learning from each other's experiences of exposures to environmental, demographic, and economic challenges. In a series of edited papers Song and Vernooy (2010) provide an in depth account, based on action research at the regional and community level of a PPB program aimed at developing a diversity of adapted maize varieties suitable to adaptive agricultural systems whilst being acceptable to local taste preferences. The action was built on the findings of an impact study (Song 1998), which showed that introduced maize germplasm from CIMMYT produced formal varieties, which were too narrowly based and unsuitable for local adoption. It was also founded on recognition of demographic change and the stresses imposed by migration to the cities of young people and male farmers as well as the fluctuations of climate change and the need for a diverse and robust varietal portfolio. Within this scenario, the participatory approach, besides empowering women's voices and engaging the best of formal and informal knowledge, stakeholders and practices has produced a set of well regarded and efficiently distributed varieties and has reinforced local farmer organisation and decision-making capacity. In short, it has supported robust adaptive capacity the influence of which has passed up to national policy level.

This initiative represents a notable continuity with other examples of the mobilisation of peasant skills in China, which argue for the bottom-up approach,

[22] http://www.bioversityinternational.org/fileadmin/bioversity/publications/pdfs/1254.pdf.

in particular the case of rice and locally produced and distributed hybrids as studied by Shen (2010).

The role of NGOs[23] in linking the formal and informal sectors should not go unremarked. An initiative which has led to widespread cooperation is the CBDC (Community Biodiversity and Development Conservation) network that has witnessed the integration of inputs from diverse actors informal and formal as well as NGOs in projects. Biodiversity International is also a significant factor in participatory programs.

Also, the resources of long-standing donors to the cause of food security like the Rockefeller Foundation have been directed to initiatives like AGRA, which includes:

> research and development of new seed varieties that are better able to resist drought. For example in May, through our grantees, more than a dozen varieties of four crop species were released—cassava, peanut, cowpea and sorghum—most of them coming from Uganda, and the result of original breeding that made use of local germplasm, plus specific traits contributed by material from the Consultative Group institutions.[24]

This is a working example of elite materials contributed by the formal sector combined with those of the informal against an established target for climatic adaptation. But it is not clear from Rockefeller's own account the degree to which the meaning of drought was assessed in relation to local patterns of water deficit or how that integrated with farmer practice.

13.3.2 Broader Sectoral Collaborations

An interesting and slightly unusual set of collaborative links relative to the context of robustness and also mitigation was embodied in an initiative for the development of allopolyploid (interspecific hybrid) wide hybrids derived from diverse accessions of *Brachiaria* species. The varieties, the first of which were named Mulato I and II were stabilised by an apomictic breeding system common in the genus, which potentially supports a high degree of internal genetic diversity within selections. The hybrid cultivars of this tropical forage grass have been commercially released and have passed into widespread cultivation as the preferred planting material for pastures in Central and South America and South-East Asia. The cultivars combine resistance to insect predation with drought tolerance, high dry matter productivity, palatability and good nutritional content relative to established *Brachiaria* cultivars. The breeding system and supporting technology required to achieve this was developed by Miles (Argel et al. 2007)[25] and his collaborators at the

[23] Commonly referred to in many quarters as Civil Society Organisations.

[24] http://www.rockefellerfoundation.org/news/speeches-presentations/bringing-resilience-agenda-climate.

[25] http://webapp.ciat.cgiar.org/forrajes/pdf/mulato_ii_ingles.pdf.

CGIAR centre CIAT in Colombia in partnership with EMBRAPA and a Japanese donor. EMBRAPA, the Brazilian Agricultural Research Corporation, constitutes a network of 38 research centres as well as transnational cooperative programs with the goal of supporting smallholders through innovation with a focus on mitigation of environmental impacts.

Early economic models envisaging the cultivation of these hybrids on 2.5–5 % of the pasture areas of Central and South American beef- and milk-producing countries predict best scenario benefits of up to 40 % of the production value, accruing both to consumers (via increased availability and price drop) and producers (as improved efficiency and overall productivity). Seed production and distribution and its influence on the rate of adoption emerge as a potential bottleneck to achieving these gains. CIAT as the holder of proprietary formal rights has selected a Public Private Partnership (PPP) via licensing to a single commercial partner (Gruppo Papalotla, Mexico) to address this issue. However, Papalotla have sponsored the decentralised multiplication of seed to feed local markets, notably in SE Asia (Hare et al. 2007).

It is not clear yet what production gains might be achieved on more marginal or less intensively managed and species-diverse pasturelands or whether the cultivars might contribute to achieving sustainable protective ground cover in more vulnerable scenarios. It is claimed however, that the cultivars are compatible with leguminous forages so perhaps they will support and have a role in species-diverse grazing. As mentioned in Sect. 13.2 there is some tension between the proponents of intensive management of pasturelands and the advocates of reestablishment of diverse native locally adapted species as a means of supporting robust occupancy of rangelands. Stipa Native Grasses Association[26] presents arguments for the latter approach but at the same time agrees that practices of grazing management modified to be more imitative of migratory herd behaviours may be required.

Nevertheless, against this complicated interplay of practices, climate mitigation imperatives and breeding innovation the Mulato introduction constitutes a very interesting experiment on two grounds. The first as discussed above relates to whether hybrid polyploid apomicts will deliver robustness across a range of agricultural settings and practices sufficient to support livelihoods and to promote carbon sequestration over time. The second is more subtle and relates to recent discoveries of heritable epigenetic markings linked to adaptation. It appears that selective silencing of particular alleles (allelic exclusion) may moderate the relationship between genotype and phenotype in hybrids, contributing to adaptive plasticity (Guo et al. 2004; Hegarty et al. 2008). It also appears that experiences of stress response may be carried forward from one generation to the next and effectively pre-adapt to a subsequent challenge (Luna et al. 2012). This raises the intriguing possibility that the locality of seed multiplication may influence adaptive capacity, in which case we have uncovered an interesting rationale for decentralised or even in situ seed multiplication.

[26] http://www.stipa.com.au/NativeGrasses/BenefitsOfNativeGrasses.html.

To put the question another way, how much robustness resides in epigenetic plasticity, or to what degree have breeders denied themselves an opportunity for enhanced adaptive potential by centralised practices and the imperatives of DUS?

13.3.3 Formal–Informal Cooperation and Resilience in the Face of Climatic Disasters

The scenarios explored above reveal contrasting locations of knowledge and materials as well as patterns of cooperation between formal and informal seed systems. Our intent was to illustrate the ways in which robust adaptive capacity, both material and epistemological, acquired close to impacts of climatic stress can be marshalled alongside the technological capacity, material resources and professional practices of the formal seed system. In this argument we have yet to look at adaptive capacity in relation to resilience in the face of disasters precipitated by extreme climatic events (IPCC 2012).

Looking at agricultural systems we can anticipate that the need for external support will depend upon the extent to which basic seed reserves are destroyed and seed supplies lost along with growing crops during the event.

There have been some attempts by academic observers in support of aid agencies to link past instances of restorative intervention to appropriate and less appropriate practices in relation to seed relief. An annotated bibliography of the accumulated literature has been assembled by Rubyogo, Sperling and Remmington (2004).[27] The literature includes several accounts of post-disaster recovery in particular that following Hurricane Mitch in Honduras where it was noted by Barbentane (2001) that coordination between local communities and external agencies was lacking and the easy path of supplying broadly adapted seed was resorted to. The emergent message seems to be that careful assessment of the nature both of the local culture and its seed systems as well as the impact of the disaster is critical and that the rush to replace lost varieties with broadly adapted high-yielding varieties from the formal sector can be counterproductive.

Longley et al. (2003) note that farmers rarely lose their reserve seed during natural disasters and that even where they are lost, seed relief is only a part of re-establishing livelihoods, which can be all the more difficult if unsuitable varieties are presented. There is an imperative under these circumstances to source material as close as possible to that which was lost. As observed by also by Nuijtens (2005) and (Longly and Richards 1999; Richards 2006) the formal seed system and its associated infrastructure may be able to provide appropriate relief by identifying and acquiring such varieties in neighbouring territories and then by multiplying up and distributing seed for farmer evaluation. In the extreme it may even be that seed is accessed from

[27] http://ciat-library.ciat.cgiar.org:8080/jspui/bitstream/123456789/6638/13/OFDA_bibliography_June_2004.pdf.

ex situ collections of the FAO undertaking. With respect to plant breeding and genomics it is interesting to note that molecular marker technologies may provide a reference framework for the reinstatement of lost varieties and variation following disasters. Resilience may thus be instrumentally augmented by anticipatory molecular categorisation of the pool of variation which accords robustness to the local agricultural system.

However, as remarked previously, under circumstances where lasting damage is inflicted on the agro-ecosystem as well as the techno-social system we might expect that novel and extreme adaptations might be required. The formal seed systems are best placed to provide these but it is arguable that farmer knowledge, tacit skills and experimentation are still important to integration of such material into robust farming practice.

13.3.4 Concluding Considerations

We are drawn to the view that bringing together the institutionalised and the socialised capacities of plant breeding in their formal and informal settings will be the test against which our best efforts to adapt and to mitigate will be judged. This will require redistribution of the loci of decision making concerning appropriate portfolios of varieties in order to generate a robust adaptive capacity and to support resilience in extremis. It will also require a sensitive levelling of the playing field with respect to the privilege accorded to diverse provenances of knowledge and material property rights in order to maximise opportunities for cooperation. This will in turn require significant institutional and regulatory readjustments (see UNEP 2012).

Genomics will have a part to play in all of this by providing support for establishing material identities and for transforming collectively prioritised breeding goals into a larger and more diverse set of accessible varieties than hitherto conceived.

> Coda
> Technologies for food security have to be robust and self-explanatory enough to withstand a good kicking in use. We need malleable components that adapt themselves to the needs and purposes of users, not entire ready-made systems. Perhaps, in fact, we need a technology revolution that emerges from the needs and purposes of the users themselves. This amounts to call for a food security revolution from within. Richards (2010).

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