CHAPTER 1

GENOMICS-ASSISTED CROP IMPROVEMENT: AN OVERVIEW

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Abstract: In recent years, a truly impressive number of advances in genetics and genomics have greatly enhanced our understanding of structural and functional aspects of plant genomes but at the same time have challenged us with many compelling avenues of investigation. The complete genome sequences of *Arabidopsis*, rice, sorghum and poplar as well as an enormous number of plant expressed sequence tags (ESTs) have become available. In the next few years, the entire genomes or at least gene space will likely be sequenced for most major crops. However, improved varieties, not sequences *per se*, contribute to improved economic return to the farmer. Functional genomics and systems biology research are facilitating the identification of gene networks that are involved in controlling genetic variation for agronomically valuable traits in elite breeding populations. Furthermore, combining the new knowledge from genomic research with conventional breeding methods is essential for enhancing response to selection, hence crop improvement. Superior varieties can result from the discovery of novel genetic variation, improved selection techniques, and/or the identification of genotypes with improved attributes due to superior combinations of alleles at multiple loci assembled through marker-assisted selection. Although it is clear that genomics research has great potential to revolutionize the discipline of plant breeding, high costs invested in/associated with genomics research currently limit the implementation of genomics-assisted crop improvement, especially for inbreeding and/or minor crops. A critical assessment of the status and availability of genomic resources and genomics research in model and crop plants, and devising the strategies and approaches for effectively exploiting genomics research for crop improvement have been presented in two volumes of the book. While Volume 1, entitled "Genomics approaches and platforms", compiles chapters providing readers with an overview of the available genomics tools, approaches and platforms, Volume 2, entitled "Genomics applications in crop improvement", presents a timely and critical overview on applications of genomics in crop improvement. An overview on the highlights of the chapters of these two volumes has been presented in the present introductory chapter.

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1. INTRODUCTION

Since the beginning of recombinant DNA technology, it has been suggested that plant molecular biology has the potential to initiate a new Green Revolution for sustainable agriculture to meet the needs of a fast-growing human population world wide. Genetic engineering has already shown the potential of plant biotechnology for crop improvement. As a result, transgenic plants with high agronomic and environmental value have been developed for several crop species such as maize, soybean, cotton, tomato, potato, tobacco, papaya, etc. (http://www.isaaa.org/). In parallel, because of their ecological and evolutionary novelty, transgenic crops have also raised a number of questions and public awareness (Wolfenbarger and Phifer 2000). In addition, the costs and uncertainties that result from the rapidly proliferating national and international regulations covering transgenic crops have significantly hindered further development of additional crops and traits (Kalaitzandonakes 2004, Bradford et al. 2005, Jank et al. 2005).

Molecular genomics, in addition to genetic engineering, is another important area of plant biotechnology that provides tools and opportunities for plant breeding. Molecular markers are one of such tools that can be used in a variety of ways to assist plant breeding activities. Some of these applications include: (i) marker-assisted selection (MAS) strategies for germplasm improvement and cultivar development, (ii) gene pyramiding for accumulating multiple genes for resistance to specific pathogens and pests within the same cultivar, and (iii) examining allelic diversity in natural populations or breeding material to select the desired genotypes (Jain et al. 2002, Gupta and Varshney 2004).

In recent years, remarkable progress has been achieved in the area of plant genomics as large-scale genome or expressed sequence tag (EST) sequencing projects were initiated in several model as well as crop plant species. In some plant species, the genome sequence data have already become available (e.g. *Arabidopsis*, rice, poplar, sorghum) and similar efforts are underway to sequence either the full genome or gene space in several other plant species such as *Medicago*, *Lotus*, tomato, soybean, maize, wheat, etc. However, sequences *per se* do not improve yield or resistance to major crop pests and abiotic stress. In fact, functional genomics studies for various agronomic traits are underway in several plant species to study gene expression and protein interactions for identifying gene networks responsible for trait phenotypes variation in the elite breeding populations. Furthermore, advances in molecular genetics and genomics provide novel approaches that can be integrated into plant breeding programmes for a more "holistic" crop improvement approach, also referred to as "genomics-assisted breeding" (Varshney et al. 2005b). The present book, therefore, aims to present in a timely and critical fashion (i) the existing information on genomics research, (ii) the prospects and approaches to apply this information to crop breeding, and (iii) the achievements and constraints of applications of genomics in crop improvement.

For the sake of convenience, the chapters of the book have been classified in two volumes. Volume 1, entitled "Genomics approaches and platforms", provides an overview on the available genomics tools, approaches and platforms, while

Volume 2, entitled "Genomics applications in crop improvement", compiles chapters that provide a critical overview on the applications of genomics for the improvement individual crops. The present introductory chapter provides an overview of all the chapters of both volumes of the book.

2. VOLUME 1: GENOMICS APPROACHES AND PLATFORMS

Due to significant progress in the area of molecular genetics during the last two decades, enormous genomic resources have been developed for major crop plant species. As an example, for most crop species a large number of molecular markers and high-density genetic maps, large-insert libraries (e.g. BAC libraries), ESTs, etc., have become available (see Phillips and Vasil 2001, Tuberosa et al. 2002, Gupta and Varshney, 2004). These tools, have been used for MAS (Koebner 2004, Varshney et al. 2006), and have facilitated mapping and cloning of genes or quantitative trait loci (QTLs) leading to sequencing and annotation of large genomic DNA fragments in several plant species (Stein and Graner 2004, Salvi and Tuberosa 2005).

Because of the availability of the above-mentioned genetic resources and significant progress in automation, robotics and bioinformatics, complete genome sequences have already become available for *Arabidopsis* (AGI, 2000), rice (Goff et al. 2002, Yu et al. 2002, 2005), poplar (http://www.eurekalert.org/pub_releases/2006-09/dgi-tft090806.php) and sorghum (http://www.phytozome.net/sorghum); similar efforts are underway for several other plant species (www.jgi.doe.gov/sequencing/index.html). Additionally, the availability of gene sequence data has made it possible to develop molecular markers directly from the genes. Such molecular markers include microsatellites or simple sequence repeats (SSR, Varshney et al. 2005a), single nucleotide polymorphisms (SNPs, Rafalski 2002) and conserved orthologous sequences (COS, Rudd et al. 2005) and very often are referred to as genic or functional molecular markers. Development and applications of such molecular markers are discussed in Chapter 2 by Rajeev Varshney and colleagues. The genic molecular markers represent a useful resource for (i) identifying the perfect or diagnostic markers, if the marker is derived from the gene(s) responsible for expression of the trait, (ii) assaying the functional diversity in the germplasm collection and (iii) comparative mapping and synteny studies. Molecular markers can play a pivotal role to enhance breeding strategies in a variety of ways. In Chapter 3, Anker Sørensen and colleagues advocate for adaptation of selection methods in breeding, towards the integrated use of genetic knowledge based on DNA markers so that the potential of molecular breeding and available germplasm resources can be better exploited. One of the main applications of molecular markers in plant breeding is to identify and map QTLs to understand the gene-to-phenotype relationships for the traits. In fact, how best to use the results of the mapping studies to enhance response to selection by MAS has always been a great concern to plant geneticists and breeders (Lande and Thompson 1990, Openshaw and Frascaroli 1997, Moreau et al. 2004, Podlich et al. 2004, Crosbie et al. 2006). In recent years, several studies have been conducted on modelling the effects of QTLs and MAS in plant breeding programmes (Hammer et al. 2005). Chapter 4, authored by Mark Cooper and colleagues, deals with illustrative examples and simulation experiments on modelling QTL effects and MAS in plant breeding.

The majority of the marker-trait association studies conducted in the past were based on linkage mapping that aimed to detect non-random association between a genotype and a phenotype. In recent years, association mapping based on linkage disequilibrium (LD), extensively used in human genetics, is becoming a very popular approach in plant genetics and breeding (Thornsberry et al. 2001). The LD-based association studies, involving associations within populations of unrelated accessions, offer a potentially powerful and rewarding approach for mapping causal genes/QTLs with modest effects and validating the role of functional candidate sequences (Hirschhorn and Daly 2005). The methodology and applications of LD-based association mappings to crop improvement have been discussed in Chapter 5 by Ersoz and colleagues. Although LD mapping offers the possibility of utilizing the potential of exotic germplasm in crop improvement, "advanced backcross (AB) QTL analysis" and "exotic libraries" are other approaches that increase the efficiency of harnessing natural biodiversity to improve yield, adaptation and quality of elite germplasm (Zamir et al. 2001). In Chapter 6, Silvana Grandillo and colleagues discuss the optimal exploitation of the naturally available genetic resources to generate new traits and improve crop performance.

Availability of EST/genome sequencing data from several crop species are being used presently to analyze the transcriptome/genomes of a species by using advanced bioinformatics tools (Zhang et al. 2004). Development of functional molecular markers from gene sequence data is an added value to the existing repository of molecular markers for their use in plant breeding. The use of sequence data by using a variety of applications in crop improvement has been discussed by Lim and colleagues in Chapter 7. Indeed, the integration of sequence-based molecular markers to the genetic maps has enhanced the resolution of comparative maps in related plant species (see Sorrells et al. 2003, Salse et al. 2004). In the case of cereals, where ca. 500,000 ESTs for each of major cereal species (e.g. wheat, maize, rice, barley) and the complete genome sequence data for rice and draft sequence for sorghum are available, comparative genomics has provided important insights on genome evolution and how to best utilize marker/gene information from one species to another. A comprehensive overview on cereal comparative genomics is presented by Jerome Salse and Catherine Feuillet in Chapter 8. Furthermore, comparative genomics allows us to isolate QTLs of agronomic interest (Salvi and Tuberosa 2005). The present status and future directions on QTL cloning have been summarized by Silvio Salvi and Roberto Tuberosa in Chapter 9.

The increasing emphasis on functional genomics and the wide accessibility of transcripts profiling has led to the establishment of various high-throughput methodologies of gene expression analysis. These methodologies include (i) EST sequencing, (ii) differential display (DD) (Liang and Pardee 1992), (ii) serial analysis of gene expression (SAGE) (Velculescu et al. 1995), (iii) nucleic acid hybridization of mRNA or cDNA fragments e.g. oligo chips (Lockhart et al. 1996), cDNA microarrays (Schena et al. 1995), (iv) cDNA-amplified fragment length polymorphism analysis (cDNA-AFLP) (Bachem et al. 1996), and (v) massively parallel signature sequencing (MPSS) (Brenner et al. 2000). These methodologies differ in principle, convenience, costs involved, number of transcripts assayed and sensitivity (Kuhn 2001). One of these approaches, i.e. SAGE, has been dealt with in detail in Chapter 10 by Prakash Sharma and colleagues. Some other important functional genomics approaches to identify candidate genes have been reviewed by Ashwani Pareek et al. in Chapter 12. Such kind of functional genomics approaches often highlight a very large number of genes associated with a trait of interest (Sreenivasulu et al. 2004). Nonetheless, pinpointing the relevant candidate genes and deploying them effectively as diagnostic markers for predicting the phenotype in plant breeding applications remain difficult and challenging tasks. In this respect, the "genetical genomics" approach, first introduced by Jansen and Nap (2001), appears quite promising. In this approach, the transcript level data generated from microarray analysis are analyzed in quantitative fashion to identify gene expression QTLs (eQTL). This approach has been referred to as "expression genetics" by Varshney et al. (2005b). In Chapter 11, Matias Kirst and Qibin Yu review the principles of genetical genomics, the results of these studies in plants and the use of this approach to dissect the genetic control of phenotypic traits of biological and agricultural interest. Although the approach is still in its infancy and remains prohibitively expensive, pioneering studies on this aspect have demonstrated its value to unravel genetic networks involved in transcription regulation, and to identify genes and pathways controlling phenotypic variation for quantitative traits. Indeed, the genetical genomics approach can help to fish out additional genes underlying the eQTLs identified for corresponding candidate genes in those plant species where genome sequence data are available.

It is important to note that the comparative analysis of the genome sequence data for *Arabidopsis* and rice is already providing important insights and understanding on the evolutionary relationships among various classes of gene families, including those representing components of hormone (especially auxin and cytokinin) signaling critical for plant development and growth. Jiten Khurana and colleagues in Chapter 13 have underlined the value of some of the auxin and cytokinin signaling components as genetic tools for manipulating agronomic traits in crops.

As a result of genome sequencing and functional genomics studies, massive sequence and expression data are being generated. Therefore, it is essential that statistical and mathematical standards, as well as guidelines for the experimental design and analysis of biological studies are upheld. Chapter 14 by Rebecca Doerge deals with past statistical issues, discusses current statistical advances that pertain to understanding complex traits, and promotes ideas about the data and statistical genomic models of the future. Such advances in the area of statistical genomics will improve the efficiency of identifying and validating the function of the most promising candidate genes. The function of candidate genes can also be verified through reverse genetics. Under these approaches, specific genes can be disrupted, and hypotheses regarding gene function can thus be directly tested *in vivo*. At present, a number of reverse genetic methods exist, many of which are limited in application because they are organism-specific, expensive, transgenic or only transiently disrupt gene function. As an alternative, TILLING (Targeting Induced Local Lesions IN Genomes; McCallum et al. 2000) deploys traditional mutagenesis and SNP discovery methods for a high-throughput, reverse-genetic strategy that is low in cost and applicable to most organisms. In fact, during the past six years, TILLING has moved from proof-of-concept to production with the establishment of publicly available services for several crop species such as maize, rice and barley. The protocols developed for TILLING have been adapted for the discovery of natural nucleotide diversity in germplasm collections and the method has been termed EcoTILLING. In Chapter 15, Bradley Till and colleagues review current TILLING and EcoTILLING technologies and discuss the progress that has been made in applying these methods to many different plant species.

It is evident from the above that there are several approaches available that can be used for applications in plant breeding. Some examples of successful utilization of genomics for crop improvement at least in some cereal species have been recently reviewed by Varshney et al. (2006). However, the anticipated success of molecular breeding has not materialized as expected. The critical factors responsible for it include the poor understanding and consideration of important genetic phenomena such as epigenetics, genome imprinting, epistasis, and regulatory variation (Morgante and Salamini 2003, Varshney et al. 2005). It has been shown that polymorphism in DNA methylation status leads to differences in gene expression and confers phenotypic effects (Ronemus et al. 1996). All these alleles having the same DNA sequence but differing in their methylation status correspond to different phenotypes and are referred to as epialleles (Kalisz and Purugganan 2004). In recent years, the polymorphism associated to the epialleles has been exploited to constitute the biomarkers. A biomarker is a substance or a process that is indicative of a phenotype or a biological event (Laird 2003) and can be used for a variety of applications. For example, in human cancer epialleles are used as biomarkers for early detection of cancer types. In plants, epigenetic inheritance is a source of polymorphism that holds great potential for selection and plant breeding (Tsaftaris et al. 2005). In Chapter 16, Marie-Véronique Gentil and Stéphane Maury describe the characterization of biomarkers using new molecular approaches and discuss the future role of biomarkers in plant breeding.

3. VOLUME 2: GENOMICS APPLICATIONS IN CROP IMPROVEMENT

The approaches and platforms presented in Volume 1 have already been utilized for crop improvement. In particular, due to the importance of cereals in the human diet and the availability of excellent genomic resources, cereal crops have been the major target of genomics approaches (see Varshney et al. 2006). In wheat, rice, maize, barley and sorghum, genomics research and MAS have already yielded fruitful results. Efforts are underway to exploit the genomics research for the improvement of several other plant species such as legumes, fruit species and trees.

Among different types of molecular markers, microsatellites have been the markers of choice for plant breeding applications until recently (Gupta and Varshney 2000). However, because of the availability of sequence data in recent years from a large number of plant species, the development and applications of single nucleotide polymorphism (SNP) markers is gaining momentum and will become more prevalent. In Chapter 1, Martin Ganal and Marion Röder review the development and applications of microsatellite and SNP markers for wheat breeding. Wheats (including durum and bread wheat) are the major foods for majority of the human population and are mainly consumed as processed products because of the unique functional properties they confer to the derived foods. Therefore, improvement of end-use quality has been a major concern to wheat breeders with an emphasis on developing cultivars for specific applications such as bread (leavened, flat, steamed, etc.), other baked goods (cakes, cookies, crackers, etc.), pasta and noodles, and a wide range of other products of restricted local uses. Processing and end-use quality of wheat-based products are influenced by several factors such as protein content and composition, starch, kernel hardness and lipids. The use of molecular markers to identify and manipulate the QTLs for important grain quality traits in wheat have been summarized in Chapter 2 by Domenico Lafiandra and colleagues. Among the factors that can affect the quantity and quality of final yield in cereal as well as in other crops abiotic stresses play a pivotal role. Of all abiotic stresses, drought ranks first in terms of economic importance and recalcitrance to breeders' efforts. The use of molecular markers and functional genomics for identifying genes/QTLs conferring tolerance to drought and their use in breeding has been discussed by Michael Baum and colleagues in Chapter 3.

After identifying the molecular markers associated with gene(s)/QTLs for traits of interest, the next step is to use molecular markers in back-crossing programmes to improve selection efficiency and to implement gene pyramiding especially for disease resistances. Applications of molecular markers in barley for marker-assisted back-crossing and gene pyramiding for several disease resistance genes have been summarized in Chapter 4 by Wolfgang Friedt and Frank Ordon. In addition to the direct use of molecular markers in breeding, high density fine mapping of genes/QTLs of interest allows for the isolation of QTLs (Salvi and Tuberosa 2005). Indeed, in several cereal species as a result of long-term efforts, a number of disease resistance genes/QTLs have been isolated. An update on cloning of important disease resistance genes/QTLs is provided in Chapter 5 by Beat Keller and colleagues.

Compared to wheat and barley, maize is a more important crop for the private sector. Therefore, the majority of genomics efforts and applications in maize genomics have been undertaken by private industries. Chapter 6 authored by Michael Lee deals with a comprehensive review on the applications of genomics and genetic engineering in maize breeding. Furthermore, this chapter presents a perspective on the requirements, pros and cons of genomics applications in maize breeding.

As a model of cereal species, rice has benefited greatly from the advances in plant genomics. For example, dense genetic maps, genome-wide physical maps and four drafts of the rice genome are available (Vij et al. 2006). These genomic resources helped the rice community to identify and apply molecular markers for the improvement of grain quality traits, disease resistance and abiotic stress tolerance. Applications of molecular markers linked with several traits of interest in MAS, gene pyramiding, breeding nurseries, etc., in rice are summarized by David Mackill in Chapter 7. Additionally, the use of the rice genome sequence, novel approaches such as candidate gene sequencing, SNP markers for rice breeding have been reviewed in Chapter 8 by Nagendra Singh and Trilochan Mohapatra.

Sorghum is an important crop that is more tolerant than other cereals to many abiotic stresses, including heat, drought, and flooding, making it an ideal crop for growing on marginal lands as the demand for food, feed and energy increases. In recent years, the use of molecular markers has been initiated for the genetic analysis and manipulation of agronomic and stress-tolerance traits important for sorghum improvement. For example, molecular markers have been identified to be associated with QTLs for many complex traits, including pre-flowering and post-flowering drought tolerance, early-season cold tolerance and resistance to the parasitic weed *Striga*. In some cases (e.g. stay green and *Striga*), efforts have been initiated to use the corresponding QTLs for the development of improved sorghum cultivars through MAS and trait introgression. Chapter 9, authored by Gebisa Ejeta and Joseph Knoll, provides an overview on development and application of molecular markers for the development of improved sorghum cultivars.

Although genomics has already provided important contributions for the improvement of major cereal species, relatively less important cereal crops such as the millets and rye have received much less attention and consequently, often lack reasonably dense genetic maps (Varshney et al. 2006). Nevertheless, in recent years, species-specific genomic resources are being generated and genomic resources from related cereal species are being transferred through comparative genomics studies. A similar case applies to grain legume crops which are very important for both human diets and animal feeds. The grain legume crops contribute 33% of human protein intake and are a major source of lipids. However, except for soybean and common bean, other legume crops have not received much attention in terms of genomics approaches. Chapter 10, authored by Rajeev Varshney and colleagues, reviews the progress in the area of molecular genetics and applications in breeding in case of three important semi-arid tropic crops, i.e. chickpea, pigeonpea and groundnut. In contrast to these species, well-saturated genetic maps, physical maps and functional genomics resources are available in soybean. The advances in the area of genomic resources and their applications in soybean improvement have been discussed in Chapter 11 by Tri Vuong and other colleagues.

In addition to cereal and legume species, advances are being made in the area of genomics-assisted crop improvement in several other species. For example, in case of forage crops, molecular breeding for quality trait has been discussed in Chapter 12 by Thomas Lübberstedt; while updates on molecular mapping, MAS and map-based cloning in tomato have been provided in Chapter 13 by Majid Foolad. Additionally, Chapter 14 by Pere Arús and Susan Gardiner reviews the progress and potential of genomics for improving Rosaceae temperate tree fruit species. In Chapter 15, Prasad Hendre and Ramesh Aggarwal discuss the development and applications of molecular markers for the genetic improvement of coffee.

Two chapters have been devoted to understand two important processes, i.e. nodulation in legumes and domestication in cereals. By using soybean as a plant system, Kyujung Van and colleagues report (Chapter 16) on the identification of nodulation mutants (e.g. non-nodulation, ineffective nodulation and super-/hypernodulation) and the genetic loci that control nodulation. These authors suggest that molecular gene identification should be combined with biochemical pathways for nodulation in order to better understand the symbiotic interactions between legume and Rhizobia. In Chapter 17, Carlo Pozzi and Francesco Salamini review several issues concerning the state of molecular knowledge of the effects induced by domestication and breeding of wheat crops. Genetic bottlenecks which have been associated to wheat domestication and breeding have also been discussed in the chapter.

Volume 2 is concluded by Chapter 18, authored by Paulo Arruda and Thaís Rezende Silva, on sugarcane improvement by using information provided by transcriptome analysis and functional genomics approaches. These authors have demonstrated the identification of genes, by analyzing EST resources, involved in biotic and abiotic stress response, disease resistance and sucrose accumulation.

4. CONCLUDING REMARKS

The two volumes of the book provide up-to-date information on genomics research including platforms, approaches, as well as the achievements of application of genomics in breeding. Although genomics holds great potential for improving breeding efficiency, the high costs associated with genomics research are a critical factor hindering further applications of genomics to crop improvement particularly for inbreeding and/or minor crop species. Nevertheless, there are several success stories on the development of improved superior cultivars. In the coming years, it is anticipated that the decreasing cost of genotyping and sequencing coupled with further advances in molecular platforms and bioinformatics, will allow genomics to become an integral part of crop breeding and to improve selection efficiency. We hope that our effort in compiling these two volumes will help and stimulate those working in crop genomics as well as conventional plant breeding to better focus their research plans for crop improvement programmes. Hopefully, the book will also help graduate students to develop a better understanding of this important aspect of modern plant science research.

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