THE CHALLENGES OF BIOTECHNOLOGIES TO IMPROVE PLANT BREEDING EFFICIENCY

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ABSTRACT

Since the development of molecular biology, the potential of molecular breeding (MB) contributing significantly to crop improvement has been controversial. With the identification of the first quantitative trait loci (QTL) in the 1980s, the ability of molecular markers to streamline the selection of complex traits has been oversold because scientists have largely underestimated the impact of gene networks and their interactions on plant phenotype. Some of these limitations have now been overcome, thanks to the development of more sophisticated statistical approaches, which allow characterizing both QTL and the QTL by environment interactions (QEI), as well as the contributions made by plant models. Today, the use of markers to track transgenes or stack favorable alleles determining a significant proportion of the phenotypic variance is routine for many crops. The number of reports asserting the successes of MB in dealing with polygenic traits has been definitely increasing. In addition, it is now generally accepted that the role of MB extends beyond the manipulation of elite alleles at a few loci in biparental segregating populations. The modern breeding concept, which includes a combination of phenotypic and molecular selection, needs to evolve new strategies to fully exploit the massive amount of information emerging from the "-omics" technologies and various genome sequencing efforts. QTL, functional genomics, and association studies are complementary approaches, which can quantify the genetic effects of specific alleles at target loci. Once the genetic gain of favorable alleles has been validated in a suitable biological context and environment, allele-based markers can be easily developed and employed. This validation step remains a major bottleneck in the establishment of a large set of markers appropriate for deployment in plant breeding. However, considering the technological and methodological progress achieved in genomics in recent years, it is clear that the potential of MB to complement phenotypic selection and improve crop productivity is set to increase significantly in the near future.

INTRODUCTION

Since the dawn of agriculture, mankind has sought to improve crops by selecting individual plants with the most desirable characteristics or traits. Although the process may have become much more sophisticated over the years, it nevertheless continues to be essentially based on observations in the field. The major objective of crop improvement is to identify those individuals within heterogeneous materials in which favorable alleles are present at the highest proportion of loci involved in the expression of key traits (Goodman et al. 1987). The classical plant breeding method is based on increasing the probability of selecting such individuals from populations generated from sexual matings. Selection has traditionally been carried out at the whole-plant level (i.e., phenotype), which represents the net result of genotype and environment (and their interactions). Phenotypic selection has delivered tremendous genetic gains in most cultivated crop species, but is severely limited when faced with traits that are heavily modulated by the environment (Cooper et al. 2006). On the top of this, the nature of some traits can make the testing procedure itself complex, unreliable, and/or expensive (or a combination of these). In these situations, which are commonplace in most crops, indirect selection, based on genetic markers, presents itself as an efficient complementary breeding tool. The rationale is that where individual genes influencing target traits can be identified and linked with one or more markers, then the marker loci can be used as a surrogate for the trait, resulting in greatly enhanced breeding efficiency as illustrated in Figure 1 (Tanksley et al. 1989).

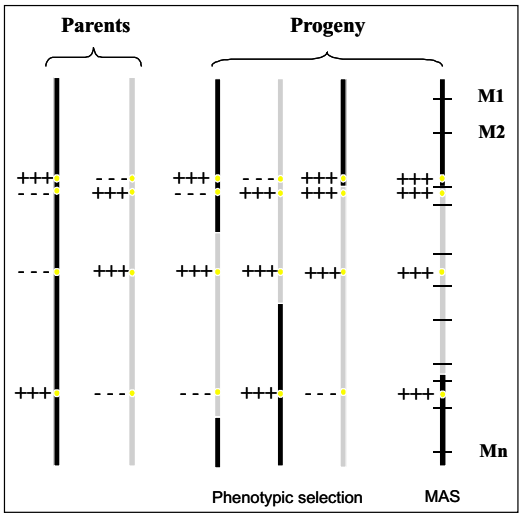

Figure 1: Molecular markers (M) allow identifying polymorphism (difference in genome composition) between parental lines permitting the identification of recombination events around genes of interest (round spots) to stack elite alleles (+++) in the segregating progeny in a single cycle of selection

It is clear that the potential of markers was greatly oversold when (during the 1980s) the first genomic regions involved in the determination of quantitative phenotypes were identified. At that time, some even talked of a revolution in plant breeding. Today, however, the value of molecular markers to complement plant phenotyping under several breeding scenarios is

no longer seriously questioned (Dekkers and Hospital 2002, Dwivedi et al. 2007a) although it remains important to recognize that there is still a ways to go before markers can be routinely used as an aid to select for complex traits (Ribaut and Ragot 2007). The challenge has been and still remains to convert the ever more plentiful supply of genetic information into a large set of markers useful for breeding, and how to integrate such markers into a sustainable breeding scheme. In essence, the priority lies in the development of efficient molecular breeding (MB) strategies aimed at plant improvement.

This paper describes the power of markers to characterize germplasm and to manipulate allele frequencies at major genes or genomic segments of interest. We emphasize the need to identify the gene functions and interactions which lead to particular genomic segments being Identified as critical, especially in the context of complex polygenic traits. Finally, the challenges involved in the development of numerous usable markers, along with some perspectives for MB, will be presented and discussed.

EVOLUTION OF MOLECULAR MARKERS

Markers are "characters" whose pattern of inheritance can be followed at the morphological (e.g., Flower color), biochemical (e.g., proteins and/or isozymes), or molecular (DNA) levels. They are so called because they can be used to elicit, albeit indirectly, information concerning the inheritance of "real" traits. The major advantages of the molecular over the other classes of markers are that their number is potentially unlimited, their dispersion across the genome is complete, their expression is unaffected by the environment and their assessment is independent of the stage of plant development (Lee 1995).

During the past two decades, DNA technology has been exploited to advance the identification, mapping and isolation of genes in a wide range of crop species. The first generation of DNA markers, restriction fragment length polymorphisms (RFLPs) was used to construct the earliest genome-wide linkage maps (Helentjaris et al. 1986) and identify the first QTL (Edwards et al. 1987; Paterson et al. 1988). During the 1990s, emphasis was switched to assays based on the polymerase chain reaction (PCR), which are much easier to use and potentially automatable (Mullis 1990). The development of simple sequence repeats (SSRs) [Senior and Heun 1993], amplified fragment length polymorphisms (AFLPs) [Vos et al. 1995] and single nucleotide polymorphism (SNPs) [Gilles et al. 1999] opened the doors for the large-scale deployment of marker technology in genome and progeny screening. Today the fingerprinting at 1500 SNP loci of 384 maize lines can be completed in two weeks at a cost of around US$ 35,000, equaling less than $0.10 per data point (Illumina technology *http://www.illumina.com)* [Walt 2005]. For a case in which a whole genome map needs to be generated in the absence of the sequence data necessary to design SNP markers, the recently developed high-throughput marker platform diversity array technology (DarT) [Jaccoud et al. 2001] is appropriate. Genome-wide maps are relevant because they provide the basis for establishing marker-trait associations, either via linkage analysis or association mapping.

USE OF MARKERS TO ACCESS PLANT DIVERSITY

Variation in the genome sequence and epigenetic activities such as gene silencing and altered chromatin structure within or across a crop species is referred to as "plant genetic diversity". This diversity provides the reason why, for example, some crop varieties are tall and some are short, why some species can survive cold temperatures while other closely related ones cannot, and why not all varieties of a crop are resistant to a particular insect pest etc. For millennia, farmers have taken advantage of genetic diversity by selecting the most favorable individuals in their own fields and using seeds harvested from these plants as the planting material for the following season.

Crop breeders need plant genetic diversity in their breeding programs to achieve genetic gain. They can exploit the allelic diversity present in their breeding materials by chromosome re-assortment and/or recombination, and/or they can import new alleles by hybridizing elite germplasm with exotic materials. The amount of genetic diversity present in a particular collection of individuals can be derived from comparisons between DNA fingerprints (the identification of alleles through molecular markers that provides an unambiguous identification of a living organism). Such estimates of the amount of genetic diversity present can be used to define phylogenetic relationships among germplasm entries, and to evaluate genetic distances separating them. A sufficient characterization of the genetic relationships among germplasm is particularly useful, as it indicates the combination of breeding parents, which is most likely to offer a high degree of allelic complementarity, contributing a large potential for genetic gain in the offspring. Thus, one of the most powerful applications of markers in breeding is the guiding of parental combinations before making crosses. Although most fingerprinting assays are based on anonymous (i.e., non-genic, or those whose function is not known) markers, the increasing pace of gene discovery and elaboration of gene function is driving a shift towards markers associated with specific genes. The advantage of such markers is that they can allow selection of parental lines based on specific traits. Fingerprinting is also valuable for establishing varietal identity and purity, and variety protection, which are real issues for the breeder, both in the commercial and the public sectors.

The potential of wild species and landraces (the earliest form of cultivar still available) as sources of genetic variation for crop improvement was recognized early in the twentieth century (Tanksley and McCouch 1997). A particularly promising application of molecular markers has been to identify novel superior alleles present in gene bank accessions (Dwivedi et al. 2007b). The CGIAR crop research centers and public research institutions around the world, including some national programs in developing countries, have spent many years collecting and conserving plant genetic material (including seeds, cuttings, and tissues) to prevent the loss of hundreds of thousands of different types of crops and their wild relatives (Spooner et al. 2005). These collections represent treasure troves of genetic diversity, valuable not just in their own right, but also as a resource for gene discovery in crop species. In addition, broad-scale genomics programs have begun to sample these genetic resources

to survey the level of phenotypic variation within species with a view to develop novel strategies for plant improvement (Fernie et al. 2006).

Although exotic germplasm has been extensively exploited as a source of variation for monogenic traits, relatively little work has been devoted to complex traits typically governed by QTL. Although the identification and introgression of favorable alleles from wild relatives have been successfully reported for several crops, with spectacular results achieved in tomato in particular (Fridman et al. 2004), much more work needs to be done to identify elite alleles in exotic germplasm. Two factors are relevant in this context viz., first, in some crops, the amount of as yet unexploited variation present in breeding material is still sufficient for genetic improvement to continue to be made without the need to identify and introgress exotic alleles; and second, the identification of favorable alleles for many agronomic traits is as challenging a task in the highly heterogeneous material typical of crop populations from subsistence environments as it is for exotic germplasm.

QTL MAPPING AND STACKING

One of the achievements of the plant biotechnology revolution for the last two decades has been the development of molecular genetics and associated technologies, which have led to the development of an improved understanding of the basis of inheritance of agronomic traits. The genomic segments or QTL involved in the determination of phenotype can be identified from the analysis of phenotypic data in conjunction with allelic segregation at loci distributed throughout the genome. Because of this, the mode of inheritance, as well as the gene action underlying the QTL, can be deduced (Lander and Botstein 1989). As with the improvement in marker technologies, the statistical tools needed for QTL mapping have evolved from a rudimentary to a very sophisticated level (Borevitz 2004). Current approaches are based on multiple regression methods, using least squares or generalized least squares estimation methods, mixed model approaches such as maximum likelihood, and Markov Chain Monte-Carlo algorithms (MCMC), which use Bayesian statistics to estimate posterior probabilities by sampling from the data. In parallel, with progress in the characterization of genetic effects at QTL and refinement of QTL peak position through meta-analysis (Chardon et al. 2004), advances have also been made in understanding the impact of the environment on plant phenotype. The mapping of QTL for multiple traits has allowed for the quantification of QEI (Jiang and Zeng 1995), and more recently, approaches using factorial regression models have been applied to model both QEI and genotype by environment interaction (GEI), using genetic and environmental covariables in the same model (Vargas et al. 2006).

A large volume of plant QTL data has been generated since 1980, and thousands of QTL now populate the various crop databases. About 150 original research papers reporting plant QTL data have been published annually from 2000-2004, covering Arabidopsis, soybean, rice,

sorghum, maize, barley and wheat. As of December 2006, over 2,200 maize QTLs have been documented in MaizeGDB (Lawrence et al. 2004), underlining the effort to identify phenotype / genotype associations in experimental crosses. In parallel, several marker-assisted selection (MAS) schemes have been proposed and tested. The power of DNA markers is their ability to select for genotypes carrying a favorable allelic composition at all marked loci. Favorable QTL alleles can be used to transfer one or more discrete genomic segments from a donor to an elite cultivar by backcrossing, or to conduct marker-assisted population improvement by the stacking of favorable alleles into individuals selected on the basis of marker genotype.

Marker-assisted backcrossing (MABC), in which a chosen allele at a marker locus is transferred from a donor to a recipient line, has been widely used to introgress favorable alleles into elite material which lacks a specific characteristic. A suite of genome-wide markers helps to expedite the progress of the backcrossing process, since it allows the simultaneous selection of the donor allele at the target locus and the rapid recovery of the recurrent parent alleles elsewhere (Tanksley et al. 1989). Although a number of parameters influence the choice of selection strategy, the design of a workable MABC program is relatively straightforward, and genetic gain can be predicted by simulation (Ribaut et al. 2002; Frisch and Melchinger 2005). MABC is an efficient means of transferring a single favorable allele (e.g., A transgene or a major QTL) into a range of genetic backgrounds, or of improving a particular genotype for a given trait (Ribaut and Ragot 2007). This latter approach is particularly important when breeding for foodstuffs, where the development of new germplasm is challenging because the new product needs to fit the requirements of local consumers and be better than products already available. For this reason, the introgression of superior alleles via MABC to improve popular cultivars for a specific trait is perhaps the most suitable application of MAS in the developing world. Although, such application of molecular markers may make its biggest short-term impact in developing countries, in the long-term the strategy is limited because its output can at best only generate an improved version of an existing genotype. To exploit the advantage of combining superior alleles from two or more parental lines, other MAS approaches need to be considered and are outlined in the following sections.

For marker-assisted population improvement, individuals selected from a segregating population based on their marker genotype are inter-mated at random to produce the following generations, at which point the same process can be repeated a number of times (Hospital and Charcosset 1997). A second approach aims direct recombination between selected individuals as part of a breeding scheme seeking to generate an ideal genotype or ideotype (Stam 1995). The ideotype is pre-defined on the basis of QTL mapping. This variety development approach is commonly referred to as marker-assisted recurrent selection (MARS) (Johnson 2004), or genotype construction. Thanks to molecular markers and the dissection of traits into QTL, an ideotype can be broken down into a mosaic of chromosomal segments / QTL from either parent. In practice, plant performance reflects

the integration of several traits, and consequently an ideotype will generally be a complex one. Stam (1995) provides the explanation as to why, in the case of bi-parental populations, ideotypes cannot be expected to occur in a selfing generation of realistic size. Similarly, because of the number of loci involved and the relative contribution of each parent, the ideotype will generally not be attainable through MABC. MARS schemes, which involve several successive generations of intercrossing selections based on molecular marker genotype, in addition to the use of multi-trait selection indices, may allow a closer approach to the ideotype (Peleman and Van Der Voort 2003). Other approaches suggest the selection to be conducted in large segregating populations (few thousands genotypes) to fix elite alleles at few selected loci in a single step of selection conducted at early stage of recombination (Ribaut and Betran 1999).

Ten years ago, the general belief was that the level of MAS efficiency was too poor for polygenic traits and the common consensus could be summarized by the statement that despite numerous reports of QTL in crops, little has been published on the implementation in breeding programs of MAS based on these QTL (Ribaut and Hoisington 1997). Fortunately, the situation has since changed, and the number of papers reporting successful MAS has increased significantly in recent years. As expected, the use of markers in breeding programs has been adopted primarily for the manipulation of simply inherited traits, for which a limited number of the most significant QTL can impact the phenotypic variance. A review of gene-marker associations for disease resistance and quality traits in various crops was recently presented by Francia et al. (2005). Other current crop-specific reviews have described the status quo in various well-studied crops, including rice (Ashikari and Matsuoka 2006), maize (Ribaut and Ragot 2007), wheat (Bonnett et al. 2005), less-studied cereals like pearl millet (Serraj et al. 2005), or legumes (Dwivedi et al. 2006; Miklas et al. 2006). Table 1, adapted from Varshney et al. (2006), presents some of the important achievements of MB, and gives some examples of genes isolated via map based cloning (MBC).

Table 1: Some important achievements in cereal genetics and breeding through molecular markers (Adapted from Varshney et al., 2006)

Cereal species	Notable examples of MAS	Examples of genes isolated through MBC
Barley	• Release of US variety Tango in 2000 that contains two QTL for adult resistance to stripe rust [1] • Advancement of a 'Sloop type' variety with CCN (cereal cyst nematode) resistance for commercial release [2] • Introgression of Yd2 gene conferring resistance to barley yellow dwarf virus (BYDV) into a BYDV susceptible background through two cycles of marker-assisted backcrossing [3]	• Powdery mildew resistance genes Mlo [4], Mla [5], Rar1 [6] • Stem rust resistance Rpg1 [7] • Barley yellow mosaic virus resistance rym3 and / or rym4 [8]

Table contd....

Table 1: Some important achievements in cereal genetics and breeding through molecular markers (Adapted from Varshney et al., 2006)

Cereal species	Notable examples of MAS	Examples of genes isolated through MBC
Maize	• Development of quality protein maize (QPM) through marker-aided transfer of opaque2 gene in backcross programmes [10] • Backcross marker-assisted selection for drought tolerance and recurrent selection for grain moisture and precocity (Ribaut and Ragot 2007) [11]	• Leaf rust resistance Rp1 D [12] • Flowering time QTL Vgt1 [13] • Root abcissic acid QTL, ABA1 (R. Tuberosa, personal Communication)
Pearl millet	• Release of a Indian pearl millet hybrid cultivar 'HHB 67 Improved' in 2005, which has resistance to downy mildew (C.T. Hash, personal communication) • Advances in marker-assisted selection for drought tolerance (Serraj et al. 2005) [14]	
Rice	• Release of two Indonesian rice cultivars 'Angke' and 'Conde', in which MAS was used to introduce xa5 into a background containing xa4 [15] • Pyramiding of disease resistance genes in rice, particularly against blight [16,17], blast [18], and both simultaneously [19] • Pyramiding of insect and blight resistance [17] quality characters [20] • The pyramiding of blight resistance with Basmati quality characters [20] • Introgression of QTL controlling root traits into Indian upland variety (Steele et al. 2006) [21]	• Bacterial blight-resistance genes Xa1 [22], xa5[23], Xa21 [24], Xa26 [25] • Rice blast-resistance genes PiB [26], Pi ta [27], Pi5 (t) [28], Pi 9 [29] • Plant architecture gene Dwarf1 [30] • A timekeeper of leaf initiation PLASTOCHRON1 [31] • Leaf spotted leaf gene Spl7 [32] • Semi-dwarf gene (sd 1) [33] • Seed shattering gene (qSH1) [34,35] • QTLs for heading Hd1 [36], Hd3a [37], Hd4 and Hd5 [38], Hd6 [39], Ehd1 [40] • QTL for grain production, Gn1a [41] • QTL for salt tolerance [42]
Sorghum	• Pyramiding of stay green QTLs in elite but drought-susceptible sorghum lines (C.T. Hash, personal communication)	• A major aluminum tolerance gene AltSB[43]

Line or population improvement achieved by exploiting linkage between markers and QTL is an expanding activity, which is proving to be effective for the manipulation of QTL and other genes having a significant impact on plant phenotype. However, the place of molecular markers in breeding for complex traits (e.g., yield or drought tolerance), where the proportion of the phenotypic variance expressed by any single QTL is of the order of 5-10% of the total, is less well-established. In some large private sector maize breeding programs, where the hybridity of the seed sold allows for high investment of capital, the manipulation of between 10 and 20 loci by MARS is close to routine (Koebner 2003, Dwivedi 2007a). Some successful MABC experiments to improve abiotic stress tolerance have been reported recently in several cereals (Ribaut 2006), but the application of such markers in public

breeding programs and in less capital-rich crops (e.g. cassava) remains limited and need to be encouraged. There is clearly a present need to identify the minor genes that underlie many complex-trait QTL and develop alternative MB strategies to permit selection at the large number of participating loci identified by genomics studies. Only then one can expect that markers will be widely deployed to improve allelic diversity at key loci in breeding populations through multi-parental crosses and then boost genetic gain by targeted allele selection at many loci simultaneously. The development of gene-based markers appears as a logical next step to boost the efficiency of MB for complex traits.

NEED TO GO FOR THE GENES

Plant genome analysis is a rapidly evolving discipline, exploiting many of the innovative technologies that have emerged from human genome research. A current and exciting approach is functional genomics, which has come to mean the development and application of genome-wide experimental approaches to assess gene function (Heiter and Boguski 1997). Its' ultimate prize is to characterize the function and expression pattern of every gene in the genome. Among the approaches currently being followed to achieve this goal are large-scale sequencing of expressed sequence tags (ESTs), insertional mutagenesis (or reverse genetics), and large-scale functional analyses of plant genes, where arrays of thousands of sequences are hybridized with mRNA.

The accelerating speed of DNA sequence acquisition, along with the vast quantity of data generated over the last decade by the application of omics technologies, has driven major advances in our understanding of gene action and interaction (for review see Science 2004). The functional testing of a gene can now be achieved by its over-expression or down-regulation using transgenesis or RNAi or by genetic complementation of known mutants. If available for the particular species of interest, reverse genetics approaches such as T-DNA or transposon-tagged populations and/or TILLing (McCallum et al. 2000) can also be exploited. These gene discovery tools can generate numerous candidate genes for the particular trait under study. For major genes, the identification of a single favorable allele is generally sufficient to achieve a significant amount of genetic gain across a broad set of germplasm, as demonstrated by the introgression of favorable allele at cloned QTL, that is, the identification of the sequence responsible for a QTL effect across germplasm (Fridman et al. 2004; Salvi and Tuberosa 2005). However, for the minor genes underlying many complex trait-QTL, it will probably be necessary to assign a value to each of the alleles present at each of the individual loci, and then adopt a selection index based on these relative values.

Association studies have significant potential to identify the genes responsible for a particular phenotype, and unlike populations derived from biparental crosses, have the power to simultaneously evaluate and compare the varying effects of many alleles

(Buckler and Thornsberry 2002). Although commonly used in human genetics to elucidate the genetic basis of hereditary diseases (Lander and Schork 1994), association approaches have only recently been applied to plant populations (Thornsberry et al. 2001). In maize, due to its rather low level of linkage disequilibrium, only polymorphisms separated from a locus responsible for the phenotypic effect by a few hundred bases are likely to be significantly associated with variation for a trait in a randomly mated population. These informative polymorphisms (or gene haplotypes) associated with a contrasting phenotype can be readily converted into markers for use in MAS experiments. Since association approaches are usually applied to a wide range of germplasm, informative markers identified through association tests can then generally be used to predict unrelated genetic backgrounds. Thus, a combination of genetic mapping and association studies is seen to have particular value for crop breeding.

Tremendous progress has been made in understanding the genetic basis of key regulatory pathways in plants in terms of gene function and allele value. But before any MB application can be considered, there is still a need to extrapolate and validate many of these discoveries in a suitable biological context. The quantification of favorable genetic effects at loci of interest in adapted germplasm under target field conditions is essential for the development of useful markers and represents one of the most severe bottlenecks limiting the extensive deployment of molecular markers. It cannot be over-emphasized that without accurate phenotypic data, no genetic data can ever be reliable. QTL validation requires multi-location evaluation of the genetic effects of specific alleles under field conditions, bringing into play the further complication of GEI. As is the case for QTL studies, the accuracy with which the prediction of the genetic effect of a candidate gene in a specific environment can be made is inversely related to the size of the genetic effect at the locus. Because breeding materials typically differ so markedly from experimental ones, the possibility of unpredicted epistatic effects or gene interactions, as well as epigenetic effects such as gene silencing, are also to be expected. These factors underline the current need to develop new MB approaches.

PERSPECTIVES FOR NEW MAS APPROACHES

The success of plant breeding is measured by crop performance in a target environment. The most critical determinants of the level of genetic gain achieved are associated with the nature of the base plant population upon which selection is practiced. These include the genetic value of the segregating alleles present (which is governed by the choice of the parental lines) and the size of the population itself. As indicated above, a number of methods are currently available to identify candidate genomic regions or genes for inclusion in a MB scheme, and markers can be dovetailed into a breeding program in a number of different ways (Figure 2). Therefore, our thinking should no longer be limited to considering what markers can do for conventional breeding. There is a need to explore alternative kinds of segregating

Populations and selection approaches which can take advantage of an increasing ability to define the characteristics of alleles at multiple target loci.

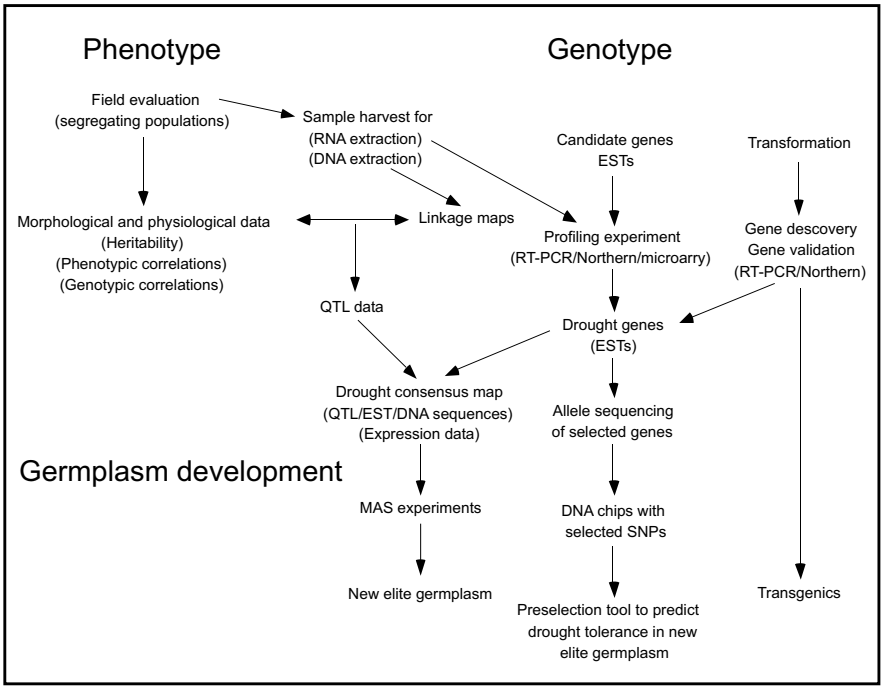

Figure 2: Multidisciplinary approaches to understand genetic basis of target traits

In such a context, novel approaches based on pre-existing genetic information, and which side-step the prior need to identify QTL or gene-based markers in every new cross, are particularly appealing. A possible approach could be to identify target genomic regions based on consensus genetic linkage maps assembled from data generated from different approaches and collected from several crosses and environments. The underlying assumption for this to succeed is that genes involved in the determination of a target trait are most likely to be located in the same position in the crop genome across cultivars, and those phenotypic differences among germplasm entries are generated by the nature/quality and interaction of the alleles at those genes. Thus, if a genomic region has been identified from a meta-analysis of diverse germplasm, then there is a high probability that the same region will be involved in the determination of the target phenotype in a new genetic background. Exploratory MB experiments aimed at improving the drought tolerance of tropical maize conducted at CIMMYT have generated some promising results along these lines. The markers used were based on gene clusters involved in drought tolerance traits identified from previous studies. The major limitations of using neutral markers for this mode of MAS are linkage drag, which can result in the unintended co-selection of deleterious alleles at loci linked to the selection markers, and the risk that contrasting alleles between the two

parental lines are not available at target loci. The next step is to directly target the genes responsible for the desirable phenotype, seeking ways to increase allelic diversity at each such locus (such as via the use of multi-parental crosses, Jourjon et al. 2005; Blanc 2006) and screening sufficiently large populations to enable critical recombinants to be recognized. This strategy is designed to permit the stacking of favorable alleles from the various parents at each of the important genes under selection.

Once the candidate genes for a target phenotype have been identified, informative polymorphisms can be assembled as gene haplotypes, and these will normally be readily convertible into a MAS assay (Camus-Kulandaivelu et al. 2006). The combination of genetic mapping and association studies has considerable potential to generate a catalogue of genetic variation, and thereby present novel opportunities for selection based on genome-wide scans (Biswas and Akey 2006). Nevertheless, it remains to be seen whether the outcome of gene interactions, particularly where significant gene networks are involved, will be sufficiently predictable across genetic backgrounds and environments. This is particularly a pressing question for those genes which have only a minor effect on phenotype, as is so frequently the case for the genes underlying complex traits. It may become necessary therefore to contemplate a strategy in which selection for favorable alleles is applied to a set of genes acting in a particular pathway, which is critical for productivity in a given environment. The overriding assumption is that if selection for the optimal allele can be based on a large number of loci, then genetic gain should be possible in any new population. Of course the absolute amount of genetic gain will always be both cross and environment-dependent.

All the strategies discussed above share a common rationale; they rely on the ability to accumulate favorable alleles, and use linked and/or gene-based markers to identify most suitable genotypes over successive cycles of recombination. In future MB selection schemes, one can anticipate that breeders will seek to predict phenotype in large population (possibly thousands) of segregating genotypes from multi-parental crosses based on the allelic constitution at many (possibly hundreds of) target loci.

INTERNATIONAL EFFORTS TO DEVELOP NEW BREEDING TOOLS: GENERATION CHALLENGE PROGRAMME AS AN ILLUSTRATION

A scientific and collaborative environment is essential to promote both gene discovery and its major downstream application i.e. the understanding of the genetic mechanisms underlying the regulation of crop productivity. There is no doubt that science and technology can make a difference in International Agriculture (Conway and Toenniessen 2003) but this implies the need for cross-cutting research platforms to facilitate the efficient application of the necessary genomic tools and knowledge required to unravel the genetic control of

Complex traits. Only a broad and integrated effort can span the full spectrum, from germplasm characterization to the deployment of molecular markers in a breeding program. Several bottlenecks in this chain have already been clearly identified, including the need to bridge the gap between upstream and applied research, and the need to ensure that the products of biotechnology will be used by plant breeders on the ground. In the public domain, most of the research resources are allocated to upstream, generally technology-oriented activities, while validation remains neglected because it is considered to be poorly compatible with scientific publication and the raising of research funding. In addition, few advanced research institutes have access to multiple-site field facilities. The challenge of validating candidate loci for MB is even bigger in the South, because of the lack of infrastructure and resources in most developing countries (Toenniessen et al. 2003).

To address some of those issues and to maximize the impact of new technologies on plant breeding, the Generation Challenge Programme (GCP) was created in 2003 by the Consultative Group on International Agricultural Research (CGIAR). The GCP is a research and capacity building network that uses plant genetic diversity, advanced genomic science, and comparative biology to develop the necessary tools and technologies to allow plant breeders in the developing world to produce better crop varieties for resource-poor farmers (www.generationcp.org). One of the strongest drivers for the creation of the GCP was the gap that currently exists in translational biology. As a result, the GCP is committed to promoting and rewarding efforts to create a strong interface between fundamental and applied research, a major challenge for modern genomic technologies to impact plant breeding and bear on the agricultural constraints of farmers in the world's poorest countries (Delmer 2005). A cornerstone of the GCP is the linking of laboratories in developed economies with user communities in developing countries to accelerate the use of elite genetic stocks and new marker technologies for crop breeding.

More than ever, varietal improvement relies on a profound understanding of the genetic basis of functional diversity, which will serve to broaden crop adaptation and improve productivity by stacking new or existing favorable alleles. The GCP's scientific approach is based on the concept that genetic resources (which provide the raw materials) should interact with both advanced biological exploration (which provides an understanding of the genetic basis of traits) and breeding programs (which realize value by applying conventional and advanced methods to produce new and improved varieties). Because of its exceptional network of partners, consisting of nine CGIAR centers, more than 30 advanced research institutes situated both in the North and in the South, and about 35 national programs, the GCP is uniquely positioned to support activities throughout the pipeline of marker development for breeding applications. Germplasm curators operate with large numbers of accessions, markers, and data points, and organize screens and funnels to optimize access to pre-existing diversity. Molecular physiologists and geneticists coupled with functional analyses at various scales between the cell, the plant, and the crop, and analyze populations in order to identify those genes responsible for useful variability. Plant breeders intercross specific germplasm accessions, recombine and tag useful alleles, and, together with

agronomists, apply the best breeding methods to enable the most effective selection under an appropriate range of field conditions.

To achieve its research agenda and to add value to GCP products and delivery, the GCP has been organized into five subprograms (SPs), which span the spectrum of research and development from genomics and bioinformatics to molecular breeding for agricultural development (Figure 3). Knowledge Generation requires the freedom to experiment with new ideas across disciplines and crops, while Product Development demands a clear road map for translating knowledge into tangible products. The GCP's research approach is based on these two pillars, which together ensure that suitable knowledge is generated and that potential products are tested and validated in target environments, within the larger global context of producing useful products for resource-poor farmers. This scheme is illustrated in the form of a set of vertically aligned activities, starting with Discovery and moving up through Validation, Application, and Use. To realize the potential of new genomic approaches, however, capacity to apply new tools must be enhanced and the setting in place of a pipeline to move results into practice is critical. Demonstrating successful outcomes for a few targeted cases in the short- to medium-term is important to help establish the road map for a broad application of these new areas of science.

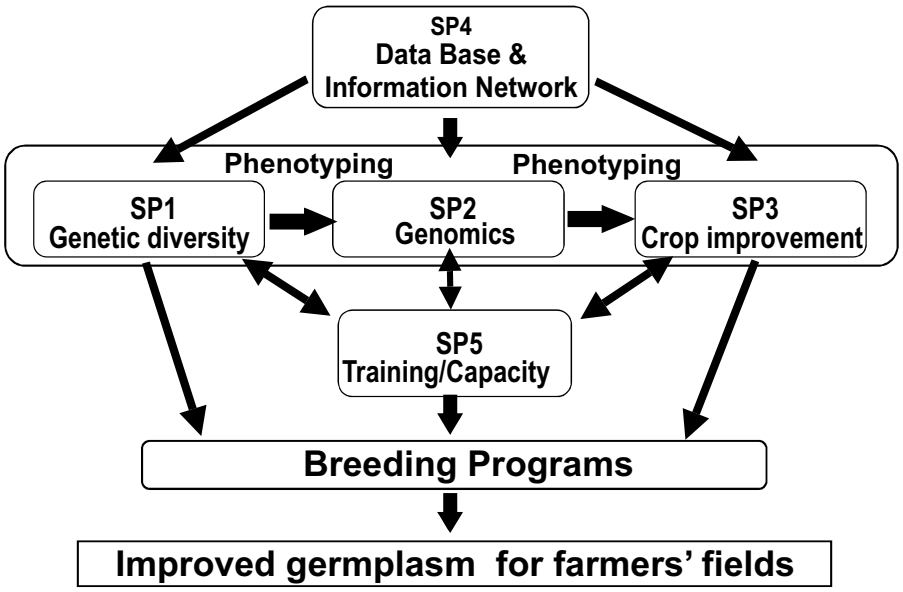

Figure 3: Generation Challenge Programme organization:
SP1: Genetic diversity of global genetic resources. This subprogram is charged with exploring the genetic diversity of the various germplasm collections of the CGIAR mandate crops. The information collected as a result of its activity forms the raw material for all the other GCP research and research products.

SP2: Comparative genomics for gene discovery. This subprogram focuses on developing genomic tools, technologies, and approaches to better understand the genetic basis of key traits in crop species important to developing countries. Its chief role is to discover and validate the function of key genes involved in stress adaptation, notably drought tolerance.

SP3: Trait capture for crop improvement. This subprogram focuses on the use of newly developed technologies, in conjunction with well proven methods, to increase the efficiency, speed, and scope of plant breeding. Its particular goal is to ensure that the GCP generates products which are actually used in breeding programmes.

SP4: Genetic resources, genomic and crop information systems, and bioinformatics. This subprogram aims to both develop information systems, analytical tools, protocols, and other products, as well as to ensure their integration into the GCP network working from a coherent and easily accessible information gateway.

SP5: Capacity building and enabling delivery. This subprogram expands researchers' capacity to accomplish the cutting-edge research agenda, seeks to bridge the technological gap between the various players from strategic research in advanced labs to its application in the field (user communities) and as such promotes the use of Generation products.

CONCLUSION

The potential of molecular breeding for complex polygenic trait improvement has, to date, been oversold, in particular because its practitioners have underestimated the complexity and variability of the gene interactions that occur under field conditions, across breeding cycles, and between locations. Recent advances in genomics (Varshney et al. 2005) and bioinformatics (Sawkins et al. 2004), however, do offer real opportunities for dissecting complex traits into their component sub-traits, which simplifies the process of developing the tools necessary to manipulate the underlying genes. The value of molecular markers as a complement to phenotyping under several breeding scenarios is largely unquestioned, as demonstrated by the increasing number of successful studies published. While there is still some way to go before markers can be used routinely and ubiquitously to breed for complex traits, it is also important not to underestimate the impact that the increasing flood of genetic data will have on breeding practices. The economical impact of MB to complement phenotypic selection is clear (Morris et al. 2003) and the criticism that MAS is an expensive indulgence is becoming less and less relevant as, driven by miniaturization and automation, the cost per marker data point falls faster than does the cost of phenotyping under field conditions. Undoubtedly, in the coming years new biotechnologies, which will further boost plant breeding and increase crop productivity under a broad range of environments, will be developed. However, to make this a reality, resources must be mobilized now to bolster plant phenotyping capacity and breeding expertise. Without this infrastructure, the task of identifying and validating the alleles and loci determining ideotype will be impossible. This vision implies a major role for both public and private breeding programs, both in the North and in the South, to guarantee that the link between genomic data and biological understanding is fully functional. Only this can ensure the continued development of tools and products, which can make a significant modernizing impact on plant breeding practices.

Annexure I

REFERENCES

Ashikari M and Matsuoka M (2006). Identification, isolation and pyramiding of quantitative trait loci for rice breeding. Trend in Plant Science, 11: 344-350.

Biswas S and Akey JM (2006). Genomic insights into positive selection. Trends in Genetics, 22: 437-446.

Blanc G, Charcosset A, Mangin B, Gallais A and Moreau L (2006). Connected populations for detecting quantitative trait loci and testing for epistasis: an application in maize. Theoretical Applied Genetics, 113: 206-224.

Bonnett DG, Rebetzke GJ and Spielmeyer W (2005). Strategies for efficient implementation of molecular markers in wheat breeding. Molecular Breeding, 15: 75-85.

Borevitz, J. (2004). Genomic approaches to identifying quantitative trait loci: lessons from Arabidopsis thaliana. In Molecular Genetics and Ecology of Plant Adaptation. Proceedings of an International Workshop held December 1113, 2002, in Vancouver, British Columbia, Canada. Edited by Q.C.B. Cronk, J. Whitton, R.H. Ree, and I.E.P. Taylor. NCR Research Press, Ottawa, Ontario, pp.53-60.

Buckler ES and Thornsberry JM (2002). Plant molecular diversity and applications to genomics. Current Opinion in Plant Biology, 5: 107-111.

Camus-Kulandaivelu L, Veyrieras JB, Madur D, Combes V, Fourmann M, Barraud S, Dubreil P, Gouesnard B, Manicacci D and Charcosset A (2006). Maize adaptation to temperate climate: relationship between population structure and polymorphism in the Dwarf8 gene. Genetics, 172: 2449-2463.

Chardon F, Virlon B, Moreau L, Falque M, Joets J, Decousset L, Murigneux A and Charcosset A (2004). Genetic architecture of flowering time in maize as inferred from quantitative trait loci meta-analysis and synteny conservation with the rice genome. Genetics, 168: 2169-2185.

Conway G and Toenniessen G (2003). Science for African Food Security. Science, 299 : 1187-1188.

Cooper M, van Eeuwijk FA, Chapman SC, Podlich DW and Löffler C (2006). Genotype-by-environment interactions under water-limited conditions. In: Ribaut J-M (ed) Drought adaptation in cereals. The Haworth Press Inc, Binghampton, NY, pp 51-95.

Dekkers JCM and Hospital F (2002). The use of molecular genetics in the improvement of agricultural populations. Nature Reviews, 3: 22-32.

Delmer, D P (2005). Agriculture in the developing world: Connecting innovations in plant research to downstream applications. PNAS,102 : 15739 - 15746.

Dwivedi SL, Upadhyaya HD, Balaji J, Buhariwalla HK, Blair MW, Ortiz R, Crouch JH and Serraj R (2006). Using genomics to exploit grain legume biodiversity in crop improvement. Plant Breeding Reviews, 26: 171-357.

Dwivedi SL, Crouch JH, Mackill D, Xu Y, Blair MW, Ragot M, Upadhyaya HD and Rodomiro O (2007a). Molecularization of public sector plant breeding: a synthesis of progress and problems. Advances in Agronomy 95, in press.

Dwivedi SL, Stalker HT, Blair MW, Bertioli D, Upadhyaya HD, Nielen S and Rodomiro O (2007b). Enhancing crop gene pools of cereals and legumes with beneficial traits using wild relatives. Plant Breeding Reviews 30, in press.

Edwards MD, Stuber CW and Wendel JF (1987). Molecular-marker-facilitated investigations of quantitative-trait loci in maize. I. Numbers, genomic distribution and types of gene action. Genetics, 116: 113-125.

Fernie AR, Tadmorand Y and Zamir D (2006). Natural genetic variation for improving crop quality. Current Opinion in Plant Biology, 9:196202.

Frisch M and Melchinger AE (2005). Selection theory for marker-assisted backcrossing. Genetics, 170: 909-917.

Francia E, Tacconi G, Crosatti C, Barabaschi D, Bulgarelli D, Dall' Aglio E and Vale G (2005). Marker-assisted selection in crop plants. Plant Cell, Tissue and Organ Culture, 82:317-342.

Fridman E, Carrari F, Liu YS, Fernie AR and Zamir D (2004). Zooming in on a quantitative trait for tomato yield using interspecific introgressions. Science, 305: 1786-1789.

Gilles PN, Wu DJ, Foster CB, Dillon PJ and Chanock SJ (1999). Single nucleotide polymorphic discrimination by an electronic dot blot assay on semiconductor microchips. Nature Biotechnology, 17: 365-370.

Goodman RM, Hauptli H, Crossway A and Knauf VC (1987). Gene transfer in crop improvement. Science, 236: 48-54.

Heiter P and Boguski M (1997). Functional genomics: Its all how you read it. Science, 278: 601-604.

Helentjaris T, Slocum M, Wright S, Schaefer A and Nienhuis J (1986). Construction of genetic linkage maps in maize and tomato using restriction fragment length polymorphisms. Theoretical and Applied Genetics, 72: 761-769.

Hospital F, and Charcosset A (1997). Marker-assisted introgression of quantitative trait loci. Genetics, 147: 1469-1485.

Jaccoud D, Peng K, Feinstein D and Kilian A (2001). Diversity arrays: a solid state technology for sequence information independent genotyping. Nucleic Acids Research, 29: e25

Jiang C and Zeng ZB (1995) Multiple trait analysis of genetic mapping for quantitative trait loci. Genetics, 140: 1111-1127.

Johnson R (2004). Marker-assisted selection. Plant Breeding Review, 24: 293-309.

Jourjon MF, Jasson S, Marcel J, Ngom B and Mangin B (2005). MCQTL: multi-allelic QTL mapping in multi-cross design. Bioinformatics, 21: 128-130.

Koebner R (2003). MAS in cereals: Green for maize, amber for rice, still red for wheat and barley. In Marker-assisted selection: a fast track to increase genetic gain in plant and animal breeding? Turin, Italy. 17-18 Oct 2003. FAO Rome pp. 12-17.

Lander ES and Botstein D (1989). Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics, 121: 185-199.

Lander ES and Schork NJ (1994). Genetic dissection of complex traits. Science 265: 2037-2048.

Lawrence CJ, Dong Q, Polacco ML, Seigfried TE, Brendel V (2004). MaizeGDB, the community database for genetics and genomics. Nucleic Acids Research, 32: 393-397.

Lee M (1995). DNA markers and plant breeding programs. Advances in Agronomy, 55: 265-344.

McCallum CM, Comai L, Greene EA and Henikoff S (2000). Targeting Induced Local Lesions IN Genomes (TILLING) for Plant Functional Genomics. Plant Physiology, 123: 439-442.

Miklas PN, Kelly JD, Beebe SE and Blair MW (2006). Common bean breeding for resistance against

biotic and abiotic stresses: From classic to MAS breeding. Euphytica, 147: 105-131.

Morris M, Dreher K, Ribaut J-M and Khairallah M (2003). Money Matters (II): Costs of maize inbred line conversion schemes at CIMMYT using conventional and marker-assisted selection. Molecular Breeding, 11: 235-247.

Mullis K (1990). The unusual origin of the polymerase chain reaction. Scientific American, April: 56-65.

Paterson AH, Lander ES, Hewitt JD, Peterson S, Lincoln SE and Tanksley SD (1988). Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. Nature, 335: 721-726.

Peleman, JD and Van Der Voort JR (2003). Breeding by design. Trends Plant Science, 7: 330-334.

Ribaut J-M and Hoisington DA (1998). Marker-assisted selection: new tools and strategies. Trends in Plant Science, 3: 236-239.

Ribaut J-M and Betrán FJ (1999). Single large-scale marker-assisted selection (SLS-MAS). Molecular Breeding, 5: 531-541.

Ribaut J-M, Jiang C and Hoisington DA (2002). Efficiency of a gene introgression experiment by backcrossing. Crop Science, 42: 557-565.

Ribaut J-M (2006). Drought Adaptation in Cereals. Haworth's Food Products Press, New York pp. 642.

Ribaut J-M and Ragot M (2007). Marker Assisted Selection to improve drought adaptation in maize: The backcross approach, perspectives, limitations, and alternatives. Journal of Experimental Botany, 58: 351-360.

Salvi S and Tuberosa R (2005). To clone or not to clone plant QTLs: present and future challenges. Trends in Plant Science, 10:297-304.

Sawkins MC, Farmer AD, Hoisington DA, Sullivan J, Tolopko A, Jiang Z and Ribaut J-M (2004). Comparative Map and Trait Viewer (CMTV): an integrated bioinformatic tool to construct consensus maps and compare QTL and functional genomics data across genomes and experiments. Plant Molecular Biology, 56: 465-480.

Science special issue: Genes in action (2004). Volume 306:557-760.

Senior ML and Heun M (1993) Mapping maize microsatellites and polymerase chain reaction confirmation of the target repeats using a CT primer. Genome, 36: 884-889.

Serraj R, Hash T, Rizvi SMH, Sharma A, Yadav RS and Bindiger FR (2005). Recent advances in marker-assisted selection for drought tolerance pearl millet. Plant Production Science, 8: 334-337.

Spooner D, van Treuren R and de Vicente MC (2005). Molecular markers for gene bank management. IPGRI Technical Bulletin No 10, International Plant Genetic Resources Institute, Rome, Italy pp. 130 .

Stam P (1995). Marker-assisted breeding. In: "Biometrics in plant breeding: applications of molecular markers. Proceedings of the ninth meeting of the EUCARPIA Section Biometrics in Plant Breeding" (J.W. Van Ooijen and J. Jansen, Eds). CPRO-DLO, Wageningen, pp 32-44

Tanksley SD and McCouch SR (1997). Seed banks and molecular maps: unlocking genetic potential from the wild. Science, 277: 1063-1066.

Tanksley SD, Young ND, Paterson AH and Bonierbale MW (1989). RFLP mapping in plant breeding: new tools for an old science. Biotechnology, 7: 257-264.

Toenniessen GH, O'Toole JC and De Vries J (2003) Advances in plant biotechnology and its adoption in developing countries. Current Opinion in Plant Biology, 6: 191-198

Thornsberry JM, Goodman MM, Doebley J, Kresovich S, Nielsen D and Buckler ES (2001). Dwarf8 polymorphisms associate with variation in flowering time. Nature Genetics, 28: 286-289.

Vargas M, vanEeuwijk F, Crossa J and Ribaut J-M (2006). Mapping QTLs and QTLenvironment interaction for CIMMYT maize drought stress program using factorial regression and partial least squares methods. Theoretical and Applied Genetics, 112: 1009-1023.

Varshney RK, Graner A and Sorrells M (2005), Genomics-assisted breeding for crop improvement Trends in Plant Science, 12:621-630.

Varshney RK, Hoisington DA and Tyagi AK (2006). Advances in cereals genomics and applications in crop breeding. Trends in Biotechnology, 24:490-499.

Vos P, Hogers R, Bleeker M, Reijans M, Lee Tho van der Hornes M, Frijters A, Pot J, Peleman J, Kuiper M and Zabeau M (1995). AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research, 23: 4407-4414.

Walt DR (2005). Miniature analytical methods for medical diagnostics. Science 308: 217-219.

Annexure II

REFERENCES FOR TABLE 1

1. Toojinda, T. et al. (1998). Introgression of quantitative trait loci (QTLs) determining stripe rust resistance in barley: an example of marker assisted line development. Theor. Appl. Genet. 96, 123-131.
2. CRCMPB Annual Report (2001/2002). Putting plant breeding into fast-forward. Cooperative Research Centre for Molecular Plant Breeding, University of Adelaide, Australia, p51.
3. Jefferies, S.P. et al. (2003). Marker assisted backcross introgression of the yd2 gene conferring resistance to barley yellow dwarf virus in barley. Plant Breed. 122, 52-56.
4. Buschges, R. et al. (1997). The barley mlo gene: A novel control element of plant pathogen resistance. Cell 88, 695-705.
5. Wei, F. et al. (1999) The Mla (powdery mildew) resistance cluster is associated with three NBS LRR gene families and suppressed recombination within a 240 kb DNA interval on chromosome 5S (1HS) of barley. Genetics 153, 1929 1948
6. Shirasu, K. et al. (1999). A novel class of eukaryotic zinc binding proteins is required for disease resistance signaling in barley and development in C. *elegans*. Cell 99, 355-366.
7. Brueggeman, R. et al. (2002). The barley stem rust resistance gene Rpg1 is a novel disease resistance gene with homology to receptor kinases. Proc. Natl. Acad. Sci. U.S.A. 99, 9328-9333.
8. Stein, N. et al. (2005). The eukaryotic translation initiation factor 4E confers multiallelic recessive bymovirus resistance in *Hordeum vulgare* (L.). Plant Jour. 42, 912-922.
9. Koebner, R.M.D. (2004). Marker assisted selection in the cereals: The dream and the reality. In Cereal Genomics (Gupta, P.K. and Varshney, R.K. eds), pp.199-252 Kluwer Academic Publishers.
10. Dreher, K. et al. (2000). Is marker assisted selection cost effective compared to conventional plant breeding methods? The case of quality protein maize. In Proc 4th Annu Conf Intern Consor on Agricultural Biotechnology Research (ICABR), The Economics of Agricultural Biotechnology, Ravello, Italy.

11. Ribaut J-M and Ragot M. (2007). Marker Assisted Selection to improve drought adaptation in maize: The backcross approach, perspectives, limitations, and alternatives. Journal of Experimental Botany, 58: 351-360.
12. Collins, N. et al. (1999). Molecular characterization of the maize Rp1 D rust resistance haplotype and its mutants. Plant Cell 11, 1365-1376.
13. Salvi, S. et al. (2002). Toward positional cloning of Vgt1, a QTL controlling the transition from the vegetative to the reproductive phase in maize. Plant Mol. Biol. 48, 601-613.
14. Serraj, R. et al. (2005). Recent advances in marker-assisted selection for drought tolerance pearl millet. Plant Production Science, 8: 334-337.
15. Toenniessen, G.H. et al. (2003). Advances in plant biotechnology and its adoption in developing countries. Curr. Opin. Plant Biol. 6, 191-198.
16. Sanchez, A.C. et al. (2000). Sequence tagged site marker assisted selection for three bacterial blight resistance genes in rice. Crop Sci. 40, 792-797.
17. Singh, S. et al. (2001). Pyramiding three bacterial blight resistance genes (xa5, xa13 and Xa21) using marker assisted selection into indica rice cultivar PR106. Theor. Appl. Genet. 102, 1011-1015.
18. He, Y. et al. (2004). Gene pyramiding to improve hybrid rice by molecular marker techniques. In New Directions for a Diverse Planet: Proc. 4th Intern. Crop Sci. Cong. Brisbane, Australia (http://www.cropscience.org.au/icsc2004/).
19. Narayanan, N.N. et al. (2002). Molecular breeding for the development of blast and bacterial blight resistance in rice cv. IR50. Crop Sci. 42, 2072-2079.
20. Joseph, M. et al. (2004). Combining bacterial blight resistance and Basmati quality characteristics by phenotypic and molecular marker assisted selection in rice. Mol. Breed. 13, 377-387.
21. Steele, K.A. et al. (2006). Marker-assisted selection to introgress rice QTLs controlling root traits into an Indian upland rice variety. Theorical Applied Genetics 112:208-221.
22. Yoshimura, S. et al. (1998). Expression of Xa1, a bacterial blight resistance gene in rice is induced by bacterial inoculation. Proc. Natl. Acad. Sci. U.S.A. 95, 1663-1668.
23. Iyer, A.S. and McCouch, S.R. (2004). The rice bacterial blight resistance gene xa5 encodes a novel form of disease resistance. Mol. Plant Microbe Inter. 17, 1348-1354.
24. Song, W.Y. et al. (1995). A receptor kinase like protein encoded by the rice disease resistance gene, Xa21. Science 270, 1804-1806.
25. Sun, X. et al. (2004). Xa26, a gene conferring resistance to Xanthomonas oryzae pv. oryzae in rice, encodes an LRR receptor kinase like protein. Plant Jour. 37, 517-527.
26. Wang, Z.X. et al. (1999). The Pib gene for rice blast resistance belongs to the nucleotide binding and leucine rich repeat class of plant disease resistance genes. Plant Jour. 19, 55-64.
27. Bryan, G.T. et al. (2000). A single amino acid difference distinguishes resistant and susceptible alleles of the rice blast resistance gene Pi ta. Plant Cell 12, 2033-2046.
28. Jeon, J.S. et al. (2003). Genetic and physical mapping of Pi5(t), a locus associated with broad spectrum resistance to rice blast. Mol. Genet. Genomics 269, 280-289.
29. Qu, S.H. et al. (2006). The broad spectrum blast resistance gene Pi9 encodes a nucleotide binding site leucine rich repeat protein and is a member of a multigene family in rice. Genetics 172, 1901-1914.

30. Ashikari, M. et al. (1999). A rice gibberellin insensitive dwarf mutant gene Dwarf 1 encodes the alpha subunit of GTP binding protein. Proc. Natl. Acad. Sci. U.S.A. 96, 10284-10289.

31. Miyoshi, K. et al. (2004). PLASTOCHRON1, a timekeeper of leaf initiation in rice, encodes cytochrome P450. Proc. Natl. Acad. Sci. U.S.A. 101, 875-880.

32. Yamanouchi, U. et al. (2002). A rice spotted leaf gene, Spl7, encodes a heat stress transcription factor protein. Proc. Natl. Acad. Sci. U.S.A. 99, 7530-7535.

33. Spielmeyer, W. et al. (2002). Semidwarf (sd 1), "green revolution" rice, contains a defective gibberellin 20 oxidase gene. Proc. Natl. Acad. Sci. U.S.A. 99, 9043-9048.

34. Konishi S. et al. (2006). An SNP caused loss of seed shattering during rice domestication. Science 312, 1392-1396.

35. Li, C. et al. (2006). Rice domestication by reducing shattering. Science 311, 1936-1939.

36. Yano, M. et al. (2000). Hd1, a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the Arabidopsis flowering time gene CONSTANS. Plant Cell 12, 2473-2484.

37. Kojima, S. et al. (2002). Hd3a, a rice ortholog of the Arabidopsis FT gene, promotes transition to flowering downstream of Hd1 under short day conditions. Plant Cell Physiol. 43, 10961105.

38. Lin, H. et al. (2003). Fine mapping and characterization of quantitative trait loci Hd4 and Hd5 controlling heading date in rice. Breed. Sci. 53, 51-59.

39. Takahashi, Y. et al. (2001). Hd6, a rice quantitative trait locus involved in photoperiod sensitivity, encodes the alpha subunit of protein kinase CK2. Proc. Natl. Acad. Sci. U.S.A. 98, 7922-7927.

40. Doi, K. et al. (2004). Ehd1, a B type response regulator in rice, confers short day promotion of flowering and controls FT like gene expression independently of Hd1. Genes Dev. 18, 926-936.

41. Ashikari, M. et al. (2005). Cytokinin oxidase regulates rice grain production. Science 309, 741- 745.

42. Ren, Z.H. et al. (2005). A rice quantitative trait locus for salt tolerance encodes a sodium transporter. Nature Genet. 37, 1141-1146.

43. Magalhaes, J.V. et al. (2006). High resolution mapping and cloning of AltSB, a major aluminium tolerance gene in sorghum. In Plant and Animal Genome Conference XIV, P61 (http://www.intl pag.org/14/abstracts/PAG14_P61.html).

44. Langridge, P. (2005). Molecular breeding of wheat and barley. In In the Wake of Double Helix: From the Green Revolution to the Gene Revolution (Tuberosa, R. et al. eds), pp. 279-286, Avenue Media, Bologna, Italy

45. Feuillet, C. et al. (2003). Map based isolation of the leaf rust disease resistance gene Lr10 from the hexaploid wheat (*Triticum aestivum* L.) genome. Proc. Natl. Acad. Sci. U.S.A. 100, 15253-15258.

46. Huang, L. et al. (2003). Map based cloning of leaf rust resistance gene Lr21 from the large and polyploid genome of bread wheat. Genetics 164, 655-664.

47. Yahiaoui, N. et al. (2004). Genome analysis at different ploidy levels allows cloning of the powdery mildew resistance gene Pm3b from hexaploid wheat. Plant Jour. 37, 528-538.

48. Griffiths, S. et al. (2006). Molecular characterization of Ph1 as a major chromosome pairing locus in polyploid wheat. Nature 439, 749-752.

49. Sutton, T. et al. (2003). The Ph2 pairing homoeologous locus of wheat (*Triticum aestivum*): identification of candidate meiotic genes using a comparative genetics approach. Plant Jour. 36, 443-456.

50. Yan, L. et al. (2003). Positional cloning of the wheat vernalization gene VRN1. Proc. Natl. Acad. Sci. U.S.A. 100, 6263-6268

51. Yan, L. et al. (2004). The wheat VRN2 gene is a flowering repressor down regulated by vernalization. Science 303, 1640-1644

52. Simons, K.J. et al. (2006). Molecular characterization of the major wheat domestication gene Q. Genetics 172, 547-555.

53. Liu, S. and Anderson, J.A. (2003). Targeted molecular mapping of a major wheat QTL for Fusarium head blight resistance using wheat ESTs and synteny with rice. Genome 46, 817-823.

54. Kota, R. et. al. (2006). Fine genetic mapping fails to dissociate durable stem rust resistance gene Sr2 from pseudo black chaff in common wheat (*Triticum aestivum* L.). Theor. Appl. Genet. 112, 492499.

55. Spielmeyer, W. et. al. (2005). Powdery mildew resistance and Lr34/Yr18 genes for durable resistance to leaf and stripe rust cosegregate at a locus on the short arm of chromosome 7D of wheat. Theor. Appl. Genet. 111, 731-735.

56. Schnurbusch, T. et al. (2004). Dissection of quantitative and durable leaf rust resistance in Swiss winter wheat reveals a major resistance QTL in the Lr34 chromosomal region. Theor. Appl. Genet. 108, 477-484.

NOTES

ABOUT THE FOUNDATION

The BARWALE FOUNDATION, formerly known as Mahyco Research Foundation is a non-profit, non-government, philanthropic organization striving for public benefit. The Foundation was established in 1986 under Section 25 of the Companies Act 1956, by the Chairman, Dr. B.R. Barwale, the winner of the World Food Prize (1998) and the recipient of Padma Bhushan Award (2001) from the Government of India for his distinguished services of high order in the field of agricultural development, seed related activities, trade and economic activity.

The Foundation works with a broader mission to promote research, technology and knowledge in the areas of agriculture, healthcare and education for human welfare.

The Foundation's vision is to work for alleviation of poverty and improve livelihoods by increased food security.

The Foundation undertakes various research programs primarily on rice improvement through molecular breeding for the development of cultivars with high yield and resistance to biotic and abiotic stresses.

The Foundation extends travel grant support for young scientists and students for attending scientific events, facilitates students for pursuing post-graduate research and training opportunities for knowledge promotion. Research based financial support for ophthalmological research and establishment of educational institutions are other notable philanthropic activities of the Foundation.

The Foundation also supports Indian Foundation Seed and Services Association (IFSSA) for popularization of public bred hybrids, utilization of quick and novel marker based techniques for seed purity assays, screening of breeding lines for specific traits and knowledge dissemination etc.

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