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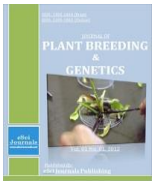

MARKER ASSISTED SELECTION IN DISEASE RESISTANCE BREEDING

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A B S T R A C T

Feeding ever-increasing population is the main challenge faced by the agricultural scientists and to meet this plant breeders have to put continuous efforts to develop new crop varieties on fast track basis. DNA based polymorphism, commonly known as DNA markers can be used for genetic improvement through selection for favourable traits such as disease resistance. Molecular markers are becoming an essential component in backcross breeding programs for tracking the resistance genes in gene pyramiding. Marker assisted selection (MAS), is expected to increase genetic response by affecting efficiency and accuracy of selection. Even though marker-assisted selection now plays a prominent role in the field of plant breeding, examples of successful, practical outcomes are rare. MAS, with few exceptions, has not yet delivered its expected benefits in commercial breeding. It is clear that DNA markers hold great promise, but realizing that promise remains elusive. The economic and biological constraints such as a low return of investment in small-grain cereal breeding, lack of diagnostic markers, and the prevalence of QTL-background effects hinder the broad implementation of MAS. Until complex traits can be fully dissected, the application of MAS will be limited to genes of moderate-to-large effect and to applications that do not endanger the response to conventional selection. Till then, observable phenotype will remain an important component of genetic improvement programmes, because it takes in to account the collective effect of all genes. In future, chip-based, high-throughput genotyping platforms and the introduction of genomic selection will reduce the current problems of integrating MAS in practical breeding programs and open new avenues for a molecular-based resistance breeding.

Keywords: Molecular markers, disease resistance, gene pyramiding, MAS.

INTRODUCTION

A rising global population requires increased crop production but some reports suggests that the rate of increase in crop yields is currently declining and hence a major focus of plant breeding efforts should be on traits related to yield, stability and sustainability. To keep the pace with increasing population and demand for food grains, there is a need to enhance the production at least by 1.5 to 2.0 M t annually in the next 10-15 years, with the back drop of declining and deteriorating resources and without adversely affecting the environment. Currently, about one billion people are in a situation of malnutrition, and nearly twice do not have access to sufficient nutrients and vitamins to meet their daily nutritional needs. Losses caused by plant diseases that manifest during pre and post-harvest treatment inevitably contribute to these deficiencies, especially in developing countries. Plant protection in general and the protection of crops against plant diseases in particular, have an obvious role to play in meeting the growing demand for food quality and quantity. In the eighties, a plague of crops in different parts of the world caused losses to half the harvests and it is still wreaking havoc in those regions. Currently, some varieties of wheat grown in the Indian subcontinent are threatened by the parasite *Puccinia graminis f. sp. tritici*, discovered in Uganda (also known as Ug99). The yield of cultivated plants is threatened by competition and destruction from pathogens, especially when grown in large-scale monocultures or with heavy fertiliser applications. In most cases, information on the

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magnitude of losses caused by diseases in plants is, however, limited. Nevertheless, it is estimated that 30 to 40% of harvests is lost each year throughout the production chain. In addition to yield losses caused by diseases, these new elements of complexity also result in post-harvest quality losses and accumulation of toxins during and after the cropping season. Disease management by using fungicides has been reported but fungicides are not eco-friendly and also pose many health problems in addition to their adverse effect on quality. Among the innovative genetic preferences, breeding for host-plant resistance is a cost effective and viable option to contribute to yield in an eco- friendly manner.

Ever since people have domesticated plants, they have noticed differences among varieties in their response to various stresses. One of the main stresses comes from attack by plant pathogens. There are references in the Bible to blights, blasts, and mildews (Haggai 2:17, 2 Chronicles 6:28, Amos 4:9). About 350 B.C., the father of botany Theophrastus was one of the first to record observations about plant diseases and noticed that plants differed with respect to their reactions to disease. However, the first demonstration of the possible genetic manipulation of plant disease resistance didn't occur until 1905, when Sir Roland Biffen showed that resistance in wheat cultivars to stripe rust was simply inherited. Harold Flor working with flax and the rust fungus *Melampsora lini*, found that host resistance to a pathogen was not only dependent on the host genetic makeup (resistance genes, *R*-genes), but also on the genetic makeup of the pathogen (avirulence genes, *Avr*-genes). With these findings Flor introduced the widely accepted concept of the gene-for-gene theory of disease resistance (often referred to as race-specific or vertical resistance), which predicts that resistance is triggered by the direct or indirect recognition of an avirulence gene product of the pathogen by a resistance gene product of the plant (Bonas and Lahaye. 2002).

Breeding plants with resistance against a specific disease requires the identification of resistant plants, which are then crossed with agronomically acceptable but susceptible plants. A program of backcrossing to the susceptible parent and selection of resistant phenotypes leads to the production of plants that are similar to the susceptible parent but having the required resistance. Typically, this process takes 10 or more years, and by this time, in some instances, the pathogen has already evolved a variant that is not recognized by the improved cultivar, leading to susceptibility. Plant breeders have developed countless cultivars with genetic resistance to possibly devastating plant pathogens. However, agriculture is a dynamic trade, changing agronomic practices and the evolution of new virulent races of pathogens, requires a persistent and continuous effort in disease management. In order to properly develop a defence against a pathogen, the breeder must understand the pathogen's life cycle (inoculation, infection, proliferation, spread, and latency), its virulence during different environmental conditions and varying stages of crop growth, along with its epidemiology. Finally, the breeder must understand the economic impacts of a particular pathogen in order to determine the amount of resources that should be directed in resolving the problem.

Breeders have successfully developed lines resistant to diseases by integrating *R*-genes into their cultivars for many years; but a durable (sometimes called Horizontal Resistance, Race non-specific resistance, or Qualitative Resistance), long lasting resistance in many cases has been difficult to achieve as pathogens quickly evolve and develop counter resistance genes that circumvent the host cultivars resistance. Breeders often spot this breakdown in resistance and hurriedly integrate a newly found effective *R*-gene into their populations. In mean time, the new *R*-gene loses its effectiveness and the boom-bust and induced co-evolution between crop and pathogen continues. Breeding for durable resistance based on minor additive genes has been challenging and often slow, for several reasons: 1) lack of sufficient number of minor genes in a single source genotype, 2) a source genotype may be poorly adapted, 3) there may be confounding effects from the segregation of both major and minor genes in the population, 4) crossing and selection schemes and population sizes are more suitable for selecting major genes, 5) reliable molecular markers for several minor genes are unavailable, and 6) the cost associated with identifying and utilizing multiple markers is high. One suggested approach is to use recurrent selection schemes to accumulate several minor genes in a single genetic background. Such selection schemes have often been more of a scientific interest than actually being applied in breeding.

Plant breeders have two options to increase the durability of their resistant cultivars. The first is High-

Dose/Refuge or Multiline strategy (Rausher 2001; Pink 2002). A multiline or refuge will reduce the selection intensity against the susceptible genotypes by providing an acceptable host for the pathogen. The selection intensities are decreased and the number of generations necessary for the new allele to become predominant increases dramatically. The multiline strategy requires, that some level of disease is acceptable and that the pathogen reproduces by sexual means. Also, multilines may not hold a necessary uniformity that many cropping systems require, which make them not feasible to practical utility. The second option for durable resistance is gene pyramiding. In theory, pyramiding several "undefeated" *R*-genes into a single cultivar will provide a more durable resistance as several mutations would need to take place, one at each of the pathogen's corresponding *Avr*-loci (McDowell and Woffenden 2003; Pink 2002). Advances in genomics have demonstrated that a considerable proportion, 1 to 2% of a plant's genome is devoted to resistance genes or genes with similar properties that could conceivably confer resistance to a pathogen possessing a complementary avirulence gene. Such genes are often clustered or occur in tandem repeats, suggesting that resistance genes with different specificities arise by gene duplication followed by intragenic and intergenic recombination, gene conversion and diversifying selection (Michelmore and Meyers. 1998). Maintenance of this genetic flux is crucial to the survival of the plants.

The modern molecular techniques make it possible to use markers and probes to track the introgression of several *R*-genes into a single cultivar from various sources during a crossing program. Although conventional breeding has had a significant impact on improving resistance cultivars, the time-consuming process of making crosses and backcrosses, and the selection of the desired resistant progeny make it difficult to react adequately to the evolution of new virulent pathogens.

In spite of optimism on conventional breeding for continued yield improvement, new technologies such as DNA markers serve as a new tool to detect the presence of allelic variation in the genes underlying the economic traits. DNA markers have enormous potential to improve the efficiency and precision of conventional plant breeding *via* marker-assisted selection (MAS) by reducing the reliance on laborious and fallible screening procedures.

TYPES OF MARKERS

Morphological markers: These are the traditional morphological mutant traits which are mapped and linked to a desirable or undesirable trait in a population which can be used in indirect selection. The major limitations with these markers are; high dependency on environmental factors, undesirable features such as dwarfism or albinism, time consuming, labour intensive and requirement of large plant population (Stuber *et al.*, 1999).

Biochemical markers: Isozymes are used as biochemical markers in plant breeding. Biochemical markers are superior to morphological markers in that they are generally independent of environmental growth conditions. The only problem with isozymes in MAS is that most cultivars (commercial breeds of plants) are genetically very similar and isozymes do not produce a great amount of polymorphism and polymorphism in the protein primary structure may still cause an alteration in protein function or expression.

Molecular markers (DNA - based Markers): Molecular markers have become important tools for genetic analysis and crop improvement. DNA-Markers, being phenotypically neutral and literally unlimited in number, have allowed scanning of the whole genome and assigning landmarks in high density on every chromosome in many plant species, which makes them fit for indirect selection.

Different types of molecular markers have been developed and evolved, including, but not limited to, restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), inter simple sequence repeat (ISSR), microsatellites or simple sequence repeat (SSR), expressed sequence tag (EST), cleaved amplified polymorphic sequence (CAPS), diversity arrays technology (DArT), and single nucleotide polymorphism (SNP) have been used in several crops (Doveri *et al.* 2008). Each marker system has its own advantages and disadvantages, and the various factors to be considered in selecting one or more of these marker systems have been described (Semagn *et al.* 2006; Panigrahi, 2011). Five conditions that characterize a suitable molecular marker are: 1) must be polymorphic, 2) co-dominant inheritance, 3) randomly and frequently distributed throughout the genome, 4) easy and cheap to detect and 5) should be reproducible.

PCR-based markers are more attractive for MAS, due to the small amount of template required and more efficient handling of large population sizes. AFLP, RAPD and Sequence tagged site (STS) are dominant markers, which limits its application for differentiation of homozygous and heterozygous individuals in segregating progenies. Among the DNA markers, the most widely used markers in major crops including cereals and legumes are SSRs or microsatellites (Li *et al*. 2008; Kumar *et al.* 2011), whereas in oilseed brassicas are RFLPs (Panigrahi *et al.* 2009). Both SSR and RFLP are highly reproducible, co-dominant in inheritance, relatively simple and cheap to use and generally highly polymorphic. The only disadvantage of SSRs is that they typically give information about a single locus per assay. This problem has been overcome in many cases by multiplexing several SSR markers in a single reaction (Kalia *et al.* 2011). STS and SCAR (sequence characterized amplified region) that are derived from specific DNA markers (e.g., RFLPs, RAPDs, etc.) that are linked to a gene or QTL are also extremely useful for MAS (Shan *et al.* 1999; Sanchez *et al*. 2000; Sharp *et al.* 2001; Collard and Mackill, 2008; Kumar *et al*. 2011). In recent years, single nucleotide polymorphisms (SNPs), i.e. single base changes in DNA sequence, have become an increasingly important class of molecular markers. The potential number of SNP markers is very high and micro-array procedures have been developed for automatically scoring hundreds of SNP loci simultaneously at a low cost per sample. Although the use of SNP markers in plants is still in its infancy, SNP markers are expected to become the marker system of choice in the near future, especially as the full sequences of more plant genomes will become available (Ganal *et al*. 2009).

Marker Assisted Selection (MAS): The development of DNA (or molecular) markers has irreversibly changed the disciplines of plant genetics and plant breeding. While there are several applications of DNA markers in breeding, the most promising for cultivar development is "marker assisted selection". MAS refers to the use of DNA markers that are tightly-linked to target loci as a substitute for or to assist phenotypic screening. By determining the allele of a DNA marker, plants that possess particular genes or quantitative trait loci (QTLs) may be identified based on their genotype rather than their phenotype. Five main considerations for the use of DNA markers in MAS (Mohler and Singrun, 2004) are;

a- Reliability: Molecular markers should co-segregate or tightly linked to traits of interest, preferably less than 5 cM genetic distance. The use of flanking markers or intragenic markers will greatly increase the reliability of the markers to predict phenotype.

b- DNA quantity and quality: Some marker techniques require large amounts and high quality DNA, which may sometimes be difficult to obtain in practice, and this adds to the cost of the procedures.

c- Technical procedure: Molecular markers should have high reproducibility across laboratories and transferability between researchers. The level of simplicity and time required for the technique are critical considerations. High-throughput simple and quick methods are highly desirable.

d- Level of polymorphism: Ideally, the marker should be highly polymorphic in breeding material and it should be co-dominant for differentiation of homozygous and heterozygous individuals in segregating progenies.

e- Cost: Molecular markers should be user-friendly, cheap and easy to use for efficient screening of large populations. The marker assay must be cost-effective in order for MAS to be feasible.

MAS SCHEMES IN PLANT BREEDING

Early generation marker assisted selection: Molecular markers can be employed at any stage of a plant breeding programme. Hence, MAS has great advantage in early generation selections by eliminating undesirable gene combinations especially those that lack essential disease resistance genes. Subsequently, the breeders can focus on a lesser number of high priority lines of desirable allelic or gene combination. MAS-based early generation selection not only selects suitable gene combinations but also ensure a high probability of retaining superior breeding lines (Eathington *et al.* 1997). An important prerequisite for successful early-generation selection with MAS are large populations and low heritability of the selected traits. The relative efficiency of MAS is greatest for characters with low heritability (Lande and Thompson 1990). This has important consequences in the later stages of the breeding program because the evaluation for other traits can be more efficiently and cheaply designed for fewer breeding lines (especially in terms of field space). However, in 2000 Barr *et al*. stated that, "this is fantasy for public sector breeders, as MAS can only be used in early generation screening for very important material", the main limitations being costs,

availability of suitable markers, and staff resources for sample and data handling. Markers are also frequently used to select parents with desirable genes and gene combinations, and marker-assisted recurrent selection (MARS) schemes involve several successive generations of crossing individuals based on their genotypes. The achievable genetic gain through MARS is probably higher than that achievable through MABC (Ribaut and Ragot 2006).

Marker-assisted backcrossing (MABC): Backcrossing is used in plant breeding to transfer favourable traits from a donor plant into an elite genotype (recurrent parent). In repeated crossings the original cross is backcrossed with the recurrent parent until most of the genes stemming from the donor are eliminated (Becker 1993). However, the donor segments attached to the target allele can remain relatively large, even after many backcrossing generations. In order to minimize this linkage drag, marker assays can be of advantage (Frisch *et al.* 1999). There are three levels of selection in which markers may be applied in backcross breeding. Markers can be used in the context of MABC to either control the target gene (foreground selection) or to accelerate the reconstruction of the recurrent parent genotype (background selection) and to select backcross progeny having the target gene with tightly-linked flanking markers in order to minimize linkage drag (recombinant selection). According to Frisch *et al.* (1999) in a computer simulation MAS can reconstruct a level of recurrent parent genome in BC_3 which would only be reached in BC_7 without the use of markers. However, the authors also state that large numbers of marker data points are required to achieve such results. MABC is especially efficient if a single allele is to be transferred into a different genetic background, for example, in order to improve an existing variety for a specific trait. To overcome the limitation of only being able to improve existing elite genotypes, other approaches like marker-assisted recurrent selection (MARS) have to be considered.

Marker-assisted recurrent selection (MARS): The improvement of complex traits via phenotypic recurrent selection is generally possible, but the long selection cycles impose restrictions on the practicability of this breeding method. With the use of markers, recurrent selection can be accelerated considerably and several selection-cycles are possible within one year, accumulating favourable QTL alleles in the breeding population (Eathington *et al.* 2007). Additionally, it is possible today to define an ideal genotype as a pattern of QTLs, all QTLs carrying favourable alleles from various parents. If individuals are crossed based on their molecular marker genotypes, it might be possible to get close to the ideal genotype after several successive generations of crossings. It is likely that through such a MARS breeding scheme higher genetic gain will be achieved than through MABC (Ribaut and Ragot 2006).

Marker assisted pyramiding: Pyramiding is the process of simultaneously combining multiple genes/QTLs together into a single genotype. This is possible through conventional breeding but extremely difficult or impossible at early generations. Using conventional phenotypic selection, individual plants must be phenotypically screened for all traits tested. Therefore, it may be very difficult to assess plants from certain population types (e.g. F_2) or for traits with destructive bioassays. DNA markers may facilitate selection because DNA marker assays are non-destructive and markers for multiple specific genes/QTLs can be tested using a single DNA sample without phenotyping. The most widespread application for pyramiding has been for combining multiple disease resistance genes.

In order to pyramid disease resistance genes that have similar phenotypic effects, and for which the matching races are often not available, MAS might even be the only practical method, especially where one gene masks the presence of other genes (Sanchez *et al.* 2000; Walker *et al.* 2002). The Barley Yellow Mosaic Virus (BaYMV) complex as an example is a major threat to winter barley cultivation in Europe. As the disease is caused by various strains of BaYMV and Barley Mild Mosaic Virus (BaMMV), pyramiding resistance genes seems an intelligent strategy. Since, phenotypic selection cannot be carried out due to the lack of differentiating virus strains. Thus, MAS offers promising opportunities. Suitable strategies have been developed for pyramiding genes against the BaYMV complex. What has to be taken into account when applying such strategies in practical breeding is the fact that the pyramiding has to be repeated after each crossing, because the pyramided resistance genes are segregating in the progeny (Werner *et al.* 2005).

Combined marker-assisted selection: The strategic combination of MAS with phenotypic screening is known as 'combined MAS' (coined by Moreau *et al.* 2004). It may have advantages over phenotypic

screening or MAS alone in order to maximize genetic gain (Lande and Thompson 1990). This approach could be adopted when additional QTLs controlling a trait remain unidentified or when a large number of QTLs need to be manipulated. In some situations a marker assay may not predict phenotype with 100% reliability. However, plant selection using such markers may still be useful for breeders in order to select a subset of plants using the markers to reduce the number of plants that need to be phenotypically evaluated. This may be particularly advantageous when the cost of marker genotyping is cheaper than phenotypic screening (Han *et al.* 1997). This was referred to as 'tandem selection' by Han *et al.* (1997) and 'stepwise selection' by Langridge and Chalmers (2005).

Simulation studies indicate that this approach is more efficient than phenotypic screening alone, especially when large population sizes are used and trait heritability is low (Hospital and Charcosset.1997). Zhou *et al.* (2003) observed in wheat that, MAS combined with phenotypic screening was more effective than phenotypic screening alone for a major QTL on chromosome 3BS for *Fusarium* head blight resistance. In practice, all MAS schemes will be used in the context of the overall breeding programme, and this will involve phenotypic selection at various stages to confirm the results of MAS as well as to select for traits or genes for which the map location is unknown.

Advantages of MAS over conventional methods: In addition to the cost and time savings, for a number of breeding scenarios, MAS methods are likely to offer significant advantages compared with conventional selection methods.

***a*- Gene stacking for a single trait:** MAS allows breeders to identify the presence of multiple genes/alleles related to a single trait, when the alleles do not exert individually detectable effects on the expression of the trait. E.g*:* when one gene confers resistance to a specific disease, breeders would be unable to use traditional phenotypic screening to add another gene to the same cultivar in order to increase the durability of resistance. In such cases, MAS would be the only feasible option, provided markers are available for such genes.

b- Early detection:MAS allows alleles for desirable traits to be detected early *i.e* in the seedling stage itself well before the trait is expressed phenotypically. This benefit can be particularly important in slow growing and long duration crops.

c- Recessive genes: MAS allows breeders to identify heterozygous plants that carry a recessive allele of interest whose presence cannot be detected phenotypically. In traditional breeding approaches, an extra step of selfing is required to detect phenotypes associated with recessive genes.

d- Heritability of traits: MAS is mainly useful in selection for traits with low heritability up to a point, gains from MAS increase with decreasing heritability.

e- Seasonal considerations: MAS offers potential savings compared with conventional selection when it is necessary to screen for traits whose expression depends on seasonal parameters. Using molecular markers, at any time of the year, breeders can screen for the presence of an allele (or alleles) associated with traits that are expressed only during certain growing seasons. For example, CIMMYT's wheat breeding station in northern Mexico is usually used for screening segregating germplasm for leaf rust resistance. However, expression of leaf rust is not uniform in all growing seasons. When there are seasons with low expression of leaf rust, markers, if available, can be a valuable alternative as a tool for screening.

f- Geographical considerations: MAS is necessary to screen for traits whose expression depends on geographical considerations. Using molecular markers, breeders in one location can screen for the presence of an allele (or alleles) associated with traits expressed only in other locations.

g- Multiple genes, multiple traits: MAS offers potential savings when there is a need to select for multiple traits simultaneously. With conventional methods, it is often necessary to conduct separate trials to screen for individual traits.

h- Biological security considerations: MAS provides a potential advantages over selection based on the use of potentially harmful biological agents (e.g. artificial viral infections or artificial infestations with pathogens), which may require specific security measures.

MAS VERSUS PHENOTYPIC SELECTION

Although cultivar development for multiple pathogen resistance in crops is a desirable goal, the process is often challenging due to the need for large-scale screening and lack of available resistance genes in a cultivated genetic background. It is often further complicated by linkage drag of unacceptable characteristics tightly linked with resistance, emergence of new disease pathogens or new races of existing pathogens, and the necessity of selecting for

resistance to multiple pathogens (Yang and Francis, 2005). Marker-assisted selection (MAS) offers an opportunity to overcome some of the problems associated with phenotypic selection and facilitates combining multiple resistance genes.

MAS will probably never replace phenotypic selection (PS) entirely. Especially for disease resistance a final testing of breeding lines is always required, regardless how tight a marker is linked to a gene or QTL (Yu *et al.* 2000). Dekkers and Hospital (2002) came to the conclusion that it is "risky to carry out selection solely on the basis of marker effects, without confirming the estimated effects by phenotypic evaluation" and Koebner and Summers (2003) claim for wheat breeding "that 'laboratory-based breeding' should remain the servant of the field breeder and not its master". According to them, large-scale MAS application could lead to an approach in breeding in which major breeding targets are attained by a single gene approach, thus possibly loosing the holistic advances that have been achieved by the PS of minor genes. The resulting varieties could become vulnerable to future changes in production systems, climate, or end use. Overall, relying only on MAS and thus excluding other potentially useful genes will rarely be the recommended approach in molecular breeding programs, and most programs involve at least one or two cycles of phenotypic evaluation during the breeding process (Dwivedi *et al.* 2007). The eventual application of these technologies in practical breeding programmes will be on the basis of economic grounds, which, along with cost-effective technology, will require further evidence of predictable and sustainable genetic advances using MAS.

A general survey on MAS revealed that among breeding strategies applied, MABC/Introgression is the main strategy with 48 publications out of 83. Regarding the breeding objective, breeding for disease/pest resistance is clearly dominating with 61 publications out of 83. Only few studies report the successful application of MAS for improved yield (8 studies), quality traits (6 studies), abiotic stress tolerance (5 studies), variety detection (2 studies), or growth character (1 study). The main marker technologies applied are microsatellite markers and RFLPs. The use of SNPs is only reported in two cases. It has, however, to be considered that the survey covers publications from 1995 to 2009. SNPs are likely to gain importance quickly and change the image significantly. MAS has increasingly been applied for the maintenance of recessive alleles in backcrossing pedigrees and for pyramiding resistance genes (Hajjar and Hodgkin 2007). Application of markers for breeding disease resistant varieties is especially interesting when breeding for resistance traits that are difficult or expensive to assess phenotypically. A prominent example is the selection for resistance to nematodes. In wheat there is extensive use of DNA markers for cereal cyst nematode (*Heterodera avenae* Woll.) resistance, Eagles *et al.* 2001; in soybean the most prominent example for MAS application in breeding is resistance to soybean cyst nematode (*H. glycines*) (Young 1999). In both cases the disease is of economic importance, the resistance is due to a single gene and the bioassay is expensive and unreliable (Eagles *et al.* 2001), thus MAS is a clear advantage. The predominance of applications for resistance traits can most likely be ascribed to the fact that many resistances are monogenic, making MAS a beneficial option in all cases where phenotypic assays are either expensive or unreliable.

DOCUMENTED RELEASES/REGISTRATIONS OF VARIETIES RESULTED THROUGH MAS BREEDING PROGRAMS

Rice, having the smallest genome of all cultivated cereals, being diploid and self pollinating, is the most extensively studied species among cereals. This is on one hand due to its global importance as a crop, on the other hand its role as a model species. Up to now, MAS in rice breeding has mainly been utilized for the pyramiding of disease resistances, namely bacterial blight and blast. The pyramided BB resistance genes, *Xa4+xa5+Xa21*, expressed strong resistance to virulent BB isolates of Korea compared with individual resistance genes that are moderately to completely susceptible (Jeung *et al*., 2006). The resistance genes *xa5*, *xa13*, and *Xa21* have been pyramided into an indica rice cultivar (PR106) using MAS that expressed strong resistance to BB races of India (Singh *et al*. 2001). Hittalmani *et al.* (2000) pyramided three major genes (Pi1, Piz-5 and Pita) using RFLP markers from three parents for rice blast into a single cultivar Co-39. Two commercially cultivated rice cultivars (Angke and Conde) were released in 2002 for cultivation in Indonesia. They possess gene pyramids *Xa4+xa5* and *Xa4+Xa7*, respectively (Bustamam *et al.* 2002). In the Philippines, two rice cultivars (NSIC Rc142 and NSIC Rc154) have the gene combination *Xa4+xa5+Xa21*. These genes have been integrated into the susceptible cultivar IR64 genetic background using MAS

(Toenniessen *et al*. 2003) and in China the photosensitive genic male sterile line 3418s (Luo *et al.* 2003), restorer lines R8006 and R1176 (Cao *et al.* 2003) and Kang 4183 (Luo *et al.* 2005) were successfully developed with a high resistance to bacterial blight by using the bacterial blight resistant gene *xa21.*

Marker-assisted backcross breeding (MABB) coupled with phenotypic selection for agronomic, grain and cooking quality traits has been used to incorporate BB resistance genes xa13 and Xa21 into 'Pusa Basmati 1' (Joseph *et al.* 2004). One of the improved lines was released as 'Improved Pusa Basmati 1' for commercial cultivation in 2007 (Gopalakrishnan *et al.* 2008), and this is one of the first product of MAS to be used in India. However, the susceptibility of 'Improved Pusa Basmati 1' and other Basmati rice varieties to rice blast and sheath blight (ShB) diseases remains a major concern. Later, Atul Singh *et al*. (2012), identified a blast resistance gene Pi54 and ShB resistance quantitative trait loci (QTL) - qSBR11-1 from a cultivar 'Tetep' to Improved Pusa Basmati 1 through MAS and the improved lines have desirable Basmati grain and cooking quality characteristics, in tandem with inbuilt resistance to BB, blast and ShB, and yield on par with 'Improved Pusa Basmati 1'.These multiple biotic stress-resistant lines will now be evaluated under multi-location trials for release to farmers as improved Basmati cultivars.

The achievements in rice blast resistant breeding program include the applications of the blast resistant genes, such as the *Pid1*, *Pib* and *Pita* pyramided to G46B (Chen *et al.* 2004), the *Pi2* introduced into Zhenshan97B (Chen *et al.* 2004) and the *Pi1*, *Pi2* and *Pi33* introgressed to Jin23B (Chen *et al.* 2008). Parallel to these efforts, the resistance breeding team at Directorate of Rice Research (DRR), Hyderabad have introgressed three bacterial blight resistance genes *Xa21, xa13* and *xa5* into the elite, high yielding, fine-grain type rice variety, Samba Mahsuri through marker-assisted breeding (Sundaram et al. 2008).

A three-gene pyramid line, RPBio-226 (IET 19046) was identified to possess high yield, good level and broad-spectrum bacterial blight resistance and excellent grain quality. Recently, this line has been released for commercial cultivation as a new variety 'Improved Samba Mahsuri'. A sister line of Improved Samba Mahsuri, RPBio- 210 (IET 19045), which has high level of BB resistance, high yield, good grain quality has been recently registered with the National Bureau of Plant Genetic Resources (NBPGR) as a novel germplasm (Sundaram *et al.* 2010). Recently, Shanti *et al.* (2010) introgressed *Xa4, xa13, xa5* and *Xa21* genes into the hybrid rice parental lines KMR3, PRR78, IR58025B, Pusa 6B and the popular cv. Mahsuri. Whereas Zhan *et al.* (2012) developed an elite restorer line R8012 carrying multiple genes (*Pi25/Xa21/xa13/xa5*) through MAS, in which all the resistance genes can confer resistance to BB and blast.

The performance of the BB-resistant version of Pusa RH10 produced by intercrossing the improved parental lines was on par with or superior to the original Pusa RH10 (Basavaraj *et al.* 2010). Importantly, we now have BB-resistant Basmati breeding lines in the genetic background of Pusa Basmati-1 (Joseph *et al*. 2004), Pusa RH10 (an aromatic hybrid, Basavaraj *et al*. 2010) and a traditional Basmati, Type-3 (Rajpurohit *et al.* 2011). Pandey *et al*. (2013) improved the two traditional BB-susceptible Basmati varieties (Taraori Basmati and Basmati 386), through the strategy of limited marker-assisted backcrossing for introgression of two major BB resistance genes, Xa21 and xa13, coupled with phenotype-based selection for improvement of their plant type and yield. This (Table.1) demonstrated the utility of molecular markers in improvement of biotic stress resistance of rice.

The University of California at Davis has developed the first wheat variety Patwin (Hard White Spring wheat) through MAS which contains the introgressed stripe rust resistance gene Yr17 and leaf rust resistance gene Lr37 (Helguera *et al*. 2005). Using RFLP markers that are tightly linked to *Pm2* and *Pm21* or co-segregate with *Pm4a*, three two-gene combinations, *Pm2 +Pm4a, Pm2 + Pm21, Pm4a + Pm21* were successfully integrated into an elite wheat cultivar 'Yang158' and double homozygotes were selected from a small F_2 population (Liu et al., 2000).

At CIMMYT, crosses have been made to combine two genes for cereal cyst nematode resistance (*Cre1* and *Cre3*) and three different genes for stem rust resistance (*Sr24, Sr26* and *Sr25*) in targeted wheat germplasm and for evaluation of leaf rust resistance genes Lr1, Lr9, Lr24, Lr47 and their introgression into common wheat cultivars by marker assisted selection (Nocente *et al*. 2007). A leaf rust resistant wheat variety from Argentina, 'Biointa 2004' (Bainotti *et al.* 2009) has been released for cultivation.

Table: 1 Examples of MAS applications in rice (adapted from Collard *et al*. 2008).

S. No.	Target trait	Gene(s)/ QTL(s)	Type/name of marker(s) used	Reference	Remarks
1	Bacterial blight (BB) resistance	*Xa21*	STS (pTA248)	Ronald *et al*., 1992	MAS applied for Marker-assisted backcross breeding
2	Bacterial blight (BB) resistance	*Xa4, xa5 & Xa10*	Gene linked RFLP and RAPD markers	Yoshimura *et al*. 1995	MAS applied for gene pyramiding
3	Bacterial blight (BB) resistance	*Xa4, xa5, xa13 & Xa21*	STS for *Xa4* CAPS for *xa5* (RG556+DraI) CAPS for *xa13* (RG136+HinfI) STS for *Xa21* (pTA248)	Huang *et al*., 1997	MAS applied for gene pyramiding
4	Bacterial blight (BB) resistance	*Xa21*	STS (pTA248)	Reddy *et al*., 1997	MAS applied for early generation selection for BB resistance
5	Bacterial blight (BB) resistance	*Xa21*	STS (pTA248)	Chen *et al*., 2000	MAS applied for Marker-assisted backcross breeding
6	Bacterial blight (BB) resistance	*xa5, xa13 & Xa21*	CAPS for *xa5* (RG556+DraI) CAPS for *xa13* (RG136+HinfI) STS for *Xa21* (pTA248)	Sanchez *et al*., 2000	MAS applied for gene pyramiding
7	Blast resistance	*Pi1, Piz-5, Pi2, Pita*	RFLP markers for *Pi1, Pi2* and *Pita* and a PCR based SAP marker for *Piz-5*	Hittalmani *et al*., 2000	MAS applied for gene pyramiding (Target variety: C039)
8	Bacterial blight (BB) resistance	*xa5, xa13 & Xa21*	CAPS for *xa5* (RG556+DraI) CAPS for *xa13* (RG136+HinfI) STS for *Xa21* (pTA248)	Singh *et al*. 2001	MAS applied for Marker-assisted backcross breeding (Target variety: PR106)
9	Bacterial blight (BB) resistance	*xa5, xa13 & Xa21*	CAPS for *xa5* (RG556+DraI) CAPS for *xa13* (RG136+HinfI) STS for *Xa21* (pTA248)	Davierwala *et al*., 2001	MAS applied for gene pyramiding
10	Bacterial blight (BB) resistance + Blast resistance	*Xa21 & Piz*	STS for *Piz,* transgene specific marker for *Xa21*	Narayanan *et al*., 2002	MAS applied for pyramiding of target traits. *Xa21* gene originally introduced into donor lines through genetic engineering (Target variety: IR50)
11	Blast resistance	*Pi1*	SSR and ISSR markers	Liu *et al*., 2003	MAS applied for backcross breeding (Target variety: Zhenshan 97A)
12	Bacterial blight (BB) resistance	*xa5*	CAPS (RG556+DraI)	Toennisen *et al*., 2003	MAS applied for Marker-assisted backcross breeding

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13	Bacterial blight (BB) resistance	*Xa4, xa5 & Xa21*	STS for *Xa4* CAPS for *xa5* (RG556+DraI) STS for *Xa21* (pTA248)	Leung *et al*., 2004	MAS applied for gene pyramiding
14	Bacterial blight	*Xa7& Xa21*	STS for *Xa7*	Zhang *et al*., 2006	MAS applied for gene
15	Bacterial blight (BB) resistance	*xa5, xa13 & Xa21*	CAPS for *xa5* (RG556+DraI) CAPS for *xa13* (RG136+HinfI) STS for *Xa21* (pTA248)	Sundaram *et al*., 2008	MAS applied for Zackcross breeding. In addition to foreground selection using the gene linked markers, background selection was also performed using parental polymorphic SSR markers (Target variety: Samba Mahsuri)
16	Bacterial blight (BB) resistance + Grain quality	*xa13 & Xa21*	CAPS for *xa13* (RG136+HinfI) STS for *Xa21* (pTA248)	Joseph *et al*., 2004 Gopalakrishnan *et al*., 2008	MAS applied for backcross breeding. In addition to foreground selection using the gene linked markers, background selection was also performed using Parental polymorphic AFLP & SSR markers. Further markers linked to grain quality traits were also used for foreground selection (Target variety: Pusa Basmati 1)
17	Bacterial blight (BB) resistance	*Xa4, X17 & Xa21*	STS for *Xa4 & Xa7* STS for *Xa21* (pTA248)	Perez *et al*., 2008	Bacterial blight (BB) resistance
18	Bacterial blight (BB) resistance	*Xa4, xa5, xa13 & Xa21*	STS for *Xa4* CAPS for *xa5* (RG556+DraI) CAPS for *xa13* (RG136+HinfI) STS for *Xa21* (pTA248)	AICRIP Progress Report. Vol. 1 (2008)	MAS applied for gene pyramiding (Target varieties: Swarna and IR64, some pre-breeding lines in the genetic background of Lalat and Tapaswini possessing BB resistance also developed by CRRI and nominated for AICRIP trials)
19	Bacterial blight (BB) resistance	*xa5* and *xa13*	CAPS for *xa13* (RG136+HinfI) STS for *Xa21* (pTA248)	Sundaram *et al*., 2009	MAS applied for backcross breeding. In addition to foreground selection using the gene linked markers, background selection was also performed using parental polymorphic SSR markers (Target variety: Triguna)
22	Blast resistance	*Pi-9(t)*	*pB8*	Wen *et al*., 2011	Introgressed the broad-spectrum blast resistant gene *Pi-9*(*t*) from the donor parent P2 into hybrid restorer Luhui17 by using MAS technique.

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21	Bacterial blight (BB) resistance	*Xa4, xa5, xa13 & Xa21*	STS for *Xa4* CAPS for *xa5* (RG556+DraI) CAPS for *xa13* (RG136+HinfI) STS for *Xa21* (pTA248)	Shanti *et al*., 2010	MAS applied for pyramiding the BB resistance genes into the hybrid rice parental lines KMR3, PRR78, IR58025B, Pusa 6B and the popular cv. Mahsuri.
22	Bacterial blight (BB) resistance + Blast resistance	*xa5, xa13, Xa21 &Pi25*	CAPS for *xa5* (RG556+DraI) CAPS for *xa13* (RG136+HinfI) STS for *Xa21* (pTA248) and STS for *Pi25* (SA7)	Zhan *et al*., 2012	MAS applied for pyramiding multiple genes (*Pi25/Xa21/xa13/xa5*) in to elite restorer line R8012 and its hybrid (Zhong 9A/R8012) playing a vital role in securing rice production in China.
23	Bacterial blight (BB) resistance + Blast resistance + sheath blight (ShB).	*xa13, Xa21 , Pi54 & qSBR11-1*	CAPS for *xa13* (RG136+HinfI) STS for *Xa21* (pTA248) SSR for *Pi54* (RM206) SSR for *qSBR11-1* (flanking markers RM224 and RM7443)	Atul Singh *et al*., 2012	The rice cultivar 'Improved Pusa Basmati 1' (carrying the BB resistance genes xa13 and Xa21) was used as the recurrent parent and cultivar 'Tetep' (carrying the blast resistance gene Pi54 and ShB resistance quantitative trait loci (QTL), qSBR11-1) was the donor and the improved lines were resistant to all three diseases and were on par with 'Improved Pusa Basmati 1' for yield, duration and Basmati grain quality..
24	Bacterial blight (BB) resistance	*xa13 & Xa21*	CAPS for *xa13* (RG136+HinfI) STS for *Xa21* (pTA248)	Pandey *et al*., 2013	Improved the two traditional BB-susceptible Basmati varieties (Taraori Basmati and Basmati 386)
25	Bacterial blight (BB) resistance + Blast resistance	*Xa21& Pi54*	STS for *Xa21* (pTA248) SSR for *Pi54* (RM206)	Hari *et al*., 2013	Marker-assisted introgression of bacterial blight and blast resistance into IR 58025B, an elite maintainer line of rice
26	Bacterial blight (BB) resistance	*Xa4, xa5, xa13 & Xa21*	STS for *Xa4* CAPS for *xa5* (RG556+DraI) CAPS for *xa13* (RG136+HinfI) STS for *Xa21* (pTA248)	Dokku *et al*., 2013	Three resistance genes *i.e*. xa5, xa13 and Xa21 were transferred from IRBB 60 through MABC to supplement the Xa4 gene present in Tapaswini, an elite cultivar having a wide coverage

Diagnostic or perfect markers (i.e. markers with complete linkage to the genes of interest with no possibility of recombination) have been developed for genes conferring resistance to different biotic stresses in wheat. CIMMYT's wheat improvement efforts use a set of diagnostic markers routinely in segregating populations to enable selective advancement of lines containing the *Cre1* and *Cre3* genes that confer resistance to Cereal cyst nematode (CCN) in wheat (Lagudah *et al.* 1997). Barley yellow dwarf virus (BYDV) resistance is derived from a chromosome segment introgressed from *Thinopyrum intermedium,*

on chromosome 7DL (Ayala *et al.,* 2001). BYDV is an important viral disease in certain wheat growing regions of the world. Environmental influence makes field screening less reliable. The diagnostic marker for the translocated chromosome segment allows the alien-derived resistance to be combined with the BYDV tolerance available in wheat.

In contrast to wheat, barley varieties have been released on the basis of MAS. In the USA the variety 'Tango', carrying two QTLs for adult resistance to stripe rust, was released in 2000 (Hayes *et al.* 2003), claiming to be the first commercially released barley variety using MAS. However, 'Tango' yields less than its recurrent parent and is therefore primarily seen as a genetically characterized source of resistance to barley stripe rust rather than a variety of its own. As a result of the South Australian Barley Improvement Program the malting variety 'Sloop' was improved with cereal cyst nematode resistance introgressed from the variety 'Chebec' and released in 2002 as 'SloopSA' (Barr *et al.* 2000; Eglinton *et al.* 2006). Another gene pyramiding example using MAS involves stacking of the resistance genes *rym4, rym5, rym9* and *rym11*for the barley yellow mosaic virus complex using molecular markers and doubled haploids (Werner *et al.* 2005).

In legumes successful identification of closely linked microsatellite markers for resistance gene *rhg1* to soybean cyst nematode (SCN) has enabled transfer of the resistance with about 99 percent accuracy (Young 1999).

In soybean MAS has been utilized in breeding for resistance to soybean cyst nematodes (*Heterodera glycines* Ichinohe) (Concibido *et al.* 1996; Cahill and Schmidt 2004; Arelli *et al.* 2006; Arelli *et al.* 2007). White bean variety 'Verano' resistant to bean golden yellow mosaic virus and carrying QTLs for common bacterial blight resistance was registered by Beaver *et al.* (2008).

There are numerous publications reporting the identification of new QTLs, however, very few of the QTLs reported (Table.2) have been used for MAS in breeding programs. Xu and Crouch stated in 2008: "It appears that the community is currently investing a large amount of time and money in generating an increasingly vast collection of publications with little impact on applied plant breeding, particularly in the public sector." These QTLs reside in journals on library shelves rather than in cultivars that have been improved through the introgression or selection of these QTL alleles (Bernardo 2008).

IMPACT OF MARKER ASSISTED SELECTION

DNA marker technology development versus conversion into practical applications: Although DNA markers have been available since the late 1980s, PCR-based markers allowing high throughput (microsatellite markers) became only available in the mid-to late 1990s. Only during the last five to ten years these markers have been widely used (Collard and Mackill 2008).

After the term "marker-assisted selection" was first used by Beckmann and Soller in 1986, it took ten years for the publication of first substantial article on the application of MAS in plant breeding (Concibido *et al.* 1996). There seems to be a time lag of about ten years between the first application of new marker technologies and their widespread use in breeding programs. If today's promises of SNP marker applications turn out to be true, a notable increase in the number of publications describing MAS has to be expected in the next ten years and beyond (Collard and Mackill 2008).

Limitations in publication of marker assisted breeding: QTL mapping is considered as a basic research process and regularly published in scientific journals. This explains the vast number of publications reporting the identification of new QTLs. However, scientists gain reputation mostly through carrying out innovative research and through publishing results within academic journals. Thus, there is little appeal to ensure that markers developed in research programs are also applied in breeding programs (Collard & Mackill 2008). For plant breeding, in contrast, the aim is not to publish results but to release new varieties. Even if the new varieties are registered, details regarding the application of markers during the breeding process are not necessarily published. In addition, in the private sector publication of results might even be discouraged due to competition reasons. New QTLs are frequently reported in scientific journals, but reconfirmation of these QTLs in other germplasm and identification of more useful markers are usually not considered novel enough to warrant new publications. The unwillingness of researchers to share data and germplasm can cause serious limitation for the advancement of MAS applications (William *et al.* 2007).

Table: 2 Examples of MAS applications in different crops.

S. No.	Target trait	Type of marker	Reference	Remarks
			WHEAT	
1	*Fusarium* head blight (FHB) resistance	SSR	Miedaner *et al.,* 2006	Introgression of three donor-QTL alleles from non-adapted sources in to an elite spring wheat background
2	*Fusarium* head blight QTL	SSR	Wilde *et al.,* 2008	Introgression of three *Fusarium* head blight QTL into an elite winter wheat breeding population and Lines with two introduced QTLs showed improved FHB resistance by ~40 %
3	Leaf rust (*Puccinia triticina*) resistance gene *Lr47*	SSR	Bainotti *et al.,* 2009	Registration of cultivar 'BIOINTA 2004'
4	Powdery mildew (*Erysiphe graminis* f.sp. *tritici*) resistance genes	RFLP	Liu *et al.,* 2000	Pyramiding of Powdery mildew resistance genes in elite cultivar 'Yang 158'
			BARLEY	
5	Barley yellow mosaic virus I-III	RFLP	Okada *et al.,* 2003	Introgression of Barley yellow mosaic virus I-III from donor line 'Y4' in to 'Mokkei 01530'. Which showed completely resistant to BaYMV I and has an acceptable level of resistance to BaYMV III
6	Resistance to cereal cyst nematode	RFLP	Barr *et al*., 2000	Transfer of resistance to cereal cyst nematode from 'Chebec' to themalting variety 'Sloop' and Release of variety 'SloopSA'
7	Barley stripe rust	RFLP RAPD AFLP	Hayes *etal.,* 2003	Release of variety 'Tango' with resistance to stripe rust
8	Stripe rust resistance gene *Rspx* and three QTLs	RFLP STS SSR	Castro *et al*., 2003	Indication that combining qualitative and quantitative resistance in the same genotype is feasible
9	Barley yellow mosaic virus complex (BaMMV, BaYMV, BaYMV-2)	RAPD SSR STS	Werner *et al.,* 2005	DH-populations carrying the pyramided resistances
10	Resistance to BYDV	CAPS SSR STS	Scholz *et al.,* 2009	Introgression of resistance to BYDV from *Hordeum bulbosum* into cv.'Igri'.

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		SOYBEAN		
11	Soybean mosaic virus (SMV)	SSR	Saghai Maroof *et al.,* 2008; Shi *et al.,* 2009	Pyramiding of Rsv1,Rsv2 and Rsv3 genes in homozygous condition in to lines. three gene pyramid lines showed resistant to all strains
12	SCN resistance	SSR	Arelli *et al*., 2007	Registration of germplasm JTN-5303
13	Resistance to frogeye leaf spot (*Cercospora sojina*)	SNP SSR	Shannon *et al.,* 2009	Registration of germplasm line S99-2281
		WHITE BEAN		
14	Bean golden yellow mosaic virus (BGYMV) and QTLs for common bacterial blight resistance.	SCAR	Beaver *et al.,* 2008	Registration of cultivar 'Verano'

Lack of conversion of publications into practical applications: A high proportion of published markers fails the translation step from research to application (Xu and Crouch 2008). Converting promising publications into practical large-scale applications in breeding programs requires different practical, economical, logistical, and genetical constraints to be resolved. Before MAS realizes its full potential in public sector breeding programs, (i) published markers need to be validated, (ii) simple, quick, and cheap technical protocols for tissue sampling need to be developed, (iii) high throughput precision phenotyping systems for QTL mapping are needed and, (iv) improved understanding of genotype by environment interaction and epistasis has to be gained (Xu and Crouch 2008).

G x E interactions and effects of genetic background: The success of marker assisted selection for complex traits will largely depend on two things: the accuracy of plant phenotyping on one hand and the understanding of genetic phenomena such as G x E interactions and epistasis on the other hand. If quantitative traits are to be improved with MAS it is essential to have information about the G x E interactions. G x E interactions impede the repeatability of QTL mapping results and consequently reduce the efficiency of selection (Koebner 2004). Especially QTLs with small effects can vary in magnitude and direction of effects, depending on environmental conditions. The extent of G x E interactions is not always known after conducting a mapping study, because such studies are usually restricted to a few years and/or locations (Collard and Mackill 2008). Epistasis is the phenomenon that genes sometimes show a certain positive or negative effect only in combination with each other. For QTLs this can lead to unpredictability of expression in genetic backgrounds other than the one in which they have been detected (Koebner 2004). Where G x E interactions or epistasis are important, it is necessary to regularly re-estimate QTL effects within the breeding program.

Economic aspects of MAS: Only few studies compare the economical aspects of conventional phenotypic selection and MAS. Landmark papers are the one by Dreher *et al.* (2003) and the companion paper by Morris *et al.* (2003). Morris *et al.* (2003) stated in their paper that "as most plant breeders well know, the cost of using DNA markers can vary greatly depending on the crop, the breeding application, the trait(s) being targeted, the availability of suitable marker technology, and other factors. This application specificity complicates economic analysis, but it does not invalidate it completely. The choice between conventional breeding and MAS involves a trade-off between time and money. They suggested that the cost-effectiveness of using MAS depends on four parameters: the relative cost of phenotypic versus marker screening; the time saved by MAS; the size and temporal distribution of benefits associated with accelerated release of improved germplasm and, finally, the availability of operating capital to the breeding programme.

Intellectual property rights (IPR) and MAS: The importance of molecular marker analyses for different applications was recognized very early, resulting in the filing of many patents in the last 10-15 years (Jorasch 2004). From the private sector, there are no reports of

the cost-benefit ratio associated with commercializing MAS-derived cultivars. However, the growing number of patent applications associated with MAS shows that the use of such approaches is seen as a comparative advantage in commercial breeding programs (Dwivedi *et al.* 2007). If companies ensure their rights through the patenting of developed markers, no matter whether they are further utilized or not, they impede the use of these markers by others. The continual assertion and protection of IPR is seen in different ways by different authors. Some opine that it has often inhibited knowledge dissemination, research and development (Stafford 2009), while in others opinion the "patents describe the latest inventions made by innovative researchers and companies and the publication of these patents guarantees their public availability. This, in turn, allows the further development and improvement of these innovative techniques" (Jorasch 2004).

CONCLUSION

Recent developments in DNA marker technology together with the concept of marker-assisted selection provide new solutions for selecting and maintaining desirable genotypes. Marker assisted selection can be performed in early segregating populations and at early stages of plant development for pyramiding the resistance genes, with the ultimate goal of producing varieties with durable or multiple disease resistance. Thus, with MAS it is now possible for the breeder to conduct many rounds of selection in a year. Molecular marker technology is now integrated into existing plant breeding programmes all over the world in order to allow researchers to access, transfer and combine genes at a faster rate and with a precision not previously possible. However, potential limitations that might restrict the wide application of MAS in breeding were high costs and non-availability of suitable markers but, not as MAS is less efficient compared to phenotypic selection. On the contrary, especially in breeding of bi- or perennial crops markers were expected to lead to a high efficiency gain. Regarding the impact of MAS on breeding in near future an increase in relevance and application is unanimously expected. New technological developments such as automation, allele-specific diagnostics and diversity array technology will make MAS based gene pyramiding more powerful and effective. Especially the increased application of SNPs and improved technologies for sequencing will contribute to an increasing impact of MAS. The MABC strategies will gain importance and more emphasis is needed on combined selection systems, rather than viewing MAS as a replacement for phenotypic or field selection. It is also critical that future endeavours in MAS are based upon lessons that have been learnt from past successes and especially failures in using MAS. Further optimization of marker genotyping methods in terms of cost effectiveness and a greater level of integration between molecular and conventional breeding represent the critical aspects for the greater adoption of MAS in crop breeding in the near future. The increase in importance of MAS is not expected to be the same for all crops, for high value crops it may be of top priority. The new tools of molecular breeding will have a better opportunity for demonstrating their true values for crop improvement, when these techniques reach a higher degree of automation; it will be possible to use molecular markers leading to "gene revolution" in the world of agriculture.

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