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# Marker-assisted Selection for Disease Resistance: Applications in Breeding (Review)

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**ABSTRACT:** Molecular markers have proven to be invaluable tools for assessing plants' genetic resources by improving our understanding with regards to the distribution and the extent of genetic variation within and among species. By using molecular markers, breeders can bypass traditional phenotype-based selection methods, which involve growing plants to maturity and closely observing their physical characteristics in order to infer underlying genetic make-up. Marker-assisted selection (MAS) provides opportunities for enhancing the response from selection because molecular markers can be applied at the seedling stage, with high precision and reductions in cost. MAS could be easily applied, but is often not necessary because the resistances are selected phenotypically. In quantitative disease resistances, MAS would be very useful, but the individual QTL often have small effects. Additionally, only a few monogenic resistances are durable and only a few QTL with high effects have been successfully transferred into elite breeding material. Further economic and biological constraints, e.g., 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. The purpose of this article is to describe the available genetic marker types and provide the status of gene mapping and marker-assisted selection in important crop species. This review highlights how genetic markers have been used in mapping genes and discusses the importance of MAS and how it can be integrated into breeding programs for enhancing selection efficiency in developing disease resistant.
***Keywords:*** Disease resistance; marker-assisted breeding; marker-assisted selection (MAS); molecular markers; quantitative trait loci (QTLs)

## INTRODUCTION

Plant breeding has a long history of development beginning with the artificial domestication of crop species. Modern plant breeding based on the fundamental principles of inheritance has become an important component of agricultural science and technology. It has features of both science and arts. Conventional breeding methodologies have extensively proven successful in development of plant cultivars and germplasm. The most renowned examples include the semi-dwarf high-yielding cultivars of cereals developed during the Green Revolution and the hybrid rice developed in 1970s. However, conventional breeding is still dependent to a considerable extent on subjective evaluation and empirical selection. Scientific breeding needs less experience and more science, i.e. practical and accurate evaluation, and effective and efficient selection. Molecular marker-assisted breeding (MAB) has brought great challenges, opportunities and prospects for conventional breeding.

Along with progress in molecular biotechnology, various types of molecular markers in crop plants were developed during the 1980s and 1990s (Xu, 2010). The rapid development of molecular markers (particularly DNA markers) and continuous improvement of molecular assays has led to the birth of a new member in the family of plant breeding - molecular marker-assisted breeding (MAB). The extensive use of molecular markers in various fields of plant science, e.g. germplasm evaluation, genetic mapping, map-based gene discovery, characterization of traits and crop improvement, has demonstrated that molecular technology is a powerful and reliable tool in genetic manipulation of agronomical important traits in crop plants (Xu, 2010; Jiang, 2013). A variety of DNA-based markers has become available over the last two decades. While the development of some of these markers has been driven directly by the requirement for better tools for genetic analysis, others have been a happy by-product of genomics research. In particular, the growing feasibility of applying high throughput platforms to molecular

marker technology has been primarily facilitated by developments in genomics research. Thus in 1996, the mapping of 5000 microsatellite loci in the human genome merited a major publication in Nature (Dib et al., 1996), but by 2003, the number of known human single nucleotide polymorphisms (SNPs) had already exceeded five million (http://www.ncbi.nlm.nih.gov/SNP/snp_summary.cgi). Therefore the expectation is that the number of SNP assays in the cereals is set to increase dramatically. With the coming together of a large number of markers and the expected development of realistic high throughput platforms, it is now timely to explore present and future prospects of using marker technology in real cereal breeding. Markers provide a tool for indirect selection, but the definition of marker-trait associations is the second element necessary for the deployment of marker-assisted selection (MAS). MAS refer 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 (Ragimekula et al., 2013). Elucidating the inheritance of quantitatively inherited traits by searching for linkage between the loci controlling them and factors which segregate in a simple Mendelian fashion is a concept that has its roots in the early days of genetics. Thus, already in 1918, Payne showed that the genetics of bristle number in Drosophilacould be simplified by the demonstration of its linkage to loci mapped to the X chromosome. Similarly, easily scorablecolour phenotypes were used to predict continuously variable traits such as seed weight in Phaseolus(Sax, 1923), and fruit size in tomato (Lindhout, 2002). But because of the lack of a sufficient supply of simply inherited morphological variants, little progress in marker assisted plant breeding was made until 1960s, when biochemical markers, based mainly on the histological staining of specific enzyme activities, were developed. At that time, the discovery of isozyme-determining loci linked to genes determining plant traits enjoyed a brief period of prominence. For instance, in maize, linkage was established between a number of such marker loci and factors determining yield (Stuber et al., 1982) and some of these markers were used to select for yield improvement (Stuber et al., 1982). However, their widespread use in breeding was limited, mainly because of difficulties in scaling up the assays to the levels needed in breeding programs.

Disease resistance is an important trait in public and commercial plant breeding. For each breeder, it is a critical decision for which diseases resistance selection should be performed because each additional trait reduces selection intensity for all other traits given a fixed population size or requires a higher budget. This decision is governed by the yield losses that are caused by the disease, the importance to the farmer, the alter-native measures for disease control,the availability of resistance sources, their durability and the input needed for selection. Molecular markers might stimulate selection gain because they can be assessed in high-throughput techniques at a very early growth stage with high heritability and they are relatively cheap since the advance of single-nucleotide polymorphism (SNP) detection platforms (Miedaner et al., 2012). Besides technical questions, the availability of markers with a linkage as close as possible to genes of high impact, ideally based on the gene sequence (“perfect marker”), is the most critical point for successful marker-assisted selection (MAS) and/or marker-assisted back-crossing (MABC) (Ribaut et al., 2010; Miedaner et al., 2012).

Plant disease resistance can be classified into twocategories: qualitative resistance conferred by a singleresistance (R) gene and quantitative resistance (QR)mediated by multiple genes or quantitative trait loci (QTLs) with each providing a partial increase in resistance. Other terms have also been used for these genetically distinguishable resistances (Hu et al., 2008; Poland et al., 2009).

***Molecular Markers***

Genetic markers are the biological features that are determined by allelic forms of genes or geneticloci and can be transmitted from one generation to another, and thus they can be used asexperimental probes or tags to keep track of an individual, a tissue, a cell, a nucleus, a chromosomeor a gene. Genetic markers used in genetics and plant breeding can be classified into two categories: classical markers and DNA markers (Xu, 2010). Classical markers include morphological markers, cytological markers and biochemical markers. DNA markers have developed into many systems based on different polymorphism-detecting techniques or methods (southern blotting – nuclear acid hybridization, PCR – polymerase chain reaction, and DNA sequencing) (Collard et al., 2005), such as RFLP, AFLP, RAPD, SSR, SNP, etc.

***RFLP***

……A restriction fragment length polymorphism, or RFLP, (commonly pronounced "rif lip"), is a variation in the DNA sequence of a genome that can be detected by breaking the DNA into pieces with restriction enzymes and analyzing the size of the resulting fragments by gel electrophoresis. It is the sequence that makes DNA from different sources different, and RFLP analysis is a technique that can identify some differences in sequence (when they occur in a restriction site). Though DNA sequencing techniques can characterize DNA very thoroughly, RFLP

analysis was developed first and was cheap enough to see wide application (Rayaa et al., 2002; Joshi et al., 2004). Analysis of RFLP variation was an important tool in genome mapping, localization of genetic disease genes, determination of risk for a disease, genetic fingerprinting, and paternity testing. The basic technique for detecting RFLPs involves fragmenting a sample of DNA by a restriction enzyme, which can recognize and cut DNA wherever a specific short sequence occurs, in a process known as a restriction digest. The resulting DNA fragments are then separated by length through a process known as agarose gel electrophoresis, and transferred to a membrane via the Southern blot procedure. Hybridization of the membrane to a labeled DNA probe then determines the length of the fragments which are complementary to the probe. A RFLP occurs when the length of a detected fragment varies between individuals. Each fragment length is considered an allele, and can be used in genetic analysis (Joshi et al., 2004). Analysis of RFLP variation in genomes was vital tool in genome mapping and genetic disease analysis. If researchers were trying to initially determine the chromosomal location of a particular disease gene, they would analyze the DNA of members of a family afflicted by the disease, and look for RFLP alleles that show a similar pattern of inheritance as that of the disease (see Genetic linkage). Once a disease gene was localized, RFLP analysis of other families could reveal who was at risk for the disease, or who was likely to be carriers of the mutant gene. RFLP analysis was also the basis for early methods of Genetic fingerprinting, useful in the identification of samples retrieved from crime scenes, in the determination of paternity, and in the characterization of genetic diversity or breeding patterns in animal populations (Vos et al.,1995; Zietkiewicz et al.,1994)

***RAPD***

Random amplified polymorphic DNA is a dominant marker based on polymerase chain reaction(PCR). It employs a single decamer primer of arbitrary sequence, which is annealed to thetemplate DNA typically at 37°C (Willcoxet al., 1990). The variation in RAPD profile is in the form of presenceor absence of a band resulting from variation in primer binding sites. A major limitation of thismarker system is non-reproducibility due to low annealing temperature. However, utility of adesired RAPD marker can be increased by sequencing its termini and designing longer primers(e.g. 24 nucleotides) for specific amplification of markers (Paran and Michelmore, 1993). Such sequenced characterizedAmplified regions (SCARs) are similar to sequence-tagged-sites [STS, (Olson et al., 1989)] in construction andapplication.

***CAPS***

Cleaved amplified polymorphic sequences are based on the restriction enzyme site variation inthe DNA fragments generated by PCR (Konieczyn and Ausubel, 1993). The source of the sequence information for the primerscan come from a gene bank, genomic or cDNA clones, or cloned RAPD bands. This marker isa co-dominant marker.

***SSRs***

Simple sequence repeats or microsatellites are ubiquitous in eukaryotes. SSR polymorphismreflects variation in the number of repeat units in a defined region of the genome. The frequencyof repeats longer than 20 bp has been estimated to occur every 33 kb in plants. Nucleotidesequence flanking the repeat is used to design primers to amplify different number of repeat unitsin different varieties. These primers are very useful for rapid and accurate detection of polymorphicloci and the information could be used for developing a physical map based on these sequencetags. This type of polymorphism is highly reproducible(Miahet al., 2013).

Since the 1990s SSR markers have been extensively used in constructing genetic linkage maps, QTL mapping, marker-assisted selection and germplasm analysis in plants. In many species, plenty of breeder-friendly SSR markers have been developed and are available for breeders. For instance, there are over 35,000 SSR markers developed and mapped onto all 20 linkage groups in soybean, and this information is available for the public (Song et al., 2010).

***AFLP***

The amplified fragment length polymorphism markers are generated by selective amplification ofDNA fragments obtained by restriction enzyme digestion (Voset al., 1995). High molecular weight DNA isdigested by two restriction enzymes: one hexacutter (e.g. EcoRI) and one tetracutter (e.g. Mse I).Adapter molecules are ligated to the ends of DNA fragments. Two primers possessing sequenceMolecular Mapping and Marker Assisted Selection of Traits 291complementarity to the adapter as well as few extra random nucleotides at their 3′ends are usedfor selective amplification of fragments employing PCR. The amplified products are separatedon sequencing gels or even ordinary PAGE and visualized by silver staining. Alternatively, theprimers are labeled either by radioisotope or fluorescent dye so that the AFLP profile can beobtained by autoradiography or by using image analysis. The

highest number of amplifiedproducts (50-100) is produced in AFLP among all the DNA profiling systems. This increases theprobability of detecting polymorphism many folds. The technique is, at present, lengthier andcostlier than other PCR based techniques. It requires good quality DNA for ensuring completedigestion by enzymes. Partial digestion of DNA results in non-reproducible variation in DNAprofiles.

***SNP***

Molecular markers are polymorphic when there is DNA sequence variation between the individualsunder study. Molecular markers are, therefore, simply an indicator of sequence polymorphism.Sequence polymorphism between individuals can take many forms, for instance, it can be dueto the insertion or deletion of multiple bases, or it can be due to single nucleotide polymorphisms[SNPs; (Brookes, 1999)]. Insertions, deletions and SNPs are important in determining sequence variationbetween individuals. SNPs are abundant in plant genomes. They are being used for genotypinghuman populations for certain genetic diseases. The cost of developing SNPs is very high, sincefor each locus DNA has to be sequenced and suitable PCR primers designed. The primers mustthen be used to amplify the corresponding fragment from all other possible genotypes. Thesefragments must then be sequenced and the sequences compared with one another to determinethe SNPs for each haplotype (Brookes, 1999). The term 'haplotype' is used in the context of SNPs insteadof the term 'allele'. There are number of methods for identifying SNPs within a genetic locusnamely direct sequencing, single-strand conformation polymorphism (SSCP), chemical cleavageof mismatches (CCM) and enzyme mismatch cleavage (EMC).

**Two classes of genes in disease resistance**

On the basis of the current model, plants respond to pathogen infection through two types of immune responses: basal resistance and race-specific resistance (Jones and Dangl, 2006)(Figure 1). Plant–pathogen recognition initiates the signal transduction pathways that interact with each other to form a complex network leading to defense responses (Panstrugaet al., 2009). We can simply divide the genes involved in disease resistance into two classes, the receptor genes, which includeRgenes and host pattern recognition receptor (HPRR) genes, and defense-responsive or defense-related genes (Figure 1). The latter are characterized by responding to a pathogen attack via changing expression levels or posttranslational modifying their encoding proteins (Eulgem, 2005; Benschopet al., 2007). There are large numbers of defense-responsive genes in a given species (Wisseret al., 2005; Benschopet al., 2007; Bogackiet al., 2008).

The encoding proteins of defense-responsive genes function either as activators or as suppressors in defense responses. The following evidence suggests that defense-responsive genes and HPRR-type genes are important resources for quantitative BSR and DR.

***Molecular bases of BSR and DR***

Defense signaling pathways leading to basal and race-specific resistance often cross-talk (Hammond-Kosack and Parker, 2003; Panstrugaet al., 2009)(Figure 1). On the basis of the current model of host–pathogen interaction and the features of the characterized genes contributing to QR, we may propose models to elucidate the molecular mechanisms of quantitative BSR and DR. Race-specific resistance QTLs have been identified in different crops (Darvishzadehet al., 2007; Balliniet al., 2008; Marcel et al., 2008; Werner et al., 2008). Thus, the quantitative BSR of a given plant may be enhanced by the cooperation of multiple genes functioning in path-ways leading to resistance against different pathogen species or different races of the same species. The above evidence also suggests that quantitative BSR can be conferred by a single gene. In this case, the gene may function in a basal-resistance pathway, in overlapping pathways between different race-specific resistances, or in the cross-talk point of different defense pathways (Figure 1). All the three situations can include both pathogen species-nonspecific resistance and race-nonspecific resistance. This one-gene model is supported by CaAMP1, OsWRKY13, andOsDR8.CaAMP1may function in basal resistance (Lee et al., 2008).OsWRKY13andOsDR8function in resistances mediated by two different Rgenes (Hu et al., 2008; Hu and Wang, 2009).

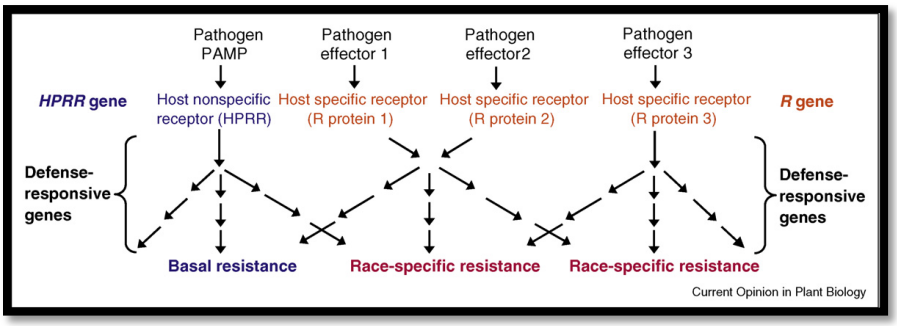


Figure 1.Cross-talks between basal and race-specific resistance pathways and between different race-specific resistance pathways. Basal resistance is initiated by the interaction between host pattern recognition receptors (HPRRs) and evolutionarily conserved pathogen-associated molecular patterns (PAMPs), which is a relative nonspecific defense response. Race-specific or gene-for-gene resistance is triggered by the direct or indirect interaction of host resistance (R) proteins and complementary pathogen effector molecules

Quantitative DR may be controlled by one or multiple genes, whose encoding proteins do not interact with rapidly evolving pathogen factors that play essential roles in pathogenicity on plants (Ma and Guttman, 2008). On the basis of this hypothesis, we may predict, to certain extent, which gene in QR may mediate DR. Cytochrome P450 enzyme StAOS2 is essential for biosynthesis of the defense signaling molecule jasmonic acid (Pajerowska-Mukhtaret al., 2008). ThusStAOS2fits this hypothesis as a candidate for quantitative DR gene to pathogens whose infection can be suppressed by host jasmonate-dependent pathway. Transcription regulator OsWRKY13 functions by directly or indirectly regulating the expression of host defense-responsive genes (Hu et al., 2008; Hu and Wang, 2009).

ThusOsWRKY13may be a quantitative DR gene. How-ever, whether the known DR QTLs,Lr34, Yr36,andpi21, also fit the above hypothesis would await the characterization of the biochemical functions of their encoding proteins. It may also be likely that a DR gene may not be durable infinitively. A new effector may become adapted to the resistance in evolutionary time (Ma and Guttman, 2008; Niks and Marcel, 2009). The polygenic control underlying QR is proposed to make it more difficult for pathogens to overcome these multiple resistances (Ayliffeet al., 2008; Palloixet al., 2009; Poland et al., 2009), which constitute more effective DR.

***Approaches for breeding BSR and DR crops***

Marker-assisted selection (MAS) has been widely applied in breeding programs for targeted transferring and pyramiding resistance loci in different crops (Kolmer, 1996;Foolad et al., 2002; Singh et al., 2005; Liu et al., 2004, Richardson et al., 2006;Asea et al., 2009;Moloney et al., 2009).MAS will provide an important approach for breeding BSR and DR corps, although in the past QR has been used in crop improvement by conventional breeding without the knowledge of resistance loci (Parlevliet and van Ommeren, 1988). With the knowledge of genes underlying resistance loci, MAS can improve the efficiency by using gene-specific or closely linked markers to avoid bringing undesired traits into an improved cultivar owing to linkage drag (Fukuoka et al., 2009;Lagudah et al., 2009). MAS may be more effective for the selection of the first and second groups of resistant loci mentioned above, whose functions depend on the specificities of encoding proteins. MAS may be less effective for selection of the third group of resistant loci, whose functions are initiated by upstream signaling thus depend on the genetic background. This prediction is supported by the reports that MAS is less effective for selection of some quantitative traits other than disease resistance (Xu and Crouch, 2008; Robbins and Staub 2009).

Characterized QR genes can also be used more efficiently for crop improvement with strategies involving transgenic.Lr34confers only adult plant resistance, which appears to be associated with its increased expression in the adult stage(Krattinger et al., 2009).Yr36-mediated high temperature-dependent resistance is due to high temperature induction ofYr36(Fu et al., 2009), suggesting that the function of Yr36 may also be associated with its expression level. Constitutive expressing Xa3/Xa26andXa21can change adult-stage resistance to whole-growth-stage resistance (Hu and Wang, 2009). It will be interesting to see whetherLr34and Yr36can confer a whole-growth-stage or temperature-independent resistance if regulated using a pathogen-induced or tissue-specific strong promoter. The use of a recessive gene in hybrid crop production is not convenient. QTLpi21is a recessive gene. However, it can be used in breeding programs by suppressing its dominant (susceptible) alleleby means of an RNA interference strategy [Fukuoka]. Thereare other advantages of improving crop resistance by manipulating the expression of QR genes using appropriate promoters. This approach may enable the utilization of QR genes

whose functions depend on upstream signaling and may enhance the effect of a single gene in QR, which has small effect driven by the native promoter, for improving BSR or DR (Hu et al., 2008).

***Marker-assisted selection for quantitative resistance loci***

Although there is a plethora of published QRLmapping studies on a wide array of diseases in numerous crop plants, far fewer papers actually demonstrate the use of MAS for QRLs in breeding for germplasm improvement and cultivar development. The reasons are varied, but include the following. Private sector seed breeding companies, which are most likely to be using MAS breeding on a large scale, rarely, publish their results due to intellectual property issues surrounding technology applications, inventions, and germplasm identities that could benefit their competitors (Bernardo, 2008; Collard and Mackill, 2008). Public breeding programs can be hampered by insufficient funds, lack of access to lab-based enabling technologies and/or technological limitations in sample throughput in employing MAS breeding for QTLs on the larger scale required for cultivar breeding programs (Young 1999; Bernardo 2008; Collard and Mackill, 2008; Fukuoka et al., 2009). In academic research institutions, more emphasis may be placed on basic research, and the process of cultivar development and cultivar release may not be considered worthy of publication and/or not valued as a scholarly contribution(Yamamoto et al., 2000; Collard andMackill, 2008). Phenotypic selection may be more cost-effective than MAS for improvement of a particular trait, so MAS may not be warranted. MAS can increase the efficiency of selection for superior genotypes in the earlier stages of cultivar development. However, the time required to test advanced breeding lines in field environments over several field seasons prior to final cultivar release cannot be shortened because the ultimate success of a cultivar depends on its consistent superior performance ingrowers' fields.There-fore, there is a significant time lag between the creation of marker-selected breeding lines and the ultimate release of a finished cultivar, which contributes to a dearth of published reports on the products of successful MAS breeding.

Because the published literature is notinclusive nor representative of all MAS breeding efforts in private and public breeding programs, I have chosen to present examples of MAS for QRLs in crop plants for which (a) the phenotypic effect of the QRLs was verified across genetically distinct populations or in different genetic backgrounds and tested in field environments prior to application of MAS; (b) MAS was employed to actually transfer beneficial alleles at one or more QRLs to create marker-assisted selected breeding lines or cultivars; and (c) themarker-assisted selected breeding lines or cultivars were evaluated phenotypically for disease resistance under field conditions typical for that crop to determine effectiveness of MAS breeding. When available, reports on released breeding lines or cultivars as MAS breeding products were included. The examples presented here are not intended to be exhaustive in scope, but illustrative of current uses of MAS for QRLs in breeding, and primarily represent public breeding efforts. The examples are organized by crop species and disease, and sorted by the categories of MAS breeding for (a) individual QRL, (b) combining (pyramiding) multiple QRLs, and (c) combining QRLs and qualitative resistance gene(s).

***MAS Breeding for Individual QRLs: Selected Examples***

Fusarium head blight (FHB) in wheat. A major QRLFhb1for resistance to FHB (causal agentFusariumgraminearum) was mapped on wheat chromosome 3B in a population derived from resistant parent cultivar 'Sumai 3', and the phenotypic effect and chromosomal location of this QRL was confirmed in a second mapping population (Anderson et al., 2001). Additional validation studies were performed in other populations and confirmed the major effect ofFhb1on resistance to FHB (Pumphrey et al., 2007). Subsequently, 19 pairs of near-isogenic lines (NILs) for the resistant 'Sumai 3' allele at QRL Fhb1were developed from 13 existing breeding populations using tightly linkedmarkers, and each pair was tested in four replicatedfieldexperiments and a green-house screen (Pumphrey et al., 2007). NILs with theFhb1resistant allele had significant average reductions of 23% in disease severity ratings and 27% for infected grain, indicating thatFhb1was effective in reducing disease. The markers tightly linked to the 'Sumai 3' allele atFhb1account for a consistently large phenotypic effect (>20%) on resistance across multiple populations (Pumphrey et al., 2007), and as a result,Fhb1has been used in wheat breeding programs worldwide (Haberle et al., 2009). Another major FHB QRL, located on chromosome 1B, has been mapped in European winter wheat and validated as a suitable target for MAS, along with 3 other QRLs on chromosomes 2B, 6A,and 7B (Haberle et al., 2009).

***Tan spot in wheat***

Tan spot caused by Pyrenophoratritici-repentis is a commonly occurring insidious disease on the Canadian prairies that regularly causes considerable losses. Because of its endemic nature, tan spot has received little attention in breeding, with other diseases that are epidemic in nature or that impart toxins on the grain such as FHB receiving most of the attention. For these reasons, MAB for resistance to this disease is appealing. Markers have

been developed for the Tsn1 locus (Singh et al., 2010). Canadian durum breeding has focused on the incorporation of Tsn1 resistance using flanking markers Xfcp620 and Xfcp394.

***Late blight in tomato***

QRLs for late blight (causal agent Phytophthorainfestans) have been mapped on all 12 tomato chromosomes in two reciprocal interspecific backcross populations, indicating the multigenic nature of resistance; the QRLs most consistently detected across replicated experiments and assays were located on chromosomes 1, 3, 4, 5, and 11 (Brouwer et al., 2004a). Subsequently, Brouwerand St. Clair (2004b) created NILs usingMASbackcrossing to transfer threeQRLs (chromosomes 4, 5, and 11) individually from wild tomato into an elite cultivar background. Three sets of recombinant sub-NILs were evaluated in replicated field experiments for dis-ease and horticultural traits. All three QRLs were fine-mapped to smaller chromosomal regions, and linkage drag for several horticultural traits was observed (Brouwer and St Clair, 2004b). In a current study (D.A. St.Clair, J.E.Haggard, E.B. Johnson, unpublished data), the QRLs on chromosomes 5 and 11 are being subjected to higher-resolution mapping using marker-selected recombinants within the previously fine-mapped QRL regions. The recombinants are undergoing evaluation in replicated field and growth chamber experiments over several years to further delineate the regions responsible for QDR and provide linked markers for MAS breeding. Horticultural trait loci linked to the QRLs are also being mapped to determine the extent and effects of linkage drag.

***Leaf rust in barley***

QRLs for resistance to leaf rust (causal agent Pucciniahordei) were mapped in two recombinant inbred line (RIL) populations (Qi et al., 2000); three of the QRLs conferring adult plant resistance were confirmed using NILs created by MAS backcrossing (Varshney and Tuberosa, 2008). Marcel *et al.* (Marcel et al., 2007a) used MAS backcrossing to transfer the same three QRLs (Rphq2, Rphq3, Rphq4) from the resistant parent 'Vada' into a susceptible background and determined that all three conferred a reduction in disease by in-creasing the latent period.Rphq2, which had the largest positive phenotypic effect, was high-resolution mapped to a 0.11 kb region, providing tightly linked markers for this QRL (Marcel et al., 2007a). A subsequent study (Marcel et al., 2007b) determined thatRphq2 was most effective in seedlings, whereasRphq3 was effective in both seedlings and adult plants and against multiple isolates. Marcel *et al.*(Marcel et al., 2007b) created a consensus map from three RIL and three double haploid populations, and divided the map into 210 sections (BINs) of 5 cM each, with each BIN delineated by specific flanking markers. Marcel *et al*. (2007b) placed 19 QRLs for leaf rust detected in several populations onto the consensus map. Each population, derived from different combinations of barley parentlines, segregated for different sets ofQRLs withonly a few shared by any pair of cultivars, indicating an abundance of QRLs for leaf rustin barley germplasm. It was concluded that continuedphenotype-based selectionby breeders against susceptibility to leaf rust over years has increased the level of QDR in cultivated barley and likely contributed to the abundance of QRLs (Marcel et al., 2007b).

***White mold in common bean***

Resistance to white mold (causal agent Sclerotiniascle rotiorium) in common bean was found to be controlled by over 10 independent QRLs (Miklas et al., 2006;Miklas 2007). Major-effect QRLs located on bean LGs B2, B7, and B8 were verified in multiple populations and environments (Ender and Kelly 2005;Miklas et al., 2006; Ender et al., 2008). Miklas et al.,(2006) performed MAS backcrossing to transfer a QRL on LG B7 into pinto bean cultivar 'Winchester' and a QRL on B8 into pinto bean cultivar 'Maverick' and great northern bean 'Matterhorn'. The resulting backcross inbred lines were evaluated in replicated field tests and greenhouse disease assays. Averaged across populations and tests, QRLs on LGs B7 and B8 reduced disease severity 15% and 17%, respectively, indicating that transfer of single QRLs effectively reduced disease in multiple genetic backgrounds (Miklas, 2007). Ender et al., (2008) used MAS to transfer two QRLs on LGs B2 and B7 in two RIL populations, derived from resistant parent 'Bunsi', and evaluated selected lines in the field over two years for white mold disease. In both RIL populations, the marker-selected RILswitch the resistance alleles atQRLs onLGs B2 andB7 had significantly lower disease severity in both years than the controls with susceptible QRL alleles, indicating the consistent effect of QRL alleles from 'Bunsi' on reducing disease (Ender et al., 2008). QRLs for white mold resistance in common bean are being used in MAS-based breeding programs for line and cultivar development, and stacking (pyramiding) of QRLs is recommended to improve overall resistance to white mold (Miklas et al., 2006;Miklas, 2007; Ender et al., 2008).

### *Marker-Assisted Selection in Gene Pyramiding*

The great opportunity offered by MAS to select superior lines based on genotype rather than phenotype becomes clearly obvious in the case of combining different simple inherited resistance genes of large effects for a given pathosystem in a single genotype (gene pyramiding), since it is difficult to select plants with multiple resistance genes based on phenotype alone as the action of one gene may mask the action of another. Pyramiding multiple qualitative disease resistance genes with different race specificities has been proposed asawayofachievingmorecomprehensive resistance (Mundt, 1990)dueto simultaneous or stepwise mutation of several avirulence genes in the pathogenthatisneededtoovercomethispyramid. Successful examples for the pyramiding of major genes in single genotypes are given for the pathosystems rice:*Xanthomonasoryzae*pv. oryzae,rice:Magnaporthegrisea,and wheat:*Blumeriagraminis*f. sp. *tritici* (Table 1). Novel approaches deal withthe implementation of transgenes in breeding programs such as the pyramiding of Btgenes cry1Ac and cry1C conferring resistance to diamondback moths in broccoli (Cao et al., 2002; Table 1).

Durable disease resistance is not associated with a distinct type or mechanism of resistance, but only refers to the number of genes involved in resistance reaction (Lindhout, 2002), for which reason pyramiding multiple quantitative or qualitative and quantitative resistance alleles in single genotypes is an approach to increase the level of disease resistance. Castro *et al.* (2003a, d) reported on the marker-assisted pyramiding of three quantitative resistance loci against barley stripe rust, caused by *Pucciniastriiformis*f. sp. *hordei*. Resistance alleles at two QTL were necessary for the seedling resistance phenotype being expressed fitting a complementary gene model, while all three QTL regions were significant determinants of adult plant stripe rust resis tance, with an additive effect of existing resistance alleles.

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 opportunitiesSuitable 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).

### *MAS Breeding for Combining QRLs and Qualitative Resistance Genes: Selected Examples*

#### *Stripe rust in barley*

Castro et al. (2003a) combined a qualitative stripe rust (causal agent Puc-ciniastriiformis f. sp. hordei) resistance gene, Rpsx, on chromosome 1 with three QRLs located on chromosomes 4, 5, and 7 that had been validated previously (Castro et al. 2003c). MAS and a series of crosses were used to create doubled-haploid (DH) lines with combinations of QRLs only and QRLs with Rpsx. The DH lines were evaluated for disease severity in replicated field experiments. Disease severity was lowest in lines with the resistance allele at Rpsx regardless of the QRLs present. The effect of the QRLs was significant only in the absence of the resistance allele at Rpsx; the presence of QTL4 and QTL5 significantly decreased disease severity whereas QTL7 did not. In the case of breakdown of Rpsx, the presence of effective QRLs such as QTL4 and QTL5 could provide reduction in disease severity (Castro et al., 2003a).

Table 1. MAS in gene pyramiding

| Crop | Trait (combination of genes) | References |
|---|---|---|
| Rice | Bacterial blight resistance (xa4+xa5+xa13+Xa21; xa5+xa13+Xa21) | Huang et al., (1997)<br>Sanchez et al., (2000)<br>Singh et al., (2001) |
| | Blast resistance (Pi1+Piz-5+Pita) | Hittalmani et al., (2000) |
| | (Pi-tq5, Pi-tq1, Pi-tq6, Pi-lm2: pyramids of 2 to 4 genes) | Tabien et al., (2000) |
| | Multiple resistance: bacterial blight (Xa21)<br>Sheath blight (RC7)<br>Yellow stem borer Btfusion gene (cry1AB/cry1Ac) | Datta et al., (2002) |
| Wheat | Powdery mildew resistance (Pm2+Pm4a; Pm2+Pm21; Pm4a+Pm21) | Liu et al., (2000) |
| Barley | Stripe rust resistance (3 QTL) | Castro et al., (2003b, d) |
| Broccoli | Diamondback moths resistance (cry1Ac+cry1c) | Cao et al., (2002) |
| Soybean | Lepidopteran resistance (cry1Ac+corn earworm QTL) | Walker et al., (2002) |

***Bean golden mosaic virus (BGMV) in common bean***

A major QRL located on linkage group B4 that reduces disease severity has been combined with qualitative resistance genebgm-1using MAS backcross breeding at the international bean breeding center in Columbia (CIAT) to create resistant bean lines (Miklas et al., 2006). Prior to use in breeding, the PCR-based markers tightly linked to the QRL on B4 and to bgm-1were shown to cosegregate for bean golden mosaic virus (BGMV) resistance in advanced breeding lines derived from multiple parents (Kelly et al., 2003). The PCR markers for these genes can be multiplexed, increasing the efficiency of MAS and enabling routine use in bean breeding programs (Miklas et al., 2006). Markers for additional resis-tance genes and QRLs are in development for application in MAS breeding (Kelly et al., 2003;Miklas et al., 2006).

***Potato virus Y (PVY) and pepper***

Carantaet al., (1997) mapped multiple QRLs for resistance to two potato virus Y (PVY) isolates in a pepper population derived from susceptible cultivar 'Yolo Wonder' (YW) and resistant line Perennial, including twomajor QRLs on chromosome P1 and one on P6. A major qualitative resistance gene,*pvr2*$^{3}$, confers pathotype-specific resistance to PVY and is located on chromosomeP4. Ina subsequent studyoncombining*pvr2*$^{3}$and PVY QRLs, Palloix et al., (2009) used MAS on DH lines derived from a cross of YW x Perennial to select DH lines that differed for alleles at*pvr2*and at the three major QRLs, PVY-P1.1, P1.2, andP6.1, such that the DH lines had either all resistant alleles (*pvr2*$^{3}$+ resistant alleles at all 3 QRLs) or a mixture: the resistant allele*pvr2*$^{3}$+susceptible alleles atall 3 QRLs, or the susceptible allele*pvr*$^{2+}$andresistant alleles at all 3 QRLs. The DH lineswere challenged with five PVY isolates, each with known (and sequenced) mutations in their *Avr*locus (VPgcistron), in a series of experiments that also tracked, identified, and quantified the specific isolates present in the host plants. The monogenic resistance provided by *pvr2*$^{3}$was overcome by PVY when present in a DH line with susceptible alleles at the three QRLs, but not when*pvr2*$^{3}$ was present in aDH line with resistant alleles at the QRLs. Furthermore, Palloix et al., (2009)determined that virus adaptation to the resistance provided by the host genetic background required multiple steps of selection, first for virulence toward *pvr2*$^{3}$, followed by adaptation to the genetic background. The ability of the virus to adapt to the genetic background suggests that deployment of QRLs should be carefully considered, and that breeding with major qualitative resistance genes should also emphasize selection of appropriategenetic backgrounds toprolong the effectiveness of resistance genes (Palloix et al., 2009).

***Marker-Assisted Selection in Backcross Breeding***

The use of molecular markers in improving backcrossing efficiency has been widely accepted and was the subject of studies dealing with the marker-assisted building of disease-resistant, abiotic stress-tolerant and quality-improved genotypes (Table 2).

Conventional backcrossing aims at introgressing a target trait that is controlled by a single gene from a usually exotic donor line into a highly adapted recipient line, the so-called recurrent parent. At each backcross cycle, molecular markers can be used to identify carriers of the target trait (foreground selection) having the closest fit to the recurrent parent genotype (background selection).

Table 2.MAS in backcross breeding of single genes and QTL alleles

| Crop | Trait (gene) | Foreground selection at | Background selection at | References |
|---|---|---|---|---|
| | Major genes | | | |
| Rice | Bacterial blight resistance (*Xa21*) | Each backcross cycle up to BC3F1 | BC3F1 (128 RFLPs) | Chen et al., (2000) |
| | Cooking and eating quality (*Waxy* gene region) | Each backcross cycle up to BC3F1 | BC1F1 and BC2F1 (129 AFLPs) | Chen et al., (2001) |
| | | Each backcross cycle up to BC3F1 | BC3F1 (118 AFLPs) | Zhou et al., (2003) |
| Barley | Barley yellow dwarf virus resistance (*Yd2*) | BC1F1 and BC2F2 | Not performed | Jiang (2013) |
| | QTL | | | |
| Rice | Root depth (1–2 QTL) | Each backcross cycle up to BC3F2 | BC3F2 (60 SSRs) | Shen et al., (2001) |
| Barley | Leaf rust (Rphq2) | Each backcross cycle up to BC3S2 | Each backcross cycle up to BC3S2 | Van Berloo et al., (2001) |
| Maize | Southwestern corn borer resistance (3 QTL) | Each backcross cycle up to BC2F2 | Each backcross cycle up to BC2F2 | Willcox et al. (2002) |

In order to minimize linkage drag, the selection of lines with the smallest introgressed segment around the target locus is usually done in tandem (Tanksley et al., 1989), i.e., selection for recombination ononesideinthefirstgenerationandselection for recombinationontheother side in the next generation. Although selection for simultaneous recombination events on both sides would save one generation of backcrossing, it is much more cost-effective due to the greater number of individuals that have to be genotyped to obtain one double recombinant. A full informativemarker-assisted backcrossing scheme can be performed with markers derived from the DNA sequence of the gene to be introgressed. Chen et al.,(2000) reported on the improvement of 'Minghui 63', a restorer line widelyused in Chinese hybrid rice production, to bacterial blight resistance, causedbyXanthomonasoryzaepv.Oryzae(Xoo), through introgression ofXa21,a broad-spectrum bacterial blight resistance gene. The PCR-based foreground selection system consisted of a marker that was part of Xa21,amarker located at 0.8 cM from the Xa21locus on one side and a marker at 3.0 cMfromthegeneontheotherside, whileatotalof128RFLPmarkers,evenly distributed throughout the rice genome, was used to recover the genetic background of the recurrent parent in the BC3F1.Theimprovedversion, 'Minghui 63(Xa21)', was exactly the same as the original except for a fragment of less than 3.8 cM in length surrounding theXa21locus. Both 'Minghui63(Xa21)' and its hybrid with 'Zhenshan 97A', 'Shanyou 63(Xa21)', showed the same spectrum of bacterial blight resistance as the donor parent. Field examination of a number of agronomic traits showed that the two pairs of versions were identical when there was no disease stress. Under heavily diseased conditions, 'Minghui 63(Xa21)' showed significantly higher grain weight and spikelet fertility than 'Minghui63', and 'Shanyou 63(Xa21)' was significantly higher than 'Shanyou 63' in grains per panicle, grain weight, and yield. In a later experiment by Chen et al., (2001), efficiency of background selection was enhanced by using the high-volume amplified fragment length polymorphism (AFLP) marker technique allowing the fast and cost-effective selection of individuals having 99.3% amount of the recurrent parent genome in BC1F1 Nearly isogenic lines for QTL (QTL NILs) were developed by repeated backcrossing of individuals from primary mapping populations carrying the desired QTL genotype to one of the parental lines (Kandemir et al., 2000; Monforte and Tanksley 2000; Willcox et al., 2002; Yamamoto et al., 2000; Shen et al., 2001; Van Berloo et al., 2001). To accelerate the creation of QTL NIL, some authors used background selection (Table 2). QTL NILs represent qualified genetic stocks for the validation of QTL effects in different environments.

They can be further used to study epistatic interactions among QTL by inter-crossing of single QTL NILs and for fine mapping of QTL for map-based cloning. Advanced backcross QTL (AB-QTL**,**Tanksley and Nelson, 1996) analysis was proposed as a general strategy for the simultaneous detection of QTL qualified for breeding purposes and cultivar development. The delay of QTL analysis until an advanced backcross generation offers advantages for QTL characterization such that the probability is reduced for the detection of QTL displaying epistatic interactions among donor alleles due to overall lower frequency of donor alleles. In fact, there will be a higher probability of detectingadditive QTL which still function in a nearly isogenic background.

***Benefits of MAS***

MAS can, in principle, increase the precision of breeding in at least fourways: first, the breeder can select on a single plant basis for a trait (or traitcombination), where this may be neither appropriate nor possible by conventional phenotypic selection (CPS) either because of poor heritability, or because the trait per se is difficult to score or cost-ineffective to analyze. Both these scenarios are commonplace in breeding programmes. Second, MAS facilitates the maintenance, and ultimately the fixation of a number of individual genetic components, which acting together, define the overall expression of the trait. This pertains even when, as is the case for many quantitative traits, each individual component may make only a relatively modest contribution to the overall determination of the phenotype. With the exception of race-specific disease resistance and a small number of quality related traits (such as waxy endosperm), most breeders' traits are under such polygenic control. Third, in segregating generations, it allows the selection of not only recessive genes, but also of those not readily amenable to CPS, without any need for validation at each generation via a progeny test. This is of particular importance in backcross programmes, where the aim is to correct an established genotype (the recurrent parent) for a single weakness (for example, susceptibility to disease) by introducing the minimum amount of genetic material from the donor of the desirable trait. Finally, markers can help in the choice of parents in crossing programmes: in some situations, this can be directed to maximise diversity, which is desirable for the exploitation of heterosis; in other situations, the aim may be to minimise it, in order to preserve intact adaptive gene complexes painstakingly built up in elite inbred germplasm.

***Prerequisites and constraints for MAS***

A critical requirement for MAS is, of course, the existence of sufficient marker polymorphism. It has long been known that the level of DNA marker polymorphism is high in maize (Evola et al., 1986), but despite this, the large-scale deployment of MAS did not gather any significant momentum until relatively recently, some 15 years after the first RFLP-based genetic map of maize was developed (Helentjaris et al., 1986). Even in the less polymorphic cereals, prominently wheat, the level of polymorphism is not likely to be the major constraint on MAS uptake, although it has been argued in the past to be so. This is because, as noted above, SNP technology is likely to remove any effective limitation on marker discovery, in an even more spectacular way than the development of microsatellite assays has already begun to do.

As noted by Lee (1995), MAS has every chance of being effective in situations where CPS can achieve genetic gain; therefore, whether or not it can be deployed will be largely determined by an analysis of the costs and benefits to the breeding effort. This translates into issues of efficiency, which include, along with the major factor of cost per assay, also considerations of throughput per unit time, and timeliness. The calculation of the unit cost of MAS is complex, and depends on what is included and what is not. Dreher etal.,(2003) recently presented a detailed analysis of CIMMYT's costings for SSR genotyping and CPS in maize, and pointed out how case-dependent these are. Throughput capacity is a critical consideration, since no matter how cheap an assay is, to be applicable to a breeding project, it must be scalable to the population sizes generally used, which is itself a function of the number of discrete genes/QTL which is likely to be segregating in the material. Finally, the timeliness factor relates to those traits, which are either phenotypable only late in the growing season (so that MAS can save resources by allowing selection early in the season), or are first amenable to CPS only in advanced generations, so that early generation MAS will result in a considerable saving of resources.

***Future Prospects of MAS***

The above review of the available literature reveals that during the last 17 years since the publication of the first paper on the use of RFLP markers for construction of linkage maps in tomato and maize in 1986, molecular markers have been extensively used for mapping and tagging of hundreds of different agriculturally important genes/QTL in various crop species. With the availability of linked markers, the first requirement for successful MAS has been fulfilled. Besides, the feasibility of MAS based on these linked markers has been demonstrated in several crops both for qualitative as well as quantitative traits as evident from the above description. However, MAS is yet to be used routinely in plant breeding programmes. Utility of the MAS in crop plants is currently limited by factors such as recombination between the marker and the target gene, low level of polymorphism between parents with contrasting traits and lower resolution of QTLs due to interaction with the environment. With the recent developments in both structural and functional genomics, it would not be difficult to find solutions to these problems. Availability of high-density genetic and physical maps will enable finding markers physically closer to the target gene that would not allow failure of MAS due to genetic recombination. Moreover, cloning and characterization of the target genes, which are possible based of their position on the linkage map, would allow development of allele-specific markers. Use of such markers would completely eliminate the possibility of breakdown of the marker-trait linkage. Besides, markers based on the sequences of the genes would facilitate allele mining in the germplasm resources, thereby leading to identification and utilization of newer alleles in crop improvement. Different alleles of a gene would differ for a number of nucleotides at different positions in their sequence that would be the basis of developing highly polymorphic single nucleotide polymorphism (SNP) markers. The problem of low level of polymorphism in narrow crosses can thus be circumvented. Use of MAS for QTL, particularly those having little effect on trait expression and highly interacting with environment would require greater amount of research effort and newer experimental strategies. Complete integration of MAS with the conventional plant breeding programmes demands consideration of two important factors: a) size of population and b) cost. Plant breeding experiment requires screening of large segregating populations routinely over generations. Genotyping of large number of samples manually is an extremely difficult task. MAS to be practicable should be amenable to automation that would allow handling of large number of samples. Development and use of PCR based markers such as STS and SCAR will be a key to success of MAS in crop improvement. As the technology develops and gets modified to analyze large number of samples, the cost will automatically go down. The investment in gene tagging, and selection based on molecular markers should be weighted against the overall cost and time involved in traditional breeding program. Even though the cost of MAS is higher at present level of estimation, its integration with traditional plant breeding is desirable because of immense possibilities it offers.

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