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Review

Progress and prospects of marker assisted backcrossing as a tool in crop breeding programs

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Marker assisted backcrossing (MAB) is one of the most anticipated and frequently cited benefits of molecular markers as indirect selection tools in breeding programs. However, routine implementations of MAB in ongoing plant breeding programs are still scarce. Currently MAB of single gene is perhaps the most powerful approach that uses DNA markers effectively. Improvement of quantitative traits loci (QTLs) through MAB resulted to variable results ranging from limited success and/or even a failure to a few highly successful stories. A major constraint to the implementation of MAB in pragmatic breeding programs has been the high relative cost compared to conventional phenotypic selection. It is a popular misconception that a 'DNA fingerprint' is always to be preferred. To be useful to plant breeders, gains made from MAB must be more cost-effective than gains through traditional breeding or MAB must generate significant time savings, which justifies the additional cost involved. Currently, most national agricultural research systems (NARS) in Africa have either no or very limited facilities, skilled manpower, and financing for integrating molecular markers as part of their breeding programs. Therefore, conventional breeding methods remain the main option for NARS for many years to come, but targeted use of MAB may become a supplement if well-validated markers are developed or available through collaboration with the international agricultural research centers. This paper provides detail review of the current literature on MAB, including requirements and selected experimental results.

Key words: Biotechnology, conventional breeding, gene introgression, marker assisted selection, molecular breeding, molecular markers.

INTRODUCTION

One main objective of plant breeding is the introgression of one or more genes from a donor into the background of an elite variety (recurrent or recipient parent) and to recover the recurrent parent genome as rapidly as possible. Such 'defect elimination' is a way to retain the qualities of a good variety from unwanted recombination, when adding desirable traits from either domesticated or wild germplasm sources (it may be pointed out that a 'good variety' may also mean an 'adapted germplasm pool'). This is usually achieved by the backcross method, but in many ways the same objective is being sought through transgenic breeding, bypassing recombination altogether but introducing a value-added trait. The development of new cultivars is the result of a cyclical process, each cycle consisting of three overlapping phases:

I. Assembling sources of genetic diversity for the major breeding activities from adapted or exotic sources and recombining these sources of genetic diversity to create new gene combinations.
II. Selection and testing to identify superior recombinants, which includes the timing of the selection (e.g., early versus late generation), the selection environment (e.g., favorable versus stress), and the number of years and locations of testing.
III. Release, distribution, and commercialization of new cultivars (Allard, 1960; Poehlman and Sleper, 1995; Hill et al., 1998).

Recurrent backcrossing is a traditional breeding method commonly employed to transfer alleles at one or more loci from a donor to an elite variety (Allard, 1960;

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Reyes-Valde´s, 2000). Traditional backcrossing programs are planned on the assumption that the proportion of the recurrent parent genome is recovered at a rate of $1 – (1/2)^{t+1}$ for each of t generations of backcrossing (Babu et al., 2004). Thus, the expected recovery of the recurrent parent genome after six generation of backcrossing would be 99.2%, a situation called near-isogenic. However, any specific backcross progeny will deviate from this expectation due to chance (stochastic or non-random positions of chiasmata) and/or linkage between a target gene from the donor parent and nearby genes (Ribaut and Hoisington, 1998). Young and Tanksley (1989a), for example, found an introgressed segments as large as 4 centimorgan (cM) in tomato cultivars developed after 20 backcrosses, and one cultivar developed after 11 backcrosses still contained the entire chromosome arm carrying the gene from the donor parent. In a study of barley lines backcrossed for 7 generations, the segments around the introgressed genes varied from about 1 cM to 14 cM (Bjørnstad et al., 2002). Therefore, the two main limitations of the backcrossing approach are:

I. The number of generations, and thus time, necessary to achieve the introgression objective.
II. The simultaneous transfer of other genes flanking the gene of interest from the donor parent (linkage drag).

Among genes carried through linkage drag could be some that control the synthesis of potentially harmful compounds or code for agronomical undesirable traits, such as low yield or disease susceptibility. Depending on the linkage distances, the size of the flanking regions can be decreased by additional backcrossing (Young et al., 1988) although breeders have not had any direct control over the size of the region or the recombination breakpoints.

During the past two decades, the developing ability to transfer target genomic regions using molecular markers resulted in extensive genetic mapping experiments aiming at the development of molecular markers for marker-assisted backcrossing (MAB) (also called marker assisted selection, marker assisted introgression or molecular breeding). Molecular markers are tools that can be used as chromosome landmarks to facilitate the introgression of chromosome segments (genes) associated with economically important traits. There is now a large amount of research that aims at identifying genomic regions of interest, from which MAB experiments are an attractive next-step. Molecular markers that are associated with economically important traits have been identified and/or used for MAB in several plant species, including maize, rice, wheat, barley, tomato, potato, sunflower, pea, bean, rye, millet, cotton, soybean, sorghum, cowpea, tobacco, turnip rape, cauliflower, sunflower, alfalfa, carrot, sugarcane, sugar beet, and grape. Molecular markers do not require genetic engineering and cultivars to be developed by MAB, are not transgenic and therefore, do not face the public resistance against transgenic crops. This paper reviews the current literature on MAB, including princeples, requirements, applications, and experimental results, and its future prospects for integration by the national agricultural research systems (NARS) in Africa.

PRINCIPLES AND REQUIREMENTS FOR MAB

MAB is the process of using the results of DNA tests to assist in the selection of individuals to become the parents in the next generation of a genetic improvement program (Figure 1). It is an approach that has been developed to avoid problems connected with conventional plant breeding by changing the selection criteria from selection of phenotypes towards selection of genes that control traits of interest, either directly or indirectly. Molecular markers are clearly not influenced by environment (unaffected by the conditions in which the plants are grown) and are detectable at all stages of plant growth. With the availability of an array of molecular markers (see Semagn et al., 2006a for review) and genetic maps, MAB has become possible both for traits governed by single gene and quantitative trait loci (QTLs) (Francia et al., 2005). The philosophy in marker development and implementation can be divided into the following steps:

1) Identify parents differing in the traits of interest.
2) Develop a population of plants segregating for the traits of interest (mapping population).
3) Screen the population for the traits of interest.
4) Construct genetic linkage maps (see Semagn et al., 2006b for review) of the cross with an adequate number of uniformly-spaced polymorphic markers to accurately locate desired QTLs or major gene(s).
5) Identify molecular markers linked to the traits of interest.
6) Test the applicability and reliability of the markers in predicting the traits in related families (also referred to as marker validation or verification).
7) Produce clear and simple protocols for assaying the markers.
8) Modify breeding strategy to optimize use of MAB relative to alternative selection techniques (develop high throughput, reproducible and user friendly genotyping facilities for screening large number of samples in a time and cost effective manner).
9) Implement into the breeding programs (use the markers directly in the breeding program to follow the introgression of desirable regions of the genome) (Gupta et al., 1999; Babu et al., 2004; Francia et al., 2005).

The above steps can be divided into three broad categories: genetic mapping, analyses of associations between molecular markers and the trait of interest, and MAB. We have provided separate reviews on the former

two categories, and only short description is given here.

The success of MAB depends upon several factors, including the distance between the closest markers and the target gene, the number of target genes to be transferred, the genetic base of the trait, the number of individuals that can be analyzed and the genetic background in which the target gene has to be transferred, the type of molecular marker(s) used, and available technical facilities (Weeden et al., 1992; Francia et al., 2005). Identification of molecular markers that should co-segregate or be closely linked with the desired trait (if possible, physically located beside or within genes of interest) is a critical step for the success of MAB. The most favourable case for MAB is when the molecular marker is located directly within the gene of interest (direct markers). MAB conducted using direct markers is called gene assisted selection (Dekkers, 2003). Alternatively, the marker is genetically linked to the trait of interest. Before a breeder can utilize linkage-based associations between a trait and markers, the associations have to be assessed with a certain degree of accuracy so that marker genotypes can be used as indicators or predictors of trait genotypes and phenoltypes. The lower the genetic distance between the marker and the gene, the more reliable is the application of the marker in MAB because only in few cases will the selected marker allele be separated from the desired trait by a recombination event. The presence of a tight linkage between desirable trait(s) and a molecular marker(s) may be useful in MAB to increase gain from selection. Based on studies by Lee (1995) and Ribaut et al. (2002b), it could be generalized that whenever a target gene is introduced for the first time from either wild or unadapted germplasm, flanking markers as close as 2 cM is considered an ideal option, while in the transfer of the same target gene in subsequent phases from elite into elite lines, positioning the flanking markers at 12 cM might be effective in reducing the required size of the backcross population.

For monogenic traits, such as a single gene-based disease resistance, the assessment of association is straightforward: mapping a monogenic trait goes along with the mapping of markers. Most traits of economic importance are, however, quantitative traits that most likely are controlled by a fairly large number of genes. Some of these genes might have a larger effect; such genes can be called major genes located at QTL. QTL constitute only some of the many genes that affect phenotype. If genetic effects at QTLs are sufficiently large, such genes could be used for MAB programs. For quantitative traits, a reliable assessment of trait-marker association requires large-scale repeated field experiments as well as statistical techniques, known as QTL mapping or QTL analysis (Figure 2). It is evident that mapping of major genes or QTLs that affect traits into approximate genomic locations using DNA markers is not enough because numerous factors can influence both the number of detected QTLs and the proportion of variance explained by each QTL. Chromosomal QTL regions are quite often large and can include many open reading frames, or favorable QTL alleles in repulsion. This situation can exacerbate introgression into elite germplasm of undesirable characters that are linked to a desirable QTL. Thus, a principal objective of QTL analysis is confining QTL to narrow chromosomal regions, which involves joint consideration of the type of experimental design or segregating population, mapping population size, informativeness and level of polymorphism of DNA markers, marker density in the genetic map, genome coverage, genetic background of the parents, scoring methods, interactions among QTLs, environment, genotype-by-environment interactions, and statistical methodologies both for linkage map construction and QTL analysis.

There are three possible errors in QTL mapping:

I. False positive or type I error that represents detection of spurious QTL, which is actually zero.
II. False-negative or type II error, which is rejection of QTL that is actually present.
III. Overestimation of the effect of QTL (Haley and Andersson, 1997).

Errors in QTL detection cause distinct reductions in responses to MAB in most cases. In maize, for example, estimates of the proportion of the phenotypic and genetic variance explained by QTLs were considerably reduced when derived from the independent validation sample as opposed to estimates from the calibration sample (Melchinger et al., 1998). The authors conclude that, unless QTL effects are estimated from an independent sample, they can be inflated, resulting in an overly optimistic assessment of the efficiency of MAB. In bread wheat, a major QTL for Fusarium head blight (FHB) resistance derived from ‘Sumai 3’ was initially reported to explain about 60% of the total variation in FHB resistance. However, the proportion of phenotypic variance explained by this QTL can be only 25% or even less due to genotype-by-environment or epistatic interactions (Liu and Anderson, 2003). Whenever possible, therefore, exp-eriments are needed to verify putative QTLs in multiple genetic backgrounds, environments, and growing sea-sons. QTL verification is defined as the repeated detec-tion of the same marker alleles at a similar position on the genetic map of a chromosome, of a QTL controlling a trait under more than one set of experimental conditions (Brown et al., 2003). Verification of QTLs is necessary to substantiate a biological basis for observed marker-trait associations, to provide precise estimates of the magni-tude of QTL effects, and to predict QTL expression at a given age or in a particular environment. Only then will sufficient experimental evidence be in place to monitor the transmission of trait genes via closely linked markers as a selection criterion (Young, 1999).

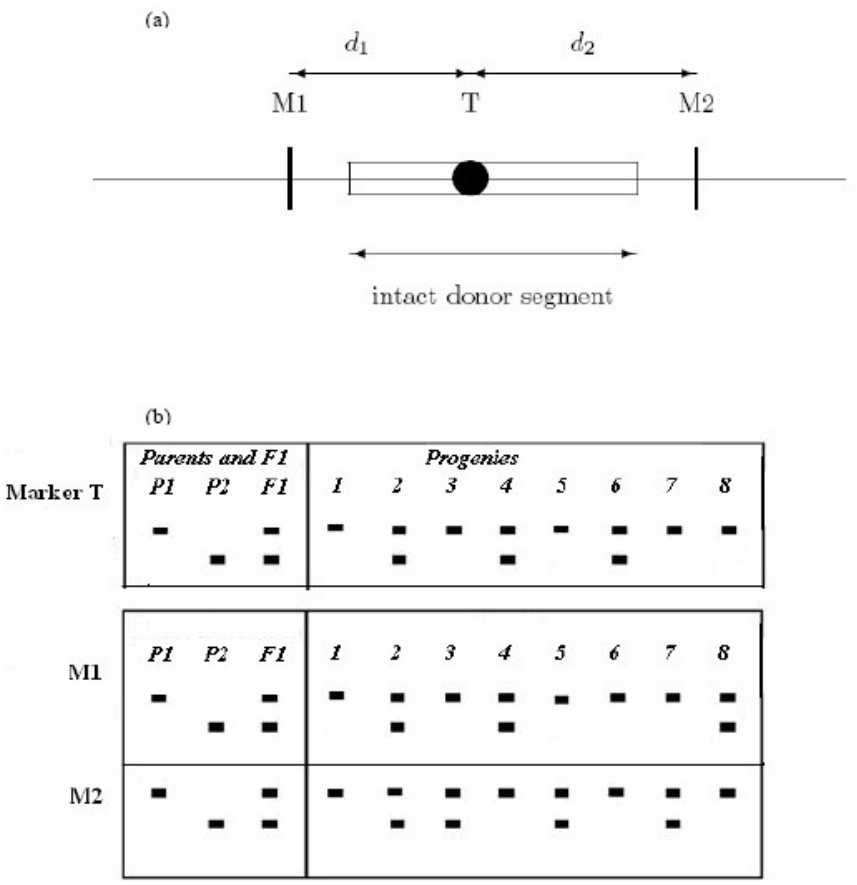

Figure 1. (a) Positions of a target locus (T) and two flanking markers loci at positions M1 and M2 on a chromosome; *d*1 and *d*2 are the map distances between the target locus and the flanking markers (Hospital, 2002). (b) Schematic representation of a codominant molecular marker for a recurrent parent (P_1), donor parent (P_2), F_1 hybrids and 8 BC_1F_1 progenies (# 1 to # 8). Three BC_1F_1 progenies (# 2, 4 and 6) are heterozygous for a recessive target allele from P_2 at the marker linked to the target trait (marker T). When the closest marker and the two flanking markers are taken into consideration, individual 6 is heterozygous for the target allele from P_2 and homozygous for P_1 alleles at both M1 and M2; individual 4 is heterozygous for the target allele from P_2, homozygous for P_1 allele at M2 but heterozygous at M1; individual 2 is heterozygous for the target allele from P_2 and heterozygous both at M1 and M2. The three individuals will then be selected to serve as parents in developing BC_2F_1 in the following order: # 6 > 4 > 2. Individuals 6 and 4 are double homozygous and single homozygous, respectively.

Frisch et al. (1999b) compared three different backcrossing selection strategies (two-stage, three-stage and four-stage) in maize in terms of how quickly they recovered a large proportion of the recurrent parent genotype, and recommended the four-stage sampling strategy as the most efficient procedure in MAB. The four-stage selection strategy involves:

I. Selecting individuals that carry the target allele (referred as foreground selection).
II. Selecting individuals homozygous for recurrent parent alleles at markers flanking the target allele.
III. Selecting individuals homozygous for recurrent parent alleles at all remaining markers on the same chromosome as the target allele.
IV. Selecting one individual that is homozygous for recurrent parent alleles at the maximum number of all markers across the whole genome.

The last three steps of the four-stage selection strategy are called background selection.

Marker assisted foreground selection

Figure 3 shows a schematic representation of the various steps of gene introgression using marker assisted backcrossing. MAB may improve the efficiency of backcross breeding programs in two ways:

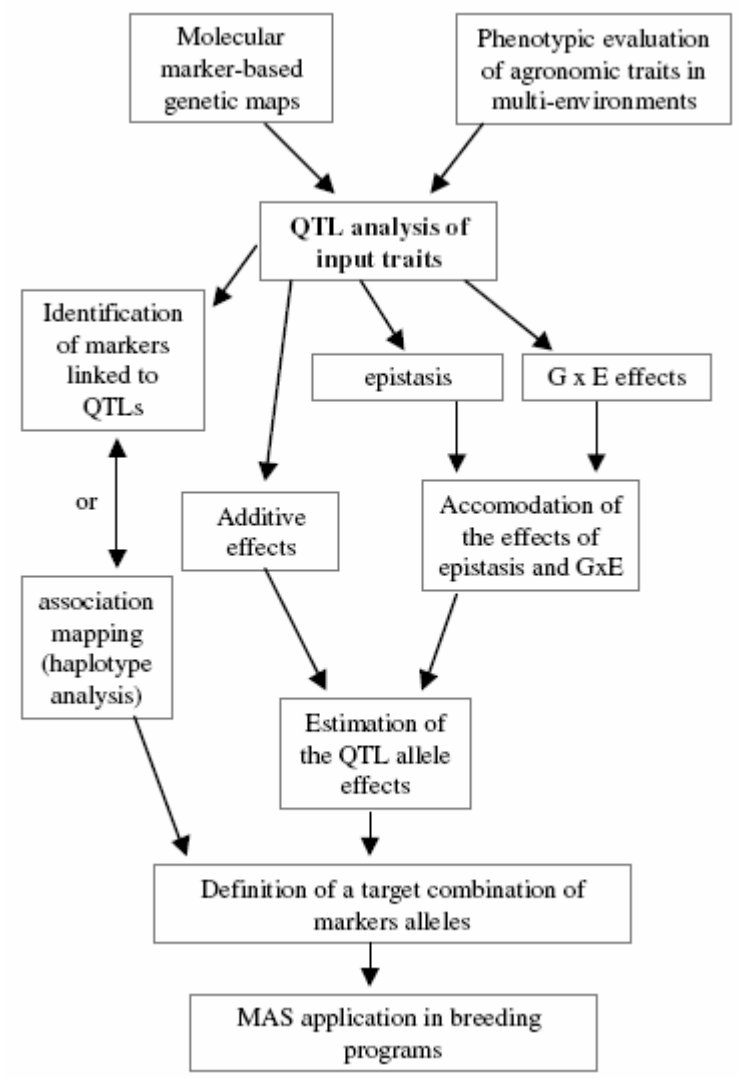

Figure 2. The various steps in the identification and characterization of quantitative trait loci (QTL) for use in marker assisted selection (Francia et al., 2005).

1) As a diagnostic tool for tracing the introgression of a target gene or allele (foreground selection)
2) For identifying individuals with a low proportion of the undesirable genome from the donor parent (background selection).

Marker-assisted foreground selection was proposed by Tanksley (1983) and investigated in the context of introgression of resistance genes by Melchinger (1990). Consider a hypothetical situation where a molecular marker M (with two alleles M1 and M2), that can be identified using a DNA assay, is known to be located on a chromosome near the DNA sequence of a disease resistant gene R (with alleles R1 and R2 contributing to resistance and susceptibility, respectively). Since the marker and the gene are close together on the same chromosome (tightly linked), they tend to be transmitted together in each generation. If a given individual in the population has the alleles M1 and R1 on one chromosome and M2 and R2 on the other chromosome, any progeny receiving the M1 allele will have a high probability (how high depends on how close M and R are to each other on the chromosome) of also carrying the favorable R1 allele, and thus would be preferred for selection purposes; those that inherit the M2 allele will tend to have inherited the unfavorable R2 allele, and so would not be preferred for selection. Such type of indirect selection is not possible with conventional approach that relies on phenotypic values. With regard to the probability of how sure one can be that M1 individuals indeed have R1 allele, there is a distinction between direct markers and linked markers. If there is no recombination between the marker and the gene or QTL (i.e., the marker exactly identifies the gene), then finding an M1 implies finding R1. A

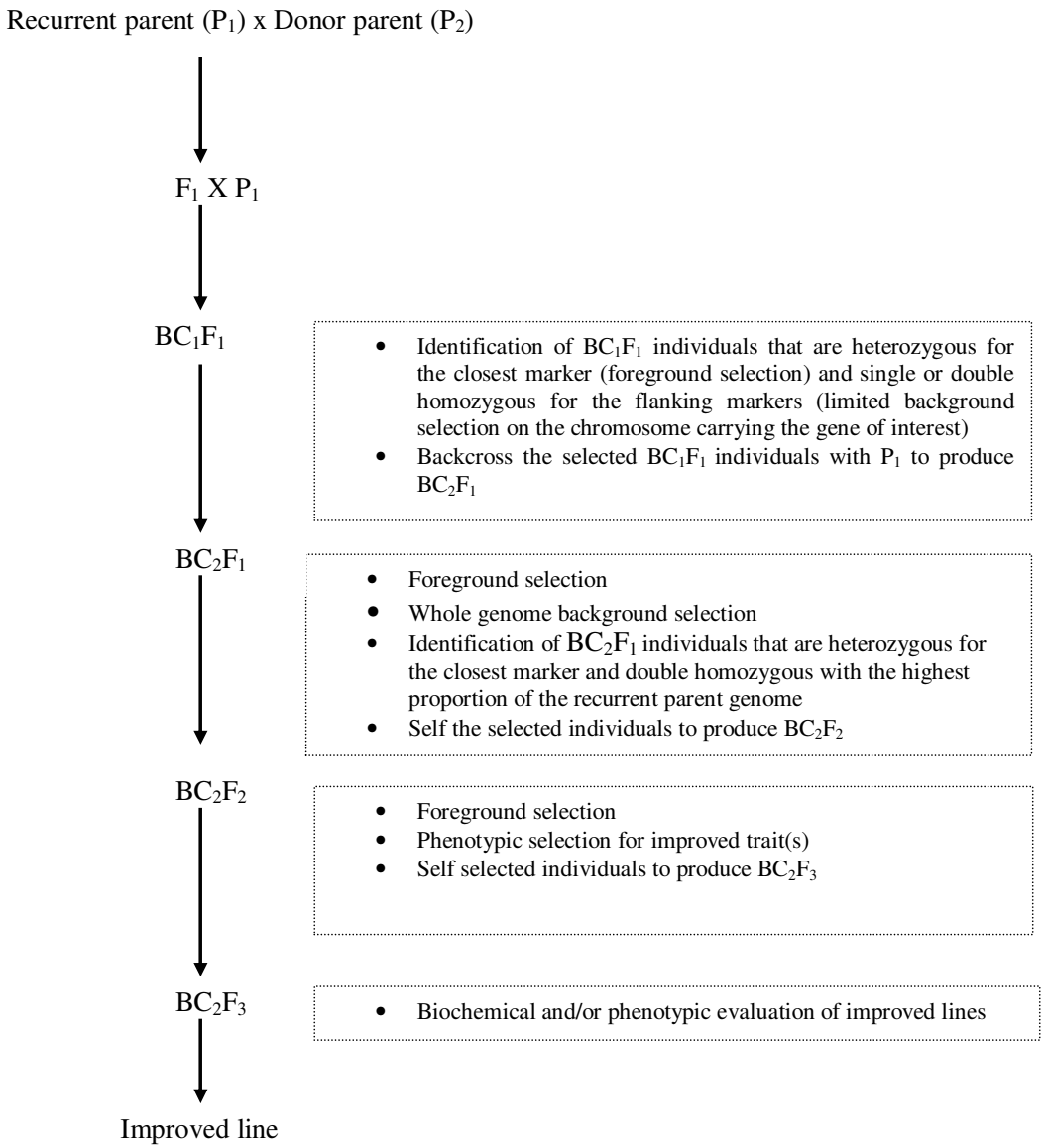

Figure 3. Schematic representation of gene introgression using marker assisted backcrossing (adapted from Babu et al., 20005).

direct marker is very convenient because the marker genotype directly indicates the trait of interest. However, finding direct markers mostly requires cloning and sequencing of the target genes and hence the identification of causative mutations, so-called Quantitative Trait Nucleotides (QTNs). Rather very few of such cases are available yet in plants. On the other hand, if M1 is a linked marker (only near R1 on the genome), M1 and R1 have a possibility to break up at meiosis; hence finding M1 individuals is not necessarily a guarantee of finding R1.

On the basis of genotypes at the target locus and two flanking markers loci (Figure 1), Frisch et al. (1999a) described five types of individuals:

a) Type 1 (an individual heterozygous for the donor allele at the target locus and homozygous for the recurrent parent alleles at both flanking markers).
b) Type 2 (an individual heterozygous for the donor allele at the target locus and homozygous for the recurrent parent allele at one of the flanking markers).
c) Type 3 (an individual heterozygous for the donor allele at the target locus and homozygous for the recurrent parent allele at one of flanking markers, irrespective of the genotype at the other flanking marker).
d) Type 4 (an individual heterozygous for the donor allele at the target locus and heterozygous for the recurrent parent alleles at both flanking markers).

e) Type 5 (an individual homozygous for the recurrent parent allele at the target locus; i.e., it is not a carrier of the target allele).

From the first backcross generation (BC_1), one individual of the most desirable type is selected in the given order: Type 1 > Type 2 or Type 3 > Type 4. If in generation BC_1 more than one individual satisfying the strongest condition is found, selection between them can be performed on the basis of analysis of other marker loci (located either on the carrier or on non-carrier chromosomes) to determine the most desirable individual for producing BC_2 (Tanksley et al., 1989; Hospital and Charcosset, 1997; Frisch et al., 1999a). If none of the BC_1 individuals carries the target allele, then the backcross program failed in BC_1.

Several authors discussed the minimum sample size required to obtain at least one most desirable individual for producing BC_2 (Hospital and Charcosset, 1997; Frisch et al., 1999a; Hospital and Decoux, 2002). Frisch et al. (1999a), for example, calculated the minimum sample size required to obtain, with 99% probability, at least one individual of Type 1, 2 or 3 for marker distances d_1 and d_2 from 5 to 20 cM. The required sample size varies from 337 to 4066 for Type 1, 32 to 100 for Type 2, and 54 to 192 for Type 3. An individual of Type 1 is expected to have the smallest proportion of donor genome, and hence it can be regarded as the final product of a gene introgression program. The other types are useful to design breeding programs that reduce the donor genome on the carrier chromosome in successive backcross generations. Hospital and Decoux (2002) developed a statistical program called “Popmin” (freely available at http://moulon.inra.fr/~fred/programs/popmin) for calculating the minimum population size required in BC_2 generation to identify with 99% probability at least one plant which is a double recombinant with heterozygosity at target locus and homozygosity for recurrent parent alleles at flanking marker loci.

Marker assisted background selection

Phenotypic selection for ‘good agronomic type’ has always been practiced along with backcross selection (Allard, 1960), but genotypic selection - monitoring the parental origin of alleles using markers throughout the genome in backcrossing - was originally proposed by Young and Tanksley (1989a) and later termed background selection (Hospital and Charcosset, 1997). The objective of the background selection is to accelerate the return to recipient parent genome outside the target gene so as to:

1) Reduce the length of the intact chromosomal segment of donor type dragged around the target gene on the carrier chromosome.
2) Reduce donor genome on the non-carrier chromosomes to the maximum extent (Young and Tanksley, 1989a; Frisch et al., 1999a, b; Hospital, 2001; Hospital and Decoux, 2002).

Reduction in donor genome content around target gene on a carrier chromosome can be achieved by selecting for individuals that are heterozygous at the target locus, and homozygous for recurrent parent alleles at two markers flanking the target locus on each side (such individuals are termed double homozygotes; Hospital and Decoux, 2002). The probability to obtain such double homozygote individuals (Figure 1) depends on the distances between the target gene and the flanking markers, total duration of the breeding program (number of successive backcross generations that are to be performed), and the number of individuals to be genotyped at each generation. Flanking markers between the target allele are necessary to remove linkage drag, and the optimal distance between a target gene and flanking markers governs the selection intensity that can be exerted. For a better reduction of linkage drag, flanking markers should be chosen as closely linked to the target locus as possible (Hospital, 2001). The probability to obtain double homozygote individuals for close markers in one single BC generation is very low. Hence, it is generally preferable to perform selection on at least two successive BC generations; i.e., selecting for a single homozygote on one side of the target in BC_1 (Figures 1 and 4), and then for a single homozygote on the other side in BC_2 (Young and Tanksley, 1989a). In order to minimize genotyping efforts, it is necessary to compute the minimal number of individuals that should be genotyped at each generation so that at least one double homozygote is obtained at the end of the program.

Basically, markers allow one to have a good idea of how much of the recurrent parent genome has been recovered in any particular BC progeny and to select for the best backcross progeny available in any generation. This ability to select for recurrent parent genotype outside of the target locus can greatly reduce the number of generations required to develop lines that possess the desired gene, but are otherwise nearly isogenic with respect to the recurrent parent. As described by Ribaut et al. (2002b), selection response for background analysis on non-carrier chromosomes depends on several factors, including the extent of saturation of the molecular marker map, availability of technical resources at a given point of time, and the required levels of line conversion. Reduction of the donor genome content other than the target gene requires DNA markers (and chiasmata) that are distributed evenly throughout the genome (Young and Tanksley, 1989a; Hospital et al., 1992; Visscher et al., 1996; Frisch et al., 1999a, b). Simulation studies by Frisch et al. (1999b) demonstrated that increasing the number of markers to more than one per 20 cM would not

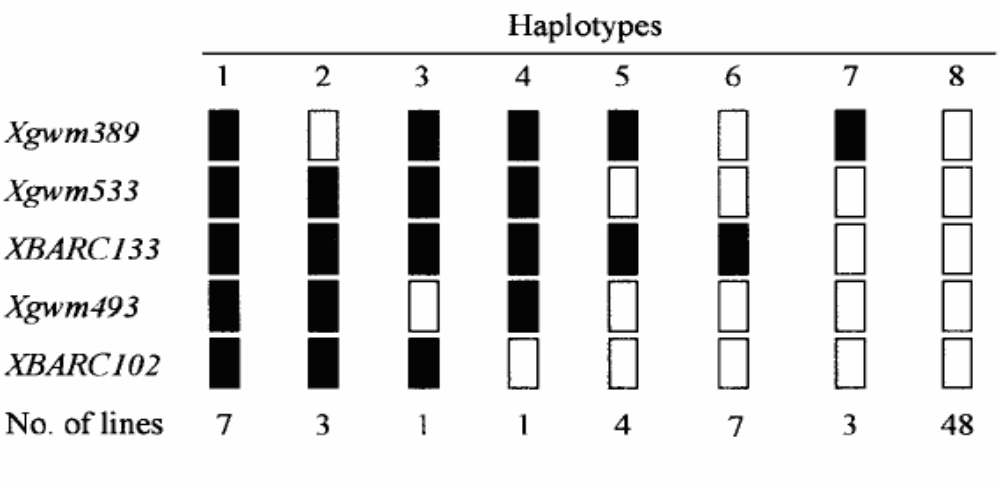

Figure 4. Eight haplotypes for 74 fusarium head blight (FHB) resistant lines based on allele type of five SSR markers near a gene associated with resistance. Xgwm533 is the marker closest to the resistance gene, and Xgwm389 and Xgwm493 are the flanking markers on either side of it. Darkened boxes represent favorable alleles from the resistant parent (Sumai 3) and open boxes represent non-favorable alleles from susceptible parent (Liu and Anderson, 2003). Individual plants in haplotypes 1 to 4 are heterozygote for Xgwm533 but those individuals in haplotype 2 and 3 will be selected to serve as parents for next backcrossing because they are homozygous to either of the flanking markers (single homozygous).

be required. Background selection has already been shown to be efficient by both theoretical studies (e.g., Hillel et al., 1990; Hospital et al., 1992; Visscher et al., 1996; Hospital and Charcosset, 1997; Frisch and Melchinger, 2001a) and experimental results. As demon-strated by Tanksley et al. (1989) with computer simulation, for example, use of molecular markers for background selection can accelerate recovery of the recurrent parent genome by 2 to 3 generations. Selection against genetic drag can save tens of generations (Young and Tanksley, 1989a; Visscher et al., 1996; Hospital and Charcosset, 1997).

A first attempt to provide a better estimate of donor genome content from molecular marker was made by Young and Tanksley (1989b), who introduced the concept of graphical genotypes, to 'portray the parental origin and allelic composition throughout the genome' (Figure 5). This takes into account distances between markers in the sense that a chromosomal segment flanked by two markers of donor type (DD) is considered as 100% donor type, a chromosomal segment flanked by two markers of recipient type (RR) is considered as 0% donor type, and a chromosomal segment flanked by one marker of donor type and one marker of recipient type (DR) is considered as 50% donor type. Such graphical genotyping could help identify:

I. The parental origin and proportion of chromosomal segment contributed by each parent (Figure 5).
II. The presence of sufficient molecular difference that leads to varieties.
III. A specific regions of the genome (chromosome segment) harboring genes responsible for desirable traits (Hayano-Saito et al., 1998; Bjørnstad et al., 2002; Foolad et al., 2001).

GGT (Graphical GenoTyping) is one of the freely available software's for such purpose and can be downloaded from the Internet (http://www.dpw.wageningen-ur.nl/pv/pub/ggt).

APPLICATIONS OF MAB

The key to successfully integrating MAB into breeding programs will lie in identifying applications in which markers offer real advantages over conventional breeding methods or complement them in novel ways. MAB offers significant advantages in cases:

1) When phenotypic screening is expensive, difficult or impossible.
2) When the trait is of low heritability (incorporating genes that are highly affected by environment).
3) When the selected trait is expressed late in plant development, like fruit and flower features or adult characters in species with a juvenile period.
4) For incorporating genes for resistance to diseases or pests that cannot be easily screened for due to special requirement for the gene to be expressed.
5) When the expression of the target gene is recessive.
6) To accumulate multiple genes for one or more traits within the same cultivar, a process called gene pyramiding (Han et al., 1997; Huang et al., 1997; Yousef

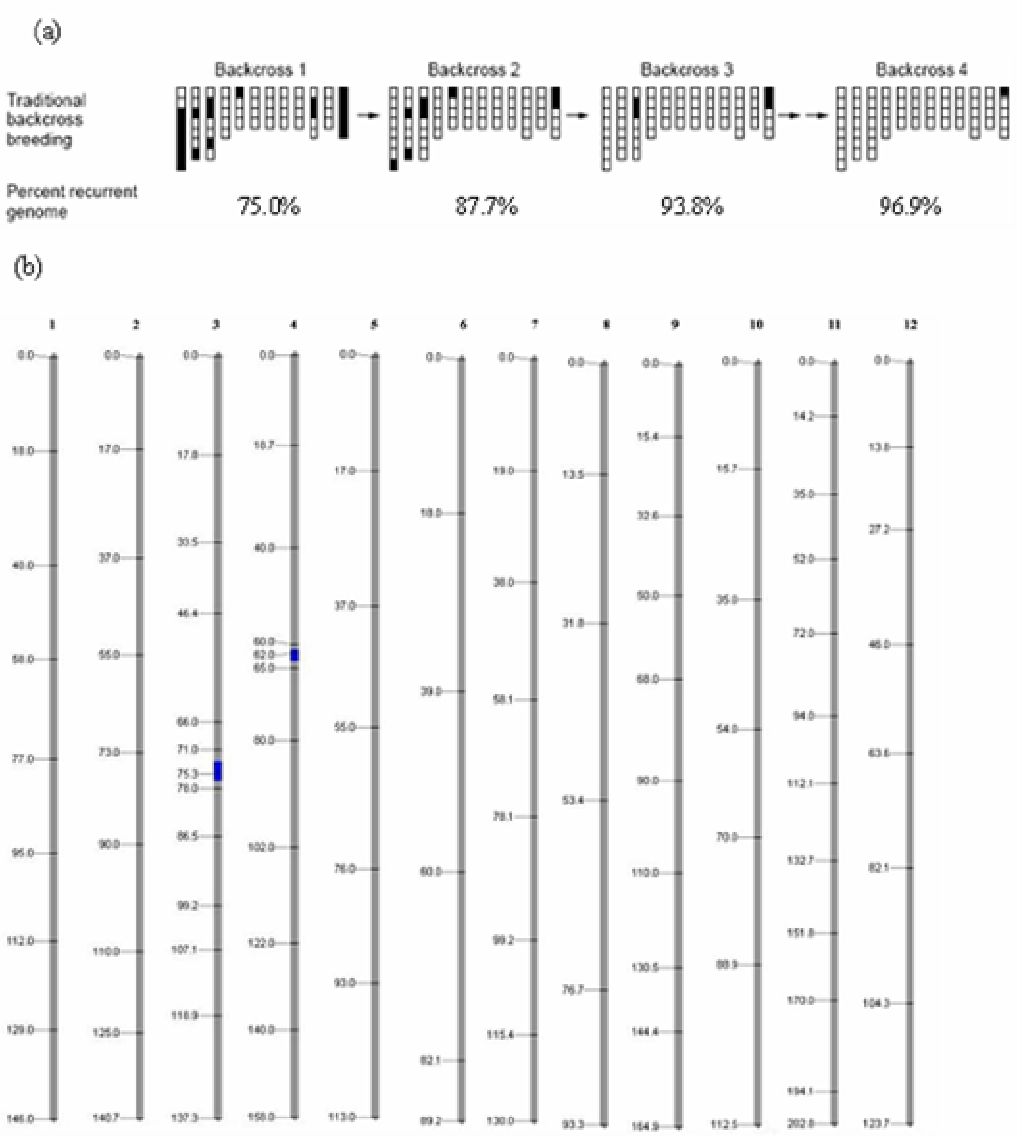

Figure 5. Graphical genotypes of 12 hypothetical chromosomes showing proportion of donor genome content (DGC) in backcross programs. (a) Graphical genotypes showing the proportion of DGC in four backcross generation for traditional backcross program. Chromosomes are indicated by vertical bars and each marker is represented by a box with those filled in black indicate the proportion of genome content from the donor parent and the others from the recurrent parent (Ribaut and Hoisington, 1998). (b) An example of graphical genotyping of a selected BC_2F_1 individual; chromosome numbers are on top, and map distances in centimorgan (cM) are on the left side of each chromosome. Each chromosome is genotyped with molecular markers at the same position as the map distances. The proportion of donor and recurrent parent genome content are shaded in blue and gray, respectively. The individual contained a target introgression from a donor parent on chromosome 4 (62 cM from the top of the chromosome) and a non-target introgression on chromosomes 3. The total genome size is 1611.8 cM, and the proportion of donor and recurrent parent genome is 6.4 cM (0.4%) and 1605.4 cM (99.6%), respectively.

and Juvik, 2001; Koebner and Summers, 2003; Dubcovsky, 2004; Joseph et al., 2004; Lecomte et al., 2004; Sharma et al., 2004; Barone et al., 2005; XiangYan et al., 2005). For example, malting quality in barley depends upon several factors, including low heritability, environment factors, genotype x environment interactions, as well as small sample sizes because of the relatively high cost of malt tests (Foster et al., 1967; Pomeranz et al., 1976; Han et al., 1997). Selection for malting quality in breeding programs by micromalting and micromashing is time- consuming and resource-intensive. The evaluation of organoleptic quality of tomato fruit requires physical,

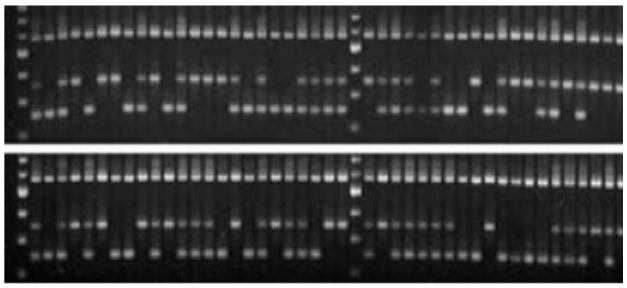

Figure 6. Agarose gel showing 96 individuals from an unselected F_2 population segregating for fragrance and analyzed using single tube allele specific amplification. The band of approximately 580 bp corresponds to the positive control present in all individuals; the 355 bp and 257 bands are present in homozygous non-fragrant and homozygous fragrant individuals, respectively; heterozygous individuals have all the three bands (Bradbury et al., 2005).

chemical and sensory analyses, which are expensive and difficult to assess. Hence, their practical use in phenotypic selection is difficult (Lecomte et al., 2004). Boron (B) toxicity has been recognized as an important problem limiting production in the low rainfall areas of southern Australia, West Asia and North Africa (Jefferies et al., 2000). Methods for screening and selection for B tolerance in breeding populations include the growing of plants in pots with soil containing toxic concentrations of B (Paull et al., 1988), by solution culture in filter paper (Chantachume et al., 1995) and in field trials conducted on toxic soils (Moody et al., 1993). Most selection methods are highly labor intensive and susceptible to experimental error.

Many important genes in breeding for resistance and quality traits are inherited recessively. In conventional backcross programs for introgression of a recessive target gene, that gene's presence or absence in a backcross individual is determined by a phenotypic assay of progeny generated either by selfing or by crossing to the donor parent (Allard, 1960). As an alternative to this time-consuming method, flanking molecular markers can be used as a diagnostic tool to trace the presence of the target gene in successive backcross generations. By this approach, presence of the target gene must be tested either by selfing or crossing to the donor only at the end of the breeding program (Frisch and Melchinger, 2001b). For example, fragrance in Basmati and Jasmine style rice is a recessive trait (Lorieux et al., 1996) which results principally from the presence of elevated levels of the compound 2-acetyl-1-pyrroline in the aerial parts of the plant. A number of sensory (e.g., tasting individual grains) and chemical methods (e.g., smelling leaf tissue or grains after heating in water or reacting with solutions of KOH or I_2-KI, gas chromatography) have been utilized to assist breeders in selecting fragrant rice but the objective evaluation of fragrance using these methods is labor intensive, difficult and unreliable (Sood, 1978; Reinke et al., 1991; Lorieux et al., 1996; Widjaja et al., 1996). Quality Protein Maize (QPM) is a genotype in which the *opaque2* gene has been incorporated along with associated modifiers and contains twice the amount of lysine and tryptophan as compared to normal maize endosperm. The *opaque2* gene is recessive and the modifiers are polygenic traits. Although conventional breeding procedures have been used to convert commercial lines to QPM forms, the procedure is highly cumbersome and not straight forward for three reasons:

I. Each conventional backcross generation needs to be selfed to identify the *opaque2* recessive gene and a minimum of six backcross generations are required to recover satisfactory levels of recurrent parent genome.
II. In addition to maintaining the homozygous *opaque2* gene, multiple modifiers must be selected.
III. Rigorous biochemical tests to ensure enhanced lysine and tryptophan levels in the selected materials in each breeding generation require enormous labor, time and material resources (Babu et al., 2005).

Gene pyramiding is a very useful approach for the introgression of genes controlling different agronomic traits to ensure that a variety may simultaneously acquire several traits. For example, genes leading to resistance to different races or biotypes to a disease or insect pest can be pyramided together to make a line with multi-race or multi-biotype resistances, which could be more durable than any single-race or single-biotype resistance (Jiang et al., 2004). The joint expression of pyramided genes was found to provide numerical increases or a broader spectrum of resistance over that conferred by single genes through gene interaction and quantitative complementation (Yoshimura et al., 1995; Singh et al., 2001). Gene pyramiding has been successfully applied in several crop breeding programs, and many varieties and lines possessing multiple attributes have been produced (Huang et al., 1997; Porter et al., 2000; Wang et al., 2001; Samis et al., 2002). Gene pyramiding is, however, difficult using conventional breeding methods due to the dominance and epistasis effects of genes governing disease resistance (the stronger resistance genes will always mask the less strong, which cannot be revealed without screening using a virulent strain on the former – in itself undesirable). Moreover, genes with similar reactions to two or more races – so called race-non specific or partial resistance - are difficult to identify and transfer through conventional approaches (Singh et al., 2001), and virtually impossible if stronger race-specific genes are present. In all the above (malting quality, fragrance, QPM, recessive gene, and gene pyramiding) and other similar cases, marker assisted backcrossing is highly jus-

tifiable. Once MAB has been completed, it may be continued as Marker Assisted Selection (MAS) within the framework of any breeding method, be it pedigree, recurrent selection, etc. The conditions for marker efficiency will be the same as in MAB, except for the backcross component.

Experimental results in MAB

Following successful marker development, MAB has been used to transfer single genes or QTLs in various species but this review provides only representative examples. The published results may be looked from simple to complex traits or successful experiments to those with limited success and even a failure (Hospital, 2003, 2005). Relatively 'simple' and certainly highly successful results start with the integration of the *Bacillus thuringiensis* (Bt) transgene into different maize genetic backgrounds (Ragot et al., 1995). *B. thuringiensis* is a species of bacterium known for its insecticidal properties. Recombinant DNA technologies have allowed the insertion of the genes responsible for production of insecticidal toxins into the maize genome and corn borer larvae that penetrate the plant tissues are killed when they ingest the toxin produced in the *B. thuringiensis* maize cells. In *B. thuringiensis* case, there is a single target, which is a well known transgenic construction, so the 'marker' equals the target without recombination (gene assisted selection). Bradbury et al. (2005) developed a single tube allele specific amplification assay, which allows discrimination between fragrant and non-fragrant rice varieties and identifies homozygous fragrant, homozygous non-fragrant and heterozygous non-fragrant individuals in a population segregating for fragrance (Figure 6). Fragrance in rice, a recessive trait, has been shown to be due to an eight bp deletion and three single nucleotide polymorphisms (SNPs) in a gene on chromosome 8, which encodes a putative betaine aldehyde dehydrogenase 2 (BAD2). Babu et al. (2005) used MAB for the incurporation of the high-lysine *opaque2* gene using gene specific markers such as *phi057* and *umc1066*, which are located within the *opaque2* gene itself.

Various other successful experiments reported the manipulation of known genes with indirect (linked) markers, including pyramiding of several major resistance genes in rice (Huang et al., 1997; Hittalmani et al., 2000; Sanchez et al., 2000; Singh et al., 2001; Jiang et al., 2004; Sharma et al., 2004), wheat (AnLi et al., 2005; XiangYan et al., 2005) and tomato (Barone et al., 2005; Yang and Francis, 2005). Several authors also reported introgression of one or more QTLs in different crops. The decision of which QTL regions to transfer with MAB and/or to consider in a selection index should be based on QTL effects verified in an independent validation sample. For example, Toojinda et al. (1998) successfully introgressed two QTLs for stripe rust resistance in barley into a genetic background different from the one used to map the QTLs; Chee et al. (2001) reported the successful transfer of QTL for grain protein concentration in wheat into a different genetic background; Ahmadi et al. (2001) successfully introgressed two QTLs for resistance to yellow mottle virus in rice; and Yousef and Juvik (2002) successfully applied three markers to introgress QTLs for seedling emergence in sweet corn. The success of introgression depends on the ability of the target gene(s) to exhibit the expected effects once introgressed in to the genetic background of the recurrent parent.

The rate of success starts to decrease for introgression of larger numbers of target QTLs. Sebolt et al. (2000) performed MAB of two QTLs for seed protein concentration in soybean but only one QTL was confirmed in $BC_3F_{4:5}$ progeny. When that QTL was introgressed in three different genetic backgrounds, it had no effect in one background. Bouchez et al. (2002) introgressed three QTLs for two traits (earliness and yield) between maize elite lines with MAB but results depend on the number of genes controlling the traits. For the simple trait (earliness), QTL effects in the progeny were in accordance with those expected from the original detection in the parental lines. For the more complex trait (yield), results were generally not as good as expected and one high-yielding allele putatively detected from the low-yielding parent finally exhibited an effect opposite to the expectation (i.e. reduced yield). Shen et al. (2001) manipulated four QTLs for drought resistance (root depth) in rice. Among the four QTLs, one exhibited the expected effect in the progeny, one was finally revealed as a false positive, one segment was shown to contain two QTLs in repulsion phase that reduced its expression, and one segment did not exhibit the expected effect. Lawson et al. (1997) introgressed four chromosomal regions containing five QTLs for pest resistance (acylsugar accumulation) from wild tomato into cultivated tomato. However, the level of acylsugar accumulation in the progeny introgressed for the five QTLs was lower than that of the interspecific F_1 hybrids.

A 'diluted' expression of an introgressed trait is common in traditional backcrossing, and what MAB can do is to identify the parts that are stably expressed in the new background. Ribaut et al. (2002a) introgressed five QTLs for drought tolerance (reduction of anthesis-silking interval (ASI)) in maize. The results depended on the condition of the phenotypic assay of the progeny: the introgressed progeny exhibited a reduced ASI under stress conditions (drought) but the introgression had no visible effect in the absence of stress (which was not expected). Lecomte et al. (2004) introgressed five QTLs strongly involved in tomato fruit quality into three different recipient lines through MAB. The breeding efficiency varied strongly with the recipient parent and significant interactions between QTL and genetic backgrounds were shown for all

the studied traits. About 50% of the QTLs were confirmed in each new background and new QTLs were detected. The QTLs with the largest effects were the most stable. Thabuis et al. (2004) transferred resistance to *Phytophthora capsici* alleles at four QTLs from a small fruited pepper into a bell pepper recipient but a decrease in the QTLs effect as well as epistatic interaction between QTLs was observed. Stuber (1995) introgressed six favorable chromosome segments but none of the improved lines had all six segments together. In several cases, the QTLs were detected after introgres-sion through MAB but their effect was reduced. In worst cases, none of the introgressed QTL showed any effect, as have been reported for three QTLs for high yield both in barley (Kandemir et al., 2000) and soybean (Reyna and Sneller, 2001) or even had opposite effect, as has been reported yield reduction in maize (Bouchez et al., 2002). The rate of unexpected results seems to increase when moving from known genes to QTLs, when increasing the number of targets and when dealing with more 'complex' traits. In fact, many of the unexpected results refer to cases where one tried to introgress multiple QTLs for yield, which is generally considered by plant breeders as one of the most 'complex' traits because it integrates most of the plant's physiological functions. The most likely reasons that contributed for unexpected results in MAB are:

1) The putative QTL may be a false positive.
2) The QTL effect might have been over estimated or the repeatability of the QTL across different environments might have not been confirmed (Melchinger et al., 1998; Schon et al., 2004).
3) There may be QTL by environment interactions (Ribaut et al., 2002a, b), which is frequent in plants.
4) The chromosomal segments associated with QTL hold not just one but several genes, and recombinetion between those genes would then modify the effect of the introgressed segments (Eshed and Zamir, 1995; Monna et al., 2002).
5) Epitasis, either between QTLs or between QTL and the genetic background.

One commonly asked question is that "since quantitative traits are controlled by several QTLs, how many QTLs are typically selected for MAB?" Theoretically, all markers that are tightly linked to QTLs could be used for MAB. However, due to the cost of utilizing several QTLs, only markers that are tightly linked to no more than three QTLs are typically used (Ribaut and Betran, 1999), although there have been reports of up to 5 QTLs being introgressed into tomato via MAB (Lecomte et al., 2004). Even selecting for a single QTL via MAB can be beneficial in plant breeding provided that such a QTL account for the largest proportion of phenotypic variance for the trait (Tanksley, 1993; Ribaut and Betran, 1999) and the QTL is stable across environments (Ribaut and Betran, 1999; Hittalmani et al., 2002).

Cost effectiveness of MAB

The use of markers in selection incurs development costs associated with the development of genetic maps, gene(s) or QTL identification and verification as well as running costs for MAB (e.g., costs of DNA extraction, genotyping, and analysis). Molecular markers are expensive to develop, and returns from the initial research can take time. Economics is, therefore, the key determinant for the application of molecular markers in genetic improvement programs. Factors that influence the cost of utilizing MAB or MAS include inheritance of the trait, method of phenotypic evaluation, field/glasshouse and labor costs, and the cost of resources. In some cases, phenotypic screening is cheaper compared to marker-assisted selection (Bohn et al., 2001; Dreher et al., 2003). In other situations, phenotypic evaluation may be time-consuming and/or difficult and therefore using markers may be cheaper and preferable (Young, 1999; Yu et al., 2000; Dreher et al., 2003). Simulation studies have shown that in some circumstances the adoption of MAB has the ability to improve selection efficiency over phenotypic selection in breeding programs (Hospital and Charcosset, 1997; Knapp, 1998; Charmet et al., 1999; Kuchel et al., 2005). Recent genetic simulation and economic analysis in wheat has shown that MAB may not only provide genetic gain but also reduce cost (Kuchel et al., 2005). One situation where MAB is frequently being implemented is in F_1 hybrid breeding. Once parents of a successful hybrid P_1 x P_2 have been identified, the maternal P_1 often needs to be endowed with cytoplasmic male sterility and the male P_2 with appropriate restorer genes. These parents are so valuable and the time factor for marketing the hybrid is so pressing that MAB is practiced routinely.

As to earlier stages in a breeding program, detailed experimental results have been published from studies at the International Maize and Wheat Improvement Center (CIMMYT) in Mexico on the relative cost-effectiveness of conventional selection and MAB for different maize breeding applications (Morris et al., 2003; Dreher et al., 2003). One of the applications considered by Morris et al. (2003) was the transfer of an elite allele at a single dominant gene from a donor line to a recipient line. At CIMMYT, neither conventional nor MAB showed clear superiority in terms of both cost and speed: conventional breeding was less expensive but MAB was quicker. For situations like this, where the choice between conventional breeding and MAB involves a trade-off between time and money, they suggested that the cost-effectiveness of using MAB depends on four parameters:

a) The relative cost of phenotypic versus marker screening.

b) The time saved by MAB.
c) The size and temporal distribution of benefits associated with accelerated release of improved germplasm.
d) The availability to the breeding program of operating capital.

All four of these parameters can vary significantly between breeding projects, suggesting that detailed economic analysis may be needed to predict the optimal selection method for a given breeding project. The other application considered at CIMMYT was identification of plants carrying a mutant recessive form of the *opaque2* gene in maize that is associated with high quality protein maize (Dreher et al., 2003). For the latter, the authors concluded that:

I. The choice between MAB and conventional selection may be complicated by the fact that the two are not always direct substitutes
II. Factors other than cost are likely to play an important role in deciding the choice of screening methods.

The studies at CIMMYT, however, did not consider the initial development cost (i.e., costs of developing molecular markers associated with the trait of interest), as it was assumed that they were already available. Development costs can be quite expensive. Koebner and Summers (2003) suggested that the current costs of MAB would need to fall considerably before it would be used widely in breeding. In practice, therefore, although MAB may lead to increased genetic responses, decision-makers need to consider whether it may be cost-effective or whether the money and resources spent on developing and applying MAB might instead be more efficiently used on adopting other new technologies or on improving existing conventional breeding programs.

Yousef and Juvik (2001) conducted 52 paired comparesons between MAB and phenotypic selection in composite populations of sweet corn (*Zea mays* L.). The average MAB and phenotypic selection gain across all composite populations and selected traits in sweet corn, calculated as percent increase or decrease from the randomly selected controls, was 10.9% and 6.1%, respecttively. A comparison between MAB and conventional greenhouse screening for introgressing bacterial blight resistant gene in common beans showed that the cost of MAB is about one-third less than that of the greenhouse tests (Yu et al., 2000).

FUTURE PROSPECTS OF MAB

MAB has generated a good deal of expectations, which in some cases has led to over-optimism and in others to disappointment because many of the expectations have not yet been realized. Although documentation is limited, the current impact of MAB on products delivered to farmers seems small (for the reasons given above, it is indirectly present in many F_1 hybrids). Often the links between breeders and researchers need to be worked out in novel ways in order for the research to become a real possibility. Currently, the cost of utilizing markers is possibly the most important factor that limits the implementation of MAB. The use DNA markers in forensic studies are possible because society is willing to invest what is needed in questions of guilty or not-guilty (innocence). In animal breeding the investments in a breeding sire are very high, even the value of a cow may defend the cost but a chicken is less likely. In plants, individuals will usually have low value to cover the cost. To be useful to plant breeders, therefore, gains made from MAB must be more cost-effective than gains through traditional breeding or MAB must generate significant time savings, which justifies the additional cost involved. However, it is anticipated that novel applications and technology improvements will result in a reduction in the cost of markers, which will subsequently lead to a greater adoption of markers in plant breeding. Valuable lessons learnt from past research are likely to encourage more researchers to develop reliable markers and plant breeders to adopt MAB. However, Young (1999) emphasized that scientists must realize the necessity of using larger population sizes, more accurate phenotypic data, different genetic backgrounds and independent verification, in order to develop reliable markers for MAB. New developments and improvements in marker technology, the integration of functional genomics with QTL mapping, and the availability of more high-density maps are the other factors that will greatly affect the efficiency and effectiveness of QTL mapping and MAB in the future.

The development of high-density maps that incorporate new marker types, such as single nucleotide polymorphisms (SNPs) and expressed sequence tags (EST) will provide researchers with a greater arsenal of tools for QTL mapping and MAB. The number of EST and genomic sequences available in databases is growing rapidly (especially from genome sequencing projects), and the accumulation of these sequences will be extremely useful for the discovery of SNPs and data mining for new markers in the future (Gupta et al., 2001; Kantety et al., 2002). It is expected that the development of high resolution maps will also facilitate the isolation of actual genes (rather than markers) via 'map based cloning' (also 'positional cloning'), which involves the use of tightly linked markers to isolate target genes by using the marker as a 'probe' to screen a genomic library (Tanksley et al., 1995). The identification of genes controlling important traits has enabled plant scientists to predict gene function, isolate homologues and conduct transgenic experiments. To enhance the efficiency of MAB, knowledge of the DNA sequence of the gene enables the design of direct or 'diagnostic' markers, which are located within the actual gene sequence, thus eliminating the possibility

of recombination between marker and gene (Ogbonnaya et al., 2001; Ellis et al., 2002). However, DNA sequences for the majority of genes controlling agronomical important traits remain unknown, and most probably, will remain unknown for sometime. In the meantime, plant scientists will continue to use QTL maps and markers that tag genes of interest for many years to come.

While the future possibilities and potential impacts of MAB are considerable in Africa, there are also obstacles to its use, including equipment, infrastructure, skilled manpower, poor private sector involvement, supplies or consumables, and lack of research on specific crops. Publicly funded agricultural research in many developing countries is sub-optimal and development priorities do not necessarily include genetic improvement programs using molecular tools. Various stages in the MAB development and application process were regarded as being costly. The most significant cost prior to MAB is the development of genetic linkage map for the species of interest and identifications of associations between genes or QTLs and economically important traits. Such cost could be significantly very high for developing countries. Once the linkage map is available and marker-trait association is reliably identified, genotyping costs could also be substantially high for public-funded projects. Even in the presence of funding, timely purchase and acquisition of consumables for molecular laboratories is frustrating in most African countries. The potential benefits (genetic, economic, etc.) of using MAB need to be critically compared to those achieved or expected from any existing conventional breeding programs. In order for MAB to be taken up breeders in developing countries, the returns to investment should be far superior compared with those developed countries, given the significant opportunity costs and various constraints associated with availability of facilities and supplies. It is hoped that through partnerships between developing and developed countries, including public-private sector collaboration, MAB costs can be reduced, resources pooled and shared and capacity be developed. With the assistance of the Consultative Group on International Agricultural Research (CGIAR) and International organizations like FAO, developing countries may benefit from MAB in the near future. Although conventional breeding programs have their limitations, they have shown over time that they can be highly successful.

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