III.1 General Considerations: Marker-Assisted Selection

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1 Introduction

Since the first reported linkage of an agronomically important trait (a quantitative trait locus affecting seed weight) to a simply controlled gene (seed color) in common bean by Sax (1923), it has taken more than 60 years for genetic markers to become a qualified tool for widely optimizing genotype building in plant breeding programs. With the advent of molecular marker technology, the identification of genetic markers displaying linkage to any genetically inherited trait became feasible. However, most types of molecular markers, though nowadays PCR-based, are still too impractical to be used in large-scale marker-assisted selection (MAS) schemes due to the complexity of the assay preventing the appropriate automation, insufficient robustness or inadequate level of detected polymorphism (Koebner and Summers 2003). Due to their high polymorphic information content, sequence-tagged microsatellite sites are presently the most appropriate marker class for MAS. The future development of single nucleotide polymorphism (SNP) markers will provide access to affordable and high-throughput genotype determination assays and automated data analyses that are crucial for breeders' acceptance of MAS. MAS will then increasingly be applied to obtain improved efficiency and effectiveness in the selection of genotypes with traits that are difficult and expensive to phenotype, for the pyramiding of disease resistance genes in single genotypes, and for the carefully directed choice of parental lines in crossing programs allowing a controlled combination of alleles targeted for selection.

2 Requirements of Markers for Marker-Assisted Selection

Key issues in successful deployment of molecular markers in MAS are as follows:

1. Markers should co-segregate or map as close as possible to the target gene (within 2 cM), in order to have low recombination frequency between the

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target gene and the marker. A better estimate of map distance between the target gene and the marker will be obtained by analysing further mapping populations which have genotypes in common with those used in the initial mapping population. Accuracy of MAS will be improved if, rather than a single marker, two markers flanking the target gene are used (Peng et al. 2000).
2. For unlimited use in MAS, markers should display polymorphism between genotypes that have and do not have the target gene.
3. Cost-effective, simple PCR markers are required to ensure genotyping power needed for the rapid screening of large populations.

Microsatellite markers, also termed sequence-tagged microsatellite site (STMS) or simple sequence repeat (SSR) markers, which use the high mutation rates of repeated short DNA motifs are presently the most complete tool for MAS. Extensive collections of mapped SSR markers from both the noncoding and expressed portion of the genome are, or will be available in the near future for all major crop species. To allow absolute allele recognition and, consequently, to exploit the full range of marker alleles at a given locus in a panel of breeding lines, SSRs need to be processed on polyacrylamide gel or capillary electrophoresis machines. However, these high demands on fragment detection can be compensated by the simultaneous electrophoresis of different SSR marker samples carrying distinguishable fluorescent dyes in a single lane/capillary.

Development is moving away from anonymous to functional and candidate gene markers as primary MAS tools since linkage relationships which limit the overall applicability of anonymous markers will no longer exist or will be reduced to a minimum. 'Perfect' markers have already been made available for the gibberellin-insensitive semi-dwarfing genes *Rht-B1b* and *Rht-D1b* (Ellis et al. 2002) and the null *Wx-B1* allele of the granule-bound starch synthase I (McLauchlan et al. 2001) of wheat and may be provided by forthcoming map-based cloning experiments and genetic association mapping studies (Rafalski 2002). Resistance gene analogs (RGAs) are a useful resource as candidate gene markers for disease resistance genes (Mohler et al. 2002; Madsen et al. 2003) since RGAs showing close genetic linkage to resistance genes often reflect physical proximity (Leister et al. 1999; Wei et al. 1999). Furthermore, a huge number of candidate gene markers for complex traits will be supplied by investigations directed at the identification of genes differentially expressed among extreme phenotypes, e.g., for potato late blight disease (Ronning et al. 2003).

The marker type by which functional alleles are discriminated from their allelic variants relies on small insertion-deletion (indel) polymorphisms or single nucleotide polymorphisms (SNPs). SNPs are of particular interest for their utilization in crop improvement, since they (1) represent the most frequent variations in the genome of any organism, thus, offering the opportunity to find informative markers for a distinct genomic region in any genetic

background and (2) can be simply treated as di-allelic markers making them amenable to automated high-throughput genotyping and data handling.

While indels can be scored by direct sizing on polyacrylamide gels, the determination of SNP genotypes can be preferentially performed using nongel-based technology platforms such as denaturing high-performance liquid chromatography (DHPLC; Oefner and Underhill 1998), the most advanced system for heteroduplex analysis, or by pyrosequencing (Ahmadian et al. 2000) which uses the reaction principle of minisequencing. Sample analyses using DHPLC are carried out sequentially with an autosampler and one analysis takes around 5 min, while minisequencing reactions are performed in a 96-well plate in an automated device and take approximately 15 min.

3 Present Status of Validated Molecular Markers for Molecular Breeding of Important Crops

Markers that have been elaborated and validated for the monitoring of agronomically important traits, most of them determining resistance to disease, and which are (or have been) used in current breeding programs are listed in Table 1. The majority of traits is detectable using simple PCR markers, however, very often they need to be tested for polymorphism between parental lines of breeding programs. SNPs that are assayed via allele-specific PCR (AS-PCR; Ugozzoli and Wallace 1991) and cleaved amplified polymorphic sequences (CAPS; Konieczny and Ausubel 1993) and SSRs provide the prevalent marker classes for MAS. However, the time-consuming post-PCR digestion step limits the application of CAPS markers for small-scale genotyping. Therefore, these markers should be genotyped in the future using more general SNP detection systems in which any sequence polymorphism, irrespective of its location with reference to restriction sites, can be assayed. RFLP markers have only been used as an MAS tool when it was profitable due to the importance of the disease, high costs and unreliability of the bioassays such as in the early-generation selection of the cereal cyst nematode resistance gene *Cre1* in wheat (Ogbonnaya et al. 2001).

4 Marker-Assisted Selection for Quantitative Trait Loci

Most agronomic traits are of a polygenic nature and it is widely accepted that molecular markers are an appropriate tool to identify loci, the so-called quantitative trait loci (QTL), having alleles that differentially affect the expression of a quantitative trait. QTL mapping has been done for yield (Stuber et al. 1987), quality (Igrejas et al. 2002; Tan et al. 2001), tolerance to abi-

Table 1. Validated markers for the monitoring of agronomically important traits in MAS programs

Crop	Trait	Gene(s)	Marker type(s)	References
Wheat	Bread making quality	*Glu-D1-x5*	Gene-specific STS	Ahmad (2000)
		Glu-D1-y10		
		Glu-B1-x7		
		Glu-D1-x5	Promotor-specific SNP (DHPLC)	Schwarz et al. (2003)
	Reduced height	*Rht-B1b*	Gene-specific AS-PCRs	Ellis et al. (2002)
		Rht-D1b		
		Rht8	SSR	Korzun et al. (1998)
	Starch quality	*Wx-B1b*	Gene-specific AS-PCR	McLauchlan et al. (2001)
	Powdery mildew resistance	*Pm1c*	AFLP	Hartl et al. (1999)
		Pm17	RFLP-derived STS	Mohler et al. (2001)
		Pm24	SSR	Huang et al. (2000)
	Barley yellow dwarf virus resistance	Ti translocation	SSR	Ayala et al. (2001)
			RAPD-derived STS	Stoutjesdijk et al. (2001)
	Cereal cyst nematode resistance	*Cre1*	RGA-RFLP	Ogbonnaya et al. (2001)
		Cre3	RGA-based STS	
	Leaf rust resistance	*Lr21*	RGA-based CAPS	Huang and Gill (2001)
		Lr47	RFLP-derived CAPS[b]	Helguera et al. (2000)
	VPM rust resistance	*Sr38/Lr38/Yr17*	RGA-based STS	Seah et al. (2001)
	Stripe rust resistance	*Yr5*	RGA-based CAPS	Chen et al. (2003)
	Fusarium head blight resistance	3BS QTL (Sumai3)	Trait-flanking SSRs	W.-C. Zhou et al. (2003)
Barley	Barley yellow mosaic virus resistance	*rym4/rym5*	SSR	Graner et al. (1999)
		rym9	RAPD-derived STS	Werner et al. (2000)
	Barley yellow dwarf virus resistance	*Yd2*	AFLP-derived STS	Paltridge et al. (1998)
			CAPS derived from a linked gene	Ford et al. (1998)
	Malting quality	QTL affecting α-amylase activity	Trait-flanking RFLP-derived STS and CAPS	Ayoub et al. (2003)
Rice	Bacterial blight	*xa-5*	Trait-flanking RFLP-derived STS and CAPS	Huang et al. (1997)
				Sanchez et al. (2000)
		xa-13	RFLP-derived CAPS	Huang et al. (1997)
		Xa-21	RAPD-derived STS	Chunwongse et al. (1993)
	Blast resistance	*Pi1*	Trait-flanking RFLPs	Hittalmani et al. (2000)
		Piz-5	RFLP-derived CAPS	
		Pita	Trait-flanking RFLPs	
Potato	Potato virus Y resistance	Ry_{adg}	RGA-based CAPS	Sorri et al. (1999)
			RGA-based STS	Kasai et al. (2000)
Soybean	Soybean cyst nematode	*Rhg4*	TaqMan assay	Meksem et al. (2001)

otic stress (Cattivelli et al. 2002; Price et al. 2002) and durable disease resistance (Lindhout 2002). The sustained utilization of QTL, which is difficult to achieve through conventional breeding, is the principal task of MAS and can be done by selecting for the presence of specific marker alleles that are linked to favorable QTL alleles. Verification of putative QTL is needed prior to application of MAS for QTL because QTL effects found in a single mapping population are generally overestimated (Lande and Thompson 1990). Aspects that should be examined are the magnitude of the bias of estimated QTL effects and the certainty of genetic linkage map positions. The simplest method is to compare phenotypic differences between individuals carrying alternative marker alleles at putative QTL, allowing the detection of significant differences for a true QTL. According to the suggestion from Lande and Thompson (1990), the validation of QTL effects can be done in an independent sample of lines within the same cross (Han et al. 1997; Melchinger et al. 1998; Romagosa et al. 1999; Igartua et al. 2000). However, to fully assess the true breeding value of a QTL, studies validating QTL alleles in different genetic backgrounds and environments have to be carried out. In barley, Toojinda et al. (1998) and Ayoub et al. (2003) succeeded in introgressing QTL alleles that confer resistance to stripe rust and affect α-amylase activity of malt, respectively, into genetic backgrounds other than the original mapping population. Moreover, W.-C. Zhou et al. (2003) validated a major QTL for wheat Fusarium head blight resistance with SSR markers in two different genetic backgrounds, while Yousef and Juvik (2002) reported on the marker-assisted introgression of a beneficial QTL enhancing seedling emergence in three different genotypes of sweet corn. However, the attempt to transfer desired QTL alleles to other genetic backgrounds using MAS can also result in the loss of QTL effects (Sebolt et al. 2001; Reyna and Sneller 2001).

A further aspect of the evaluation and verification of QTL is the complexity of the trait, e.g., grain yield is a more complex trait to handle than disease resistance. Several studies reported that the number of QTL associated with grain yield and yield-related traits depends on the genotypes and the variance created by the cross (e.g., Melchinger et al. 1998; Ajmone Marsan et al. 2001). The effect of an added QTL allele on grain yield in an elite genotype is more difficult to estimate than it is, for example, for a QTL on disease resistance in a susceptible elite genotype. For introgression of a QTL allele influencing grain yield into diverse elite genotypes, it must have superior value to all other alleles at this QTL or to alleles at all grain yield QTL that are present in the gene pool (Reyna and Sneller 2001).

Comparative studies exist about the benefit of MAS versus phenotypic selection (van Berloo and Stam 1999; Yousef and Juvik 2001). The benefit depends on the heritability of the trait and the population size. When the heritability is high, the cost involved in genotyping many plants may not outweigh the expected benefits from phenotypic selection. As calculated for recombinant inbred lines, a benefit can be expected within a range of heritability of 0.1–0.3 (van Berloo and Stam 1998). If the value is less than 0.1, it is

not possible to detect the QTL with the accuracy required to rely on flanking markers for selection (van Berloo and Stam 1999).

5 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 as a way of achieving more comprehensive resistance (Mundt 1990) due to simultaneous or stepwise mutation of several avirulence genes in the pathogen that is needed to overcome this pyramid. Successful examples for the pyramiding of major genes in single genotypes are given for the pathosystems rice:*Xanthomonas oryzae* pv. *oryzae*, rice:*Magnaporthe grisea*, and wheat:*Blumeria graminis* f. sp. *tritici* (Table 2). Novel approaches deal with the implementation of transgenes in breeding programs such as the pyramiding of *Bt* genes *cry1Ac* and *cry1C* conferring resistance to diamondback moths in broccoli (Cao et al. 2002; Table 2).

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

Table 2. 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); Sanchez et al. (2000); 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*) Sheath blight (*RC7*) Yellow stem borer *Bt* fusion 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. (2003a, b)
Broccoli	Diamondback moths resistance (*cry1Ac+cry1c*)	Cao et al. (2002)
Soybean	Lepidopteran resistance (*cry1Ac*+corn earworm QTL)	Walker et al. (2002)

an approach to increase the level of disease resistance. Castro et al. (2003a, b) reported on the marker-assisted pyramiding of three quantitative resistance loci against barley stripe rust, caused by *Puccinia striiformis* 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 resistance, with an additive effect of existing resistance alleles.

6 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 3).

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). 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 on one side in the first generation and selection for recombination on the other 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 informative marker-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 widely used in Chinese hybrid rice production, to bacterial blight resistance, caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), through introgression of *Xa21*, a broad-spectrum bacterial blight resistance gene. The PCR-based foreground selection system consisted of a marker that was part of *Xa21*, a marker located at 0.8 cM from the *Xa21* locus on one side and a marker at 3.0 cM from the gene on the other side, while a total of 128 RFLP markers, evenly distributed throughout the rice genome, was used to recover the genetic background of the recurrent parent in the BC_3F_1. The improved version, 'Minghui 63(*Xa21*)', was exactly the same as the original except for a fragment of less than 3.8 cM in length surrounding the *Xa21* locus. Both 'Minghui 63(*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 'Minghui 63', 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 BC_1F_1.

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; Yamamoto et al. 2000; Shen et al. 2001; van Berloo et al. 2001; Willcox et al. 2002). To accelerate the creation of QTL NIL, some authors used background selection (Table 3). 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 intercrossing 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

Table 3. 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 BC_3F_1	BC_3F_1 (128 RFLPs)	Chen et al. (2000)
		Each backcross cycle up to BC_3F_1	BC_1F_1 and BC_2F_1 (129 AFLPs)	Chen et al. (2001)
	Cooking and eating quality (*Waxy* gene region)	Each backcross cycle up to BC_3F_1	BC_3F_1 (118 AFLPs)	P.H. Zhou et al. (2003)
Barley	Barley yellow dwarf virus resistance (*Yd2*)	BC_1F_1 and BC_2F_2	Not performed	Jefferies et al. (2003)
	QTL			
Rice	Root depth (1–2 QTL)	Each backcross cycle up to BC_3F_2	BC_3F_2 (60 SSRs)	Shen et al. (2001)
Barley	Leaf rust (Rphq2)	Each backcross cycle up to BC_3S_2	Each backcross cycle up to BC_3S_2	van Berloo et al. (2001)
Maize	Southwestern corn borer resistance (3 QTL)	Each backcross cycle up to BC_2F_2	Each backcross cycle up to BC_2F_2	Willcox et al. (2002)

displaying epistatic interactions among donor alleles due to overall lower frequency of donor alleles. In fact, there will be a higher probability of detecting additive QTL which still function in a nearly isogenic background.

7 Conclusions

The future of MAS aims not only at utilizing perfect markers for improving existing breeding schemes, e.g., backcrossing, but also controlling all allelic variation for all genes of agronomic relevance. To build superior genotypes in silico, Peleman and van der Voort (2003) introduced a concept, 'Breeding by Design' that requires the knowledge of the map positions of all loci of agronomic importance, the allelic variation at those loci, and their contribution to the phenotype. Although great efforts have to be made to gather all this information, the starting position looks promising: molecular marker technology is very well developed, precise genetic stocks such as introgression line libraries (Eshed and Zamir 1995) for mapping all relevant traits are available for several crop plants and allelic variation at any locus in the genome can be assessed by establishing haplotypes of multiple tightly linked markers. This all-embracing approach has to be addressed immediately to make molecular markers an accepted and irreplaceable tool for developing better crop plants.

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