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Marker Assisted Selection: A Novel Approach for Crop Improvement

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Abstract: Integrating molecular marker technologies such as MAS into breeding strategies could become increasingly important in the coming years, to realize genetic gains with greater speed and precision. The promise of MAS for improving polygenic traits in a quick time-frame and in a cost-effective manner is still elusive. There is a wider appreciation that simply demonstrating that a complex trait can be dissected into QTLs and mapped to approximate genomic locations using DNA markers would not serve the ultimate goal of trait improvement. In facing the challenge of improving several lines for quantitative traits, MAS strategies use DNA markers in one key selection step to maximize their impact. The present paper discusses the basic requirements and the potential applications of MAS in crop plants, recent developments in MAS strategies and genotyping techniques and the significance of integrating MAS into conventional plant breeding programmes.

Key words: Disease resistance · Molecular markers · Plant breeding · QTL

INTRODUCTION

Conformist plant breeding is primarily based on phenotypic selection of superior individuals among segregating progenies resulting from hybridization. Although significant strides have been made in crop improvement through phenotypic selections for agronomically important traits, considerable difficulties are often encountered during this process, primarily due to genotype-environment interactions. Besides, testing procedures may be many times difficult, unreliable or expensive due to the nature of the target traits (e.g. abiotic stresses) or the target environment.

Most of the traits considered in plant genetic improvement programmes are quantitative, i.e. they are controlled by many genes together with environmental factors and the underlying genes have small effects on the phenotype observed. Milk yield and growth rate in animals or yield and seed size in plants are typical examples of quantitative traits. In classical genetic improvement programmes, selection is carried out based on observable phenotypes of the candidates for selection and/or their relatives but without knowing which genes are actually being selected. The development of molecular markers was therefore greeted with great enthusiasm as it was seen as a major breakthrough promising to overcome this key limitation. As Young wrote: "Before the advent of DNA marker technology, the idea of rapidly uncovering the loci controlling complex, multigenic traits seemed like a dream. Suddenly, it was difficult to open a plant genetics journal without finding dozens of papers seeking to pinpoint many, if not most, agriculturally relevant genes" [1].

Molecular marker-assisted selection, often simply referred to as marker-assisted selection (MAS) involves selection of plants carrying genomic regions that are involved in the expression of traits of interest through molecular markers. With the development and availability of an array of molecular markers and dense molecular genetic maps in crop plants, MAS has become possible for traits both governed by major genes as well as quantitative trait loci (QTLs).

The potential benefits of using markers linked to genes of interest in breeding programmes, thus moving from phenotype based towards genotype-based selection, have been obvious for many decades. However, realization of this potential has been limited by the lack of markers. With the advent of DNA-based genetic markers in the late 1970s, the situation changed and researchers could, for the first time, begin to identify large numbers of

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markers dispersed throughout the genetic material of any species of interest and use the markers to detect associations with traits of interest, thus allowing MAS finally to become a reality. This led to a whole new field of academic research, including the milestone paper by Paterson *et al*. [2]. This showed that with the availability of large numbers of genetic markers for their species of interest (tomato), the effects and location of marker-linked genes having an impact on a number of quantitative traits (fruit traits in their case) could be estimated using an approach that could be applied to dissect the genetic make-up of any physiological, morphological and behavioural trait in plants.

MOLECULAR MARKERS

All living organisms are made up of cells that are programmed by genetic material called DNA. This molecule is made up of a long chain of nitrogen-containing bases (there are four different bases-adenine [A], cytosine [C], guanine [G] and thymine [T]). Only a small fraction of the DNA sequence typically makes up genes, i.e. that code for proteins, while the remaining and major share of the DNA represents non-coding sequences, the role of which is not yet clearly understood. The genetic material is organized into sets of chromosomes (e.g. five pairs in *Arabidopsis thaliana*) and the entire set is called the genome. In a diploid individual (i.e. where chromosomes are organized in pairs), there are two alleles of every gene-one from each parent.

Molecular markers should not be considered as normal genes as they usually do not have any biological effect. Instead, they can be thought of as constant landmarks in the genome. They are identifiable DNA sequences, found at specific locations of the genome and transmitted by the standard laws of inheritance from one generation to the next. They rely on a DNA assay, in contrast to morphological markers that are based on visible traits and biochemical markers that are based on proteins produced by genes.

Different kinds of molecular markers exist, such as restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNA (RAPDs) markers, amplified fragment length polymorphisms (AFLPs), microsatellites and single nucleotide polymorphisms (SNPs). They may differ in a variety of ways-such as their technical requirements (e.g. whether they can be automated or require use of radioactivity); the amount of time, money and labour needed; the number of genetic markers that can be detected throughout the genome; and the amount of genetic variation found at each marker in a given population. The information provided to the breeder by the markers varies depending on the type of marker system used. Each has its advantages and disadvantages and, in the future, other systems are likely to be developed.

From Markers to MAS: The molecular marker systems described above allow high-density DNA marker maps (i.e. with many markers of known location, interspersed at relatively short intervals throughout the genome) to be constructed for a range of economically important agricultural species, thus providing the framework needed for eventual applications of MAS.

Using the marker map, putative genes affecting traits of interest can then be detected by testing for statistical associations between marker variants and any trait of interest. These traits might be genetically simple-for example, many traits for disease resistance in plants are controlled by one or a few genes [1]. Alternatively, they could be genetically complex quantitative traits, involving many genes (i.e. so-called quantitative trait loci [QTL]) and environmental effects. Most economically important agronomic traits tend to fall into this latter category. For example, using 280 molecular markers (comprising 134 RFLPs, 131 AFLPs and 15 microsatellites) and recording populations of rice lines for various plant water stress indicators, phenology, plant biomass, yield and yield components under irrigated and water stress conditions, Babu *et al.* detected a number of putative QTL for drought resistance traits [3].

Having identified markers physically located beside or even within genes of interest, in the next step it is now possible to carry out MAS, i.e. to select identifiable marker variants (alleles) in order to select for non-identifiable favorable variants of the genes of interest. For example, consider a hypothetical situation where a molecular marker M (with two alleles M_1 and M_2), identified using a DNA assay, is known to be located on a chromosome close to a gene of interest Q (with a variant Q_1 that increases yield and a variant Q_2 that decreases yield), that is, as yet, unknown.

If a given individual in the population has the alleles M_1 and Q_1 on one chromosome and M2 and Q2 on the other chromosome, then any of its progeny receiving the M1 allele will have a high probability (how high depends on how close M and Q are to each other on the chromosome) of also carrying the favourable Q1 allele and thus would be preferred for selection purposes. On the other hand, those that inherit the M2 allele will tend to

have inherited the unfavourable Q2 allele and so would not be preferred for selection. With conventional selection which relies on phenotypic values, it is not possible to use this kind of information.

The success of MAS is influenced by the relationship between the markers and the genes of interest. Dekkers distinguished three kinds of relationship [4]:

- The molecular marker is located within the gene of interest (i.e. within the gene Q, using the example above). In this situation, one can refer to gene-assisted selection (GAS). This is the most favourable situation for MAS since, by following inheritance of the M alleles, inheritance of the Q alleles is followed directly. On the other hand, these kinds of markers are the most uncommon and are thus the most difficult to find.
- The marker is in linkage disequilibrium (LD) with Q throughout the whole population. LD is the tendency of certain combinations of alleles (e.g. M_1 and Q_1) to be inherited together. Population wide LD can be found when markers and genes of interest are physically very close to each other and/or when lines or breeds have been crossed in recent generations. Selection using these markers can be called LD-MAS.
- The marker is not in linkage disequilibrium (i.e. it is in linkage equilibrium [LE]) with Q throughout the whole population. Selection using these markers can be called LE-MAS. This is the most difficult situation for applying MAS.

The universal nature of DNA, molecular markers and genes means that MAS can, in theory, be applied to any agriculturally important species. Indeed, active research programmes have been devoted to building molecular marker maps and detecting QTLs for potential use in MAS programmes in a whole range of crop, livestock, forest tree and fish species. In addition, MAS can be applied to support existing conventional breeding programmes. These programmes use strategies such as: recurrent selection (i.e. using within-breed or within-line selection, important in livestock); development of crossbreds or hybrids (by crossing several improved lines or breeds) and introgression (where a target gene is introduced from, or example, a low-productive line or breed (donor) into a productive line (recipient) that lacks the target gene (a strategy especially important in plants). MAS can be incorporated into any one of these strategies (e.g. for marker assisted introgression by using markers to accelerate introduction of the target gene). Alternatively, novel breeding strategies can be developed to harness the new possibilities that MAS raises.

Current Status of Applications of MAS in Agriculture: Most of the traits of agronomic importance are complex and regulated by several genes. Unlike the case of simply inherited traits that are controlled by one or a few major genes, improvement of polygenic traits through MAS is a complex endeavour [5]. The difficulty in manipulating quantitative traits is related to their genetic complexity, mainly the number of genes involved in their expression and interactions among genes (epistasis). Because several genes are involved in expression of a quantitative trait, these genes, in general, have smaller individual effects on the phenotype and the effect of the individual genes is not easily identifiable. This warrants repetitions of field tests to characterize accurately the effects of QTLs and to evaluate their stability across environments. Below is a brief summary of the current status regarding application of MAS in the different agricultural sectors (Table 1).

Table 1: Selected examples of gene-marker associated for important traits in major crops

Crop	Trait	Gene/QTL	Linked marker (s)
Rice	Blast resistance	Pi-1	RZ 536 and r 10
		Pi-2	RG 64
	Bacterial blight resistance	Xa-1	XNpb 235
		Xa-3	XNpb 181
	Rice tugro virus resistance	RTSV	RZ 262
	Gall midge resistance	Gm-2	RG 329
		Gm-4	R 1813
	Brown plant hopper resistance	Bph-1	XNpb 248
		Bph-(1)	RZ 404
	Green leaf hopper resistance	Grh-1	R 566
	Submerge tolerance	Sub-1	RZ 698
	Salt tolerance	OSA-3	RG 457
	Wide compatibility	S-5	RG 213
	Temperature sensitive male sterility	TGMS	RM 257
	Grain aroma	Fgr	RG 28
	Amylose content	Wx	Wx

Table 1: Continued

Crop	Trait	Gene/QTL	Linked marker (s)
	Photoperiod sensitivity	Se-1	RG 64
	Semi-dwarf stature	Sdg	R 2182
	Shattering resistance	Sh 4	R 250
	Northern corn blight resistance	Hm-1	Umc 117
Maize	Cytoplasmic male sterility	T, C and S cytoplasms	M 112582, S 81074 and AF 008647
	Enhanced lysine and tryptophan (QPM)	opaque-2	umc 1066, phi 112
	Days to pollenshed	QTL	Taqman probes
	Cyst nematode resistance	Ccn-D1	Cs E 20-2
	Leaf rust resistance	Lr 24	6 RFLP markers
	Powdery mildew resistance	Pm-1, Pm-2	RFLP marker
	Hessian fly resistance	H 6	Op AF 08, Op B01
	Root lesion nematode resistance	Rlnn1	Xedo 347-7A
Wheat	Earliness per se	Eps-Am1	Xwg 241
	Loose smut resistance	T 10	SCAR marker
	Vernalization requirement	Vrn-B1	(TG)3 primers
	Coleoptile colour	Rc-A1, Rc-D1	Xgwm 913
	Flour colour	Major QTL	STS marker
Sorghum	Head smut resistance	shs	RFLP probes
	Fertility restoration	rf4	LW 7, LW 8
Soybean	Cyst nematode resistance	rhg 1	LW 7, LW 8
	Soybean mosaic virus resistance	Rsv	Sat 309 (SSR)
	Linolenic acid content	Fan	pB 194-1, pB 124
	Super nodulation ability	nts	pA-132
Chickpea	Double-podding	s	TA 80 (STMS)
Pea	Nodulation ability	Sym 9	A 5/14, A 5/16
	Powdery mildew resistance	er	p 236 (RFLP)
	Fusarium wilt resistance	Fw	RFLP marker
Tomato	Meloidogyne incognita resistance	Mi	RAPD marker
	Yellow leaf curl virus resistance	Ty 1	RFLP marker
	Black mold resistance	QTL	RFLP markers

CONCLUSIONS

Molecular marker maps, the necessary framework for any MAS programme, have been constructed for the majority of agriculturally important species but the density of the maps varies considerably among species. Currently, MAS does not play a major role in genetic improvement programmes in any of the agricultural sectors. Enthusiasm and optimism remain concerning the potential contributions that MAS offers for genetic improvement.

However, this seems to be tempered by the realization that it may be more difficult and therefore take longer than originally thought before genetic improvement of quantitative traits using MAS is realized. The conclusions from the review by Dekkers and Hospital [6] are a good reflection of this: "Further advances in molecular technology and genome programmes will soon create a wealth of information that can be exploited for the genetic improvement of plants and animals. High-throughput genotyping, for example, will allow direct selection on marker information based on population wide Linkage Disequilibrium. Methods to effectively analyse and use this information in selection are still to be developed. The eventual application of these technologies in practical breeding programmes will be on the basis of economic grounds, which, along with cost-effective technology, will require further evidence of predictable and sustainable genetic advances using MAS. Until complex traits can be fully dissected, the application of MAS will be limited to genes of moderate-to large effect and to applications that do not endanger the response to conventional selection. Until then, observable phenotype will remain an important component of genetic improvement programmes, because it takes account of the collective effect of all genes."

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