

Challenges for effective marker-assisted selection in plants

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Abstract The basic principle of Marker-Assisted Selection (MAS) is to exploit Linkage Disequilibrium (LD) between markers and QTLs. With strong enough LD, MAS should in theory be easier, faster, cheaper, or more efficient than classical (phenotypic) selection. I briefly review the major MAS methods, describing some ‘success stories’ where MAS was applied successfully in the context of plant breeding, and detailing other cases where efficiency was not as high as expected. I discuss the possible causes explaining the difference between theoretical expectations and practical observations. Finally, I review the principal challenges and issues that must be tackled to make marker-assisted selection in plants more effective in the future, namely: managing and controlling QTL stability to apply MAS to complex traits, and integrating MAS in traditional breeding practices to make it more economically attractive and applicable in developing countries.

Keywords Marker-Assisted Selection · Plant breeding · DNA markers · Quantitative trait loci · Linkage disequilibrium · Gene effects · Epistasis · Genotype by environment interactions

Introduction

One may wonder why the effectiveness of Marker-Assisted Selection (MAS) is still challengeable in the year 2007, while the basic idea of using (molecular) markers to aid in selection breeding programs is probably as old as the idea of

QTL detection (see J. B. Walsh, this issue). In fact, a quick literature survey shows that the number of bibliographic references containing any MAS-related keyword started to explode in 1990—just after the publication of one of the first ‘modern’ QTL studies (Paterson et al. 1988)—and then regularly increased up to 337 per year in 2007 (source: ISI Web of Knowledge, search limited to articles in English). Note, however, that very often MAS is only mentioned as a future perspective of QTL or molecular studies, even in the most recent publications... To my knowledge, the earliest references to MAS are by Neimann-Sorenson and Robertson (1961) and Smith (1967), followed by a series of well known and often cited papers in the late 70s and early 80s (Soller and Plotkinhazan 1977; Soller 1978; Soller and Beckmann 1983; Beckmann and Soller 1983; Tanksley and Rick 1980; Tanksley et al. 1981; Stuber 1982). The latter two references are notable in that they supposedly refer to the first real MAS experiments. As stated earlier (J. B. Walsh, this volume), availability of numerous enough markers was one of the main limitations to the development of modern QTL studies. Technical as well as theoretical developments have removed this limitation in the past 15 years, and now the results of numerous QTL detection experiments in various species are available. So, having found the QTLs, surely MAS should be readily effective?

Before we go further, let us recall briefly MAS principles, methods, and expected advantages. The basic principle of MAS is to exploit Linkage Disequilibrium (LD) between markers and QTLs, i.e. the fact that marker alleles are not randomly associated with QTL alleles. If there is strong enough LD, then one can select (directly) on the markers in order to (indirectly) increase the frequencies of linked QTL alleles of interest. Compared to classical (phenotypic) selection, MAS should then be: (i) easier (e.g., when phenotypes are difficult to record); (ii) faster

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(when phenotyping is long, e.g., progeny testing; also, MAS permits selection at an early stage of development); (iii) cheaper (if the cost of genotyping is lower than the cost of phenotyping); and finally (iv) more efficient (because ‘heritability’ at the marker itself (not the linked QTL) is 1 if there is no genotyping error). Though items (i) to (iii) are not really in question and mostly depend on biological or technical particularities of the species and/or breeding scheme considered, the increased ‘efficiency’ (not heritability) in item (iv) is more in doubt, because the ultimate goal of selection is obviously not the marker itself, but rather the linked QTL, or even better the trait. Thus, a good efficiency of MAS presupposes that LD between markers and QTLs does not vanish, and that QTL effects are well estimated and sustained (e.g. over time, or environmental conditions). The latter is indeed a more controversial matter, and in fact the basis of the current paper.

It is noteworthy that in this paper the ‘efficiency’ of MAS is considered from a breeder’s point of view. One may wish to use molecular techniques to help decipher the genetic architecture of complex traits for the purpose of obtaining fundamental knowledge. In such a case, the cost of the information provided by markers does not matter much (in the limits of available research grants). But, the picture is different from an applied breeding point of view, because selection methods have already existed for a long time, based on the record of phenotypic performance in more-or-less sophisticated designs, combined with more-or-less sophisticated statistical analyses. If such methods were not effective already, surely plant and animal breeding would not have been so successful in the past century. Hence, from a breeder’s point of view, MAS must not only be efficient, but *more* efficient than already available breeding methods on an economical scale, i.e. when comparing return to investment. Thus, one must keep in mind that we are always comparing the efficiency of MAS to that of standard phenotypic selection.

Moreover, we focus in this paper on MAS in plants (except trees), and note that plant populations and breeding practices present a number of differences from those used with animals. Plant populations are in general inbred, as opposed to outbred, which means that variation is rather more between populations (or between lines) than within populations. Plant populations exhibit in general larger effective population sizes (N_e), which provide possibly more power for QTL detection, more selection intensity, but also a faster decrease of LD with recombination rate. Conversely, the samples studied are in general smaller than in animal breeding, which gives less accuracy for QTL detection, and environmental variation is higher. Finally, an important and useful property of most plant species is that they allow self-mating to produce fixed inbred lines that can later be used in replicated trials.

A so-called ‘genealogical’ plant breeding scheme typically starts with a bi-parental cross (between two inbred lines), followed by the rapid fixation of progenies by repeated selfing, then selection of the best(s) progeny line(s). Such lines may be released in the production process if of good enough agronomic value, and/or intercrossed with line(s) selected from other bi-parental crosses to pursue the selection process further. Such schemes generally involve multiple starting bi-parental crosses with small progeny samples, and strong selection intensity. As we will see further, such traditional plant breeding practices are contradictory to optimal molecular breeding conditions, which poses the first challenge to the efficiency of MAS.

Major MAS breeding methods include the following: (i) Marker-Assisted Introgression (MAI) or Marker-Assisted Back-cross (MAB), where one gene from a donor line is introgressed into the genetic background of a recipient parent by repeated back-crossing to the recipient parent. Here, markers are used either to control the presence of the target gene and/or to accelerate the return of background genome to recipient type. (ii) The simple screening of populations (e.g. F_2 , F_3 , Recombinant Inbred Lines, Doubled Haploids, ...) for genotypes of interest based on markers (*population screening*). (iii) *Gene Pyramiding* schemes, where two (or more) parent line(s), each hosting one (or more) gene(s) of interest are crossed, then the offspring population is screened for individuals carrying both (all) genes of interest. The process can be iterated further to combine more genes. More complex methods are: (iv) Marker-Based Recurrent Selection (several generations of selection on markers with random mating), and finally (v) selection on an index combining molecular and phenotypic score.

Success stories

Success stories in Marker-Assisted Selection, i.e. successful experiments published so far, were obtained essentially from methods (i) to (iii) above.

Marker-assisted back-cross breeding (MAB) is always successful, except of course when the effect of the target gene is unstable (e.g. a QTL of low effect on a complex trait, see below). This method has been a matter of many theoretical optimization studies, and the efficiency of selection on markers to accelerate the return to background genome is one of the best examples where selection experiments meet theory. MAB is routinely used, especially in private companies (but those works are most often unpublished). Examples of commercially released genetic material include for example Patwin (Hard White Spring wheat), the first variety developed by MAS released by the University of California at Davis (<http://www.plantsciences.ucdavis.edu/>

[plantbreeding/main/history.htm](#)), which contains the introgressed stripe rust resistance gene Yr17 and leaf rust resistance gene Lr37 (Helguera et al. 2003).

Population screening is almost always successful, as anticipated, and does not request much of theoretical optimization when screening only in one generation (i.e. not intercrossing the selected individual in recurrent selection schemes).

Ashikari et al. (2005) provide a good example of successful gene pyramiding experiments. First, the introgression of one QTL for grain number and one QTL for plant height separately in the same genetic background improved both traits. Second, the lines generated by pyramiding both QTLs in the same genetic background exhibited trait values slightly lower than expected based on single introgression lines, but overall the addition of genetic loci was still beneficial and permitted improvement of the yield of a strain of rice.

There are many other successful examples in numerous species, including: pyramiding of Xa7 and Xa21 for the improvement of disease resistance to bacterial blight in hybrid rice (Zhang et al. 2006); marker-assisted pyramiding of two cereal cyst nematode resistance genes from *Aegilops variabilis* in wheat (Barloy et al. 2007); micro-satellite-assisted backcross selection in maize (Benchimol et al. 2005); development of uniform double-crossed varieties using near-isogenic lines produced by marker-assisted selection in radish (Park et al. 2007); and evaluation of leaf rust resistance genes Lr1, Lr9, Lr24, Lr47 and their introgression into common wheat cultivars by marker-assisted selection (Nocente et al. 2007).

Other stories

In some cases MAS is not as efficient as expected. Most of the time, this depends on how stable are QTL effects, which may be altered in different ways. In some cases, the QTL effect ‘vanishes’ after MAS or introgression (Shen et al. 2001). One can then wonder whether the QTL was a false positive (‘ghost QTL’), or a true positive for which the effect (expression) depended on one or several of the interactions listed below. There is also a tendency for supposedly ‘additive’ QTL effects not to really sum up! One example was given above for two traits. The same phenomenon was observed for QTLs for a single trait and coined ‘less than additive epistasis’ by Eshed and Zamir (1996). More generally, many sources of negative interactions may reduce the efficiency of MAS. Either QTL $\times$ QTL, QTL $\times$ Genetic background, and/or QTL $\times$ Environment interactions may reduce QTL effects after MAS compared to what was expected from single-gene effects (see also Jannink et al. this volume). Those can even obliterate the QTL effect (in which

case it may look like a false positive), or even invert it (as was observed by Bouchez et al. 2002 for grain yield in maize).

At this stage, a remark about epistasis is necessary. Epistasis may be more general than originally thought (based on strong influence of the linear model in Quantitative Genetics), see for example T.F.C. Mackay (this volume). Epistasis is very difficult to detect, so in general it is difficult to prove its influence. But from a general point of view, it is worth noting that epistasis can have very contrasting consequences. On the one hand, epistasis may sometimes completely annihilate the response to MAS. Conversely, in other cases, MAS could sometimes be the *only* way to select for favorable epistatic combinations (once they are identified) as demonstrated by Ahmadi et al. (2001) for resistance to yellow mottle virus in rice. In such cases, MAS is *very* rewarding.

Finally, the issues on QTL stability can be nicely summarized through the results of a series of marker-assisted introgression experiments for QTLs controlling fruit quality traits in tomato (Lecomte et al. 2004; Chaib et al. 2006; Causse et al. 2007). In a Recombinant Inbred Line (RIL) population derived from the line cross C $\times$ L, they detected five QTL regions involved in the genetic variation of numerous traits associated with fruit quality. These QTLs were introgressed into the L, B, and D lines backgrounds by a Marker-Assisted Backcross scheme that produced BC3S1 lines having one to five QTLs. This nice design permits checking of interactions between QTLs, between QTLs and background, and of the effect of the environment and population. The results indicate that over the years and progenies (i.e. the RIL versus BC3S1 populations, having different genetic structure and being grown in different years), approximately 60% of the QTLs were stable (18/30, all traits together), and 20% were no longer detected, which can be due to environmental or epistatic interactions. In addition, they observed 20% ‘new’ QTLs in the BC3S1 population that were not detected in the RIL population, which can be due to dominance. One original feature of these studies is that QTLs were introgressed in three different genetic backgrounds: one (L) was a parent of the original cross that produced the RIL population where the QTL detection took place, which is the classical case, and two other backgrounds (B and D) were ‘new’, in that the QTL effects were never estimated in these backgrounds. When considering the stability of the QTLs between the genetic backgrounds, comparison of background L. vs. B indicated that 53% (16/30) of the QTLs were stable, 23% were no longer detected in the new background, and 23% were new (new allelic differences). The picture was approximately the same for the other comparison (L vs. D). Finally, comparing lines that were introgressed for different numbers of QTLs, the results indicate that, although a large part of the genetic variation

for the six traits was under additive control, interactions between QTLs leading to epistatic effects were common. All the significant epistatic interactions had a sign opposite to those of the additive effects (“less than additive epistasis”), and epistasis frequently involved a region where main effect QTLs had been detected.

State of the art and future challenges

As a preliminary conclusion on this on-going research topic, we can first identify clear-cut cases where the value of MAS is not questionable. Those are the cases where MAS, compared to classical phenotypic selection, either (i) provides an increased genetic gain per unit of time, which is the case when MAS permits selection at DNA level at an early stage in the development and/or when it permits reduction of the generation interval (most trees and animals, crops with breeding based on progeny testing), or (ii) provides an increased genetic gain per unit of cost (molecular genotyping cheaper than phenotypic evaluation, disease resistances,...). A good example combining both advantages is certainly when phenotypic recording is destructive, killing the individual before it can reproduce and thereby complicating phenotypic selection (as in silage maize, root or fresh weight measure during growth, etc.), whereas marker-based selection permits selection at an early stage, before reproduction.

In less clear-cut cases, the success of MAS experiments (in plants) appears to depend mostly on the complexity of the genetic architecture of the trait. To take contrasting cases, MAS must surely be more efficient when the trait is simple (e.g., resistance) as opposed to complex (e.g., yield), controlled by few QTLs (as opposed to many QTLs), when the QTL effects are large (not small), when there are no $G \times E$ interactions, and in general when there are no epistatic interactions (although a counter example was given above). Also, MAS would be more attractive if it improves cost efficiency. In such favourable cases, population screening, gene pyramiding, or introgression assisted by markers would be efficient. In the other (opposite) cases, selection on a marker-phenotype index (see Lande and Thompson 1990), should be favored to try to control QTL effects stability from one generation to the next. Also, more theoretical and experimental work would be necessary to optimize such MAS methods to make them economically competitive to classical phenotypic methods.

Finding new sources of major genes

Even in the most favorable case described above (a trait controlled by a small number of QTLs with large, stable effects), the efficiency of MAS may suffer some

limitations. The first one is that, if MAS is really effective in cumulating favorable genes, then at some point (not in the very near future though) a limiting factor may be the availability of new ‘big’ genes to pursue genetic progress, when all genes already segregating in commercial varieties have been used. One solution to this could be the apparently huge potential of plant genetic resources to provide new genes of agronomic interest. This use of molecular markers to ‘unlock’ wild genetic variation already suggested ten years ago (Tanksley and McCouch 1997) has gained a renewal of interest with the experimental demonstration of its potential: in tomato, a pyramid of three independent yield-promoting genomic regions introduced from the drought-tolerant green-fruited wild species *Solanum pennellii* increased yield of hybrids by more than 50% compared to a control market leader variety (Gur and Zamir 2004; Fridman et al. 2004). The latter result has been made possible after the creation of a library of ‘Introgression Lines’ between the wild and cultivated plants. Such work is on-going in various species (Canady et al. 2005; Szalma et al. 2007; Finkers et al. 2007).

A future challenge in this emerging field is to integrate metabolic, phenotypic and genomic databases to allow a wider view of the plant metabolome and the application of this knowledge within genomics-assisted breeding (Fernie et al. 2006). In particular, the elucidation of the fundamental mechanisms of heterosis and epigenetics, and their manipulation, has great potential. Eventually, knowledge of the relative values of alleles at all loci segregating in a population could allow the breeder to design a genotype *in silico* and to practice whole genome selection (Gur et al. 2004; Varshney et al. 2005), even though this may seem unrealistic at the moment. This is now also a matter for dedicated companies (e.g. <http://naturesourcegenetics.com>).

Another way to find new sources of variation within cultivated material is to broaden genetic base by using multi-parental crosses (Blanc et al. 2006), see below.

Accelerated methods of gene pyramiding

Gene pyramiding was described above as one of the ‘best’ MAS methods currently available (along with marker-assisted introgression, which is complementary since its aim is slightly different). But, even such a ‘best’ method can accumulate only a couple of major genes from two parents, and requires a couple of generations. If large sources of major genes were really to be unlocked, as wishfully anticipated in the previous section, then an efficient marker-assisted gene pyramiding scheme would need to tackle multiple, possibly linked, genes, from multiple parents. Methodological developments in this area are only starting (Servin et al. 2004; Wang et al. 2007; Ye et al. 2007) and still need more work.

Managing and controlling QTL stability

As seen above, the (in-)stability of QTL effects (across environments, genetic background...), even for QTLs with reasonably large effects, is one of the main causes of unexpected results of MAS experiments. Not surprisingly, QTL stability and QTL by environment interactions have become a major concern, and there are numerous corresponding projects in various species: study of QTL x environment effects for grain yield and related traits in maize (Moreau et al. 2004), stability of QTLs for alpha-acid content in hop (*Humulus lupulus* L.) (Cerenak et al. 2006), heterosis in maize when mapped under several stress conditions (LeDeaux et al. 2006), testing the effects and stability of QTLs for morphological and agronomical traits from the wild species *Aegilops tauschii* (Pestsova et al. 2006), and QTLs with stable and major effects for rice grain length (Wan et al. 2006). However, assessing the effects of each QTL in each environment is an immense task.

Dealing with polygenic complex traits

At the beginning of the ‘QTL revolution’, there was a belief that molecular breeding could *remove* the frontier between ‘Mendelian’ and ‘Statistical’ Genetics, i.e. between ‘those who count’ and ‘those who measure’ (Crow 1993). This turned out not to be completely true. In some cases indeed, some traits have been turned from ‘complex’, quantitative, traits into ‘simple’ Mendelian traits after discovering that they were controlled by a surprisingly low number of QTLs with large effects. A nice example is the work on morphological traits that distinguish maize and its progenitor teosinte (Doebley 2004). Although most of the traits are under the control of multiple genes and exhibit quantitative inheritance, a few loci of large effect appear to represent key innovations during maize domestication. One is the locus for the maize mutant teosinte branched1 (tb1) (Doebley et al. 1995).

But, this does not hold for the vast majority of traits, so the frontier has only moved towards more Mendelian traits (note however that for those traits at the border, the change has been considerable, including in terms of breeding, as was the case for many resistance traits in plants). But, for the still complex traits, a large part of the variation may be due to QTL with small, unstable effects. The question now (and challenge) is whether MAS is also profitable for improving complex polygenic traits? The answer is still a matter of debate, and an interesting one from both an applied and a fundamental point of view. There is a paradox here, which is well summarized by the following quote from Rocha et al. (2004): “Traits with low heritability demand considerably larger sample sizes to achieve effective power of QTL detection. This is unfortunate as traits with low heritability are among those that could most

benefit from QTL-complemented breeding and selection strategies...”. Put in extreme terms, this means that on the one hand MAS would be most effective for traits with high heritability—but for those traits classical phenotypic selection is already efficient, so MAS can add little gain if any—conversely MAS would provide a large additional gain compared to phenotypic selection for traits with low heritability—but in that case MAS efficiency is low, due to a low power of detection of QTL effects, unless very large sample sizes are used that then make MAS unaffordable ...

One way to tackle this “Squaring the circle” problem is to optimize the model selection process and find a compromise between the power of detection, the risk of false positives and of over fitting the predictive model. Actually, the efficiency of MAS is maximized when using an ‘optimum’ type I error risk value, which corresponds to a *higher* type I error risk than those usually considered in QTL detection experiments (0.1%). This optimum type I error for MAS may be at least 5% for low heritability traits (Moreau et al. 1998; Hospital et al. 1997) or even between 10% and 20% depending on trait heritability (Bernardo 2004). This corresponds to a large False Discovery Rate (Bernardo 2004). One can also aim to balance type I and type II error risk in MAS (Moerkerke et al. 2006).

In animal breeding there is a lot of interest now in ‘genomic selection’ (Meuwissen et al. 2001; Goddard and Hayes 2007), with the emphasis on using all markers to label regions rather than pick out specific QTL. The same interest exists in plant breeding, although investigations are probably at a less advanced stage. Using a Bayesian regression method Xu (2003) was able to handle a large number of markers, even larger than the number of observations, where most of the markers may have negligible effects. As a result, it is possible to evaluate the distribution of the marker effects. This was applied to data from the North American Barley Genome Mapping Project in double-haploid barley, and to simulated data sets of F2 and backcross (BC) families. Interest of such approaches for MAS were investigated by simulations by Bernardo and Yu (2007) for a population of doubled haploids derived from a cross between two lines. They showed that this method was more efficient than methods based on a selected subset of makers. From an economic point of view, such methods are of interest only if the cost of genotyping is low enough (Laurence Moreau, pers. comm.).

There seem to be no clear lower limit for the size of QTLs that can be found and used for MAS, some private companies claiming to apply MAS for QTLs explaining less than 10% of phenotypic variation (Laurence Moreau, pers. comm.).

Integrating MAS in traditional breeding practices

The other way to tackle the “Squaring the circle” problem above is to incorporate MAS in existing breeding schemes, in

order to provide additional gain at limited cost. Here, it is necessary to recall that up to date, optimal molecular breeding conditions are somehow antagonistic to traditional plant breeding practices (Moreau et al. 1999). For example, an optimal MAS design would consist of one replicate of a large population, whereas breeders would traditionally use multiple small populations derived from multiple crosses. If MAS is more costly than phenotypic selection, as is generally the case for selection on a marker-phenotype index (see Moreau et al. 2000), this is a disincentive to its use.

One solution to the need to increase sample size at low cost is to use existing pedigrees, as opposed to designing new specific crosses (Crepieux et al. 2004). More generally, that would be almost equivalent to considering the whole set of available commercial and wild resources in a species as a vast (structured) population, and applying strategies already undertaken in outbred populations (animals) and humans. However, locating quantitative trait loci is statistically more challenging and considerable research is needed to provide robust and computationally efficient methods (Boitard et al. 2006), so whole-genome association analysis may be more difficult than first anticipated (see Hyten et al. 2007 for an example in soybean).

More generally, the use of MAS would certainly be facilitated if it was better integrated into traditional breeding practices, rather than considered as a substitute. Such a topic is still a matter of intense consideration (Stuber et al. 1999; Kuchel et al. 2005; Bonnett et al. 2005; Hu 2007) that will likely continue for some time because there is probably no globally optimal strategy, and each case must be investigated separately depending on the particularities of the species, breeding objectives and traditions.

Applications in developing countries

The new science of genomics has an important contribution to make to the development of agriculture in the ‘Third World’ and thus to improving the livelihoods of many of the poorest people on earth. However, although some claim that “developing countries are already benefiting and should continue to benefit significantly from advances in plant biotechnology” (Toenniessen et al. 2003), others notice that “the persistence of poverty demonstrates, however, that this contribution is not yet being made” (Reece and Haribabu 2007). Compared to historical evidence from the Green Revolution, the present situation is different in several ways, which raise not only scientific but also economic and political issues. So far, it is mostly the industrialized world that is benefiting from the technology, with two main consequences: (i) Most studies are of the crops important in the industrialized world, leaving ‘orphan crops’ in developing countries. These crops, including *tef* (*Eragrostis tef* (Zucc.)), millet, cowpea, and indigenous

vegetables, fruits, roots, and tubers, tend to be locally important, but receive little public or private investment (Naylor et al. 2004). (ii) The large private-sector investment in biotechnology research was directed more to breeders and farmers in the industrialized world, and relatively little contribution of the technology was made to increasing food production, nutrition, or farm incomes in less-developed countries (Herdt 2006). In parallel, public investment has been reduced. Hence, some advocate that the strategic leadership role of the public sector is strengthened, research functions are reallocated to the private sector, and new policy and organizational mechanisms are used to stimulate pro-poor research in and for developing-country agriculture (Spielman 2007), or even recommend policy measures that allow transfer of current technology to the poor and generate more biotechnology research focused on problems of the poor (Pray and Naseem 2007).

Conclusions

After all that has been said, it is obviously rather difficult to draw a definite conclusion, except on the few—but certainly economically important—clear and non ambiguous cases that have been identified. At this stage, the general conclusions that have been drawn are a matter of belief (Price 2006), pessimism (Bernardo 2001), or ‘cautious optimism’ (Young 1999). The practical answer will surely come from accumulation of real-life experiments and practice in various species.

In any case, the issues raised by marker-assisted selection clearly show that more quantitative genetics theory is needed, in particular to address selection for most of the traits that (still) remain complex and polygenic. Such theory must integrate the knowledge that has emerged from molecular studies. Particularly relevant would be a model that could accurately (at least) explain or (if at most possible) predict the (sustained) response to selection that is observed for traits controlled by both a few QTLs of large effects, and many genes of smaller, non detectable, effects.

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