MAS IN CEREALS: GREEN FOR MAIZE, AMBER FOR RICE, STILL RED FOR WHEAT AND BARLEY

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Summary
This paper reviews the uptake of marker assisted selection in the major cereals maize, wheat, rice and barley, and contrasts the growing and substantial use of MAS in maize with the slow pace of uptake in wheat breeding. The difference largely reflects the fact that maize varieties are predominantly F_1 hybrid, whereas wheat and barley varieties are almost exclusively pure breeding inbreds. The value of hybrid seed is much higher than that of inbred, as the former, but not the latter, can be fully protected from farm-saved seed and competitor use. Until the unit assay cost of MAS falls by at least an order of magnitude, it is doubtful that MAS will have a substantial impact on the conventional wheat and barley breeding paradigm.

Keywords
Wheat, maize, rice, barley, SNP

Abstract
The explosion over the last decade in the availability of molecular markers has been a happy by-product of 'big biology' genomics research. Just seven years ago, the definition of 5,000 microsatellite loci in the human genome merited a major publication in *Nature*, but the number of known human single nucleotide polymorphisms (SNPs) now runs into millions. Now that marker availability, potentially at least, is no longer limiting in crops, it is timely to explore the extent to which marker technology has to date - and is likely in future to - affect the practices of cereal breeding.

Understanding the inheritance of traits by exploiting linkage with factors which segregate in a simple Mendelian fashion is a concept almost as old as genetics itself. In plants, simple colour traits were applied in the 1920's to predict seed weight in *Phaseolus*, and fruit size in tomato. But because simply inherited morphological variants are very rare, and/or are largely irrelevant to breeding germplasm, marker assisted plant breeding remained of theoretical interest until the development, in the 1960s, of biochemical markers. In this period, a number of isozyme-determining loci in maize were linked to factors determining yield, and some were even used to select for yield improvement in experimental populations. However, their widespread use in breeding was restricted by the difficulty in scaling-up the assays to a level sufficient to be useful in a realistic plant breeding situation.

The clear potential benefits of marker deployment to plant breeding are undisputed, and have been described at length elsewhere. Despite this, the uptake of marker aided selection (MAS) in practical breeding programmes, among the cereals and elsewhere, has been patchy. Only relatively recently has it begun to make more than a marginal impact on breeding methodology. The level of DNA marker polymorphism is high in maize; however, large-scale deployment of MAS did not gather any significant momentum until over 15 years since the publication of the first RFLP-based maize genetic map. Even in the less genetically variable cereals, prominently wheat, the level of polymorphism is not likely to represent the

major constraint on MAS uptake, although this has been argued in the past to be the case. What has changed now is that current marker technology, particularly SNPs and other parallel genotyping technologies, are starting to remove any effective limitation on marker discovery, in an even more spectacular way than the development of microsatellite technology has already done.

Maize

Maize breeding in developed countries is dominated by a small number of large private sector companies (which do not freely, for understandable competition reasons, publicise their procedures). This contrasts with the situation in the other major cereal species, where breeding is more commonly carried out by public sector organisations. Globally, maize production is dominated by F_1 hybrids. The control over individual varieties that this allows the breeder has far-reaching consequences on the financial returns of breeding: first, no revenue is lost as a result of the use of farm-saved seed; and second, the inbred components of a successful hybrid are not available to competitors to use as parental material for their own varietal improvement programmes.

Following the development of the maize RFLP genetic map, the late 1980s saw the exploration of opportunities for the commercial application of MAS in maize breeding, while various studies set out to demonstrate the selection efficiency of MAS, particularly where the MAS targeted variation at QTL. A typical conclusion of these experiments was that because markers are usually able to significantly increase the precision with which superior advanced selfed-generation lines can be identified, MAS will work in most situations where CPS (conventional phenotypic selection) has been able to achieve progress. Thus, in situations where QTLs are themselves unreliable, either because of epistasis (leading to unpredictability of expression in genetic backgrounds other than in the one where they have been detected) or to genotype x environment interactions (so that the effect is environment-dependent), MAS directed at QTL variation is also unreliable; but where such interactions are insignificant, genetic progress is predictable and MAS, if economically justifiable, is advantageous.

A major investment in MAS infrastructure has occurred and is continuing within the large private sector maize breeding companies (in the USA primarily Pioneer Hi-Bred, Syngenta and Monsanto; in Europe, in addition to these, KWS and Limagrain). As an example, in Monsanto's US operation, the past five years have seen 'the development of thousands of new marker assays, a 17-fold increase in the acquisition rate of marker data, and a decrease in unit data point cost of 75%' (M. Edwards, Monsanto Company, St. Louis USA, pers. comm.). Much of this scale-up is based around the automation of the whole process of marker genotyping, and is increasingly reliant on SNPs as a technology platform. Yield-directed MAS typically targets around 20 QTL, and is achieved by incorporating three generations of purely marker-based selection within one year, thereby adding one year to the conventional five-year cycle of inbred line production. This protocol is claimed to generate a doubling in breeding gain over CPS, and to produce a saving of one to two years in the time required to backcross a specific trait into an elite inbred. Such a level of commitment to MAS technology is likely to be self reinforcing – as experience and infrastructure related to the technology and its deployment grow, so its use will become more pervasive.

Wheat

In contrast to maize, wheat is a naturally inbreeding species. Although a level of heterosis can be demonstrated, difficulties in enforcing cross-pollination in a reliable and cost-effective way (largely associated with poor pollen dispersal from the male parent to the male sterile female parent) have hindered the development of any significant contribution of F_1 hybrids to the variety pool. Most varietal development programmes are therefore based on versions of the long-established pedigree breeding system, where large F_2 populations are generated, and CPS is carried out in early generations for highly heritable, qualitative traits (such as disease resistance) and in later ones for the quantitative traits (primarily yield and quality). Thus most varieties are bred and grown as inbred, pure breeding lines, and although the breeder is afforded some protection by Plant Breeders' Rights, this neither prevents the widespread use of farm-saved seed, nor the incorporation of successful releases into a competitor's breeding programme**.** As a result, although the volume of seed sale may be comparable with that of maize, the value of the wheat seed market is much smaller, and thus the economic margin for the breeder is far less. This has far-reaching implications on the economics of MAS deployment.

The use of MAS to date has a history of about 20 years, and until very recently involved the exploitation of just two non-DNA based assays. The first, which has been retained with only slight modifications, ever since its inception, exploits a correlation between bread-making quality and allelic status at the *Glu-1* (endosperm storage protein subunit) loci, which uses electrophoretic profiles (obtained by the straightforward, robust and cheap procedure SDS-PAGE) from crude seed protein extracts to be partially predictive of end-use quality. The second predicts, as a result of tight genetic linkage, the presence/absence of a gene conferring a high level of resistance a stem base disease, which is difficult to score by conventional pathology methods. The assay involves isoelectric focussing separation of the isozymes of endopeptidase. However, in the last few years, the number of loci for which DNA-based assays have been generated has increased dramatically, the majority using PCR as a technology platform (Table 1).

Table 1
Published DNA based assays for wheat genes and QTLs, 1996-2003

Trait	Gene	Marker type	Trait	Gene	Marker type
Yellow (stripe) rust resistance	*Yr5*	SSR	Eyespot resistance	*Pch1*	SSR
	Yr7	SSR		*Pch2*	RFLP
	Yr9	RGA	Karnal bunt resistance		SSR, AFLP
	Yr10	SSR	Loose smut resistance		STS from AFLP
	Yr15	RAPD, SSR	Bunt resistance	*Bt10*	STS from RAPD
	Yr17	RGA, STS from RAPD	*Septoria nodorum* resistance		STS from RAPD
	Yr18	SSR	*Pyrenophora tritici repentis* resistance		RFLP
	Yr26	SSR	BYDV resistance	*Bdv2*	STS from RAPD SSR
	Yr28	RFLP	WSMV resistance	*Wsm1*	STS from RAPD
	Yr29	SSR	WSSMV resistance	*Wss1*	RFLP
	YrH52	SSR	CCN resistance	*Cre1*	STS (perfect)

	YrMoro	STS from AFLP		*Cre3*	STS (perfect)
	YrKat	SSR		*Cre6*	STS (perfect)
	Unspecified adult	SSR	RLN resistance	*Rlnn1*	RFLP
Brown (leaf) rust resistance	*Lr3*	RFLP	RKN resistance	*Rkn-mn1*	RAPD
	Lr19	STS from AFLP	RWA resistance	*Dn1*	SSR
	Lr26	RGA		*Dn2*	SSR, STS from RAPD, RFLP
	Lr28	STS from RAPD		*Dn4*	SSR, RFLP
	Lr35	STS from RFLP		*Dn5*	SSR
	Lr37	RGA		*Dn6*	SSR
	Lr39	SSR		*Dn8*	SSR
	Lr47	STS from RFLP		*Dn9*	SSR
	Lr50	SSR		*Dnx*	SSR
	Unspecified	SSR		*Unspeci-fied*	STS from RAPD
Black (stem) rust resistance	*Sr2*	SSR	Hessian fly resistance	*11 loci*	RAPD
	Sr30	RGA, SSR	Wheat curl mite resistance	*Cmc3*	SSR, RFLP
	Sr31	RGA		*Cmc4*	SSR, RFLP
	Sr36	SSR	Greenbug resistance	*Gb3*	SSR, AFLP
	Sr38	RGA	Waxy endosperm	*Wx-1*	STS (perfect)
Powdery mildew resistance	*Pm1*	AFLP	Endosperm protein quality	*Glu-1*	STS (perfect)
	Pm3	SSR	Endosperm colour		STS from AFLP, SSR
	Pm4	AFLP	Grain protein content		STS from AFLP, SSR, RFLP
	Pm5	SSR	Grain hardness		STS (perfect), AFLP
	Pm6	RFLP	Dormancy		SSR
	Pm8	STS from RFLP	Dwarf stature	*Rht-1*	STS (perfect)
	Pm13	STS from RFLP		*Rht8*	SSR
	Pm17	STS from RFLP	Vernalisation requirement	*Vrn-B1*	AFLP, SSR, STS from RFLP
	Pm21	STS from RAPD	Heading time		SSR, RFLP
	Pm24	AFLP, SSR	Ear morphology		SSR, RFLP
	Unspecified adult	SSR, RFLP	Manganese efficiency		RFLP
Fusarium head blight	*Sumai3 QTL*	STS from AFLP SSR, RFLP	Boron toxicity		RFLP, AFLP

Although the potential for take-up is now much wider than in the past, progress has nevertheless been slow, albeit measurable. The critical issue remains the unit assay cost. Using a capillary sequencing platform, a single microsatellite data point, excluding the cost of both the PCR itself and the acquisition of DNA template, has been estimated to cost around US$0.40 to generate, while a more extensive published calculation puts its full economic cost at between US$1 and US$2. At such a level of cost, uptake for the moment remains restricted to low volume applications, such as genotype construction by backcrossing, and to the development of niche genotypes such as waxy wheats, which can command a price premium. It is instructive that exactly the same considerations are seen to be relevant for the

widely heralded exploitation of human DNA polymorphisms to predict differential drug responses. Thus it has been reported (Roses 2002) that although the average cost of a SNP assay has fallen from US$1.00 to US$0.10 over a 12 month period, a further order of magnitude reduction to US$0.01 per assay will be required before wide-scale usage of the technology will become feasible.

Other cereals: barley and rice

Barley breeding closely resembles that of wheat in both structure and economics. The species is inbreeding, and selection methods are very similar to those used by wheat breeders – varieties worldwide to date have been exclusively released as pure breeding inbred selections. However, the world's first F_1 hybrids have been recently launched in the UK, entering official trials in 2000, with commercial seed expected to be available in autumn 2003. Like wheat, the end value of the crop is low. Molecular breeding is 'not widely used, other than as a marker for BaYMV (barley yellow mosaic virus) resistance' (Thomas 2003), even though extensive collections of microsatellite assays have been assembled. The two genes *rym4* and *rym5* are both marked, and these markers are in wide use by European breeders. As for wheat, many of the proposed MAS targets relate to genes for disease resistances, most of which are well catered for with efficient phenotypic screens. Proposed QTL targets for MAS breeding have included malting quality, a trait which attracts a substantial price premium and adult plant resistance to stripe rust, which is difficult to handle by CPS.

Although rice is also a self-pollinating species, there has been a sustained effort to develop F_1 hybrid breeding, and this has led to an increasing presence and use of F_1 hybrid varieties (particularly in China). Interestingly, this has not been the trend in Japan, where because of a strong consumer preference for *japonica* types, the use of *japonica* x *indica* hybrids is restricted. Genetic maps and molecular marker collections are well developed. Uniquely, rice's status as a genomic model should promote the application of MAS in breeding, as much of the gene cloning activity in monocot species will be driven by the availability of the whole rice genome sequence and a worldwide investment in technologies associated with large scale gene isolation. Much of the progress in rice MAS to date has centred on the pyramiding of disease resistance genes, particularly to blight and blast. The potential for MAS to aid in resistance breeding with respect to both diseases has been recently demonstrated, and 2002 saw the release of two Indonesian cultivars, in which MAS had been employed to introduce *xa5* into an *xa4*-containing background (Toenniessen *et al.* 2003). The *Xa21* gene is particularly significant since it has been cloned and this has facilitated the development of perfect within-gene markers for its tracking in segregating materials, and its ready transfer between genotypes by transgenesis. Thus its selection by MAS features in a number of programmes. A number of quality and breeders' QTL traits are currently also being targeted.

Conclusion

In 1999, Young set out his 'cautiously optimistic vision' for marker-assisted selection. Four years on, the situation is starting to crystallise. The technology itself is no longer limiting. With respect to marker availability, SNPs will soon represent a source of plentiful within-gene markers, and are set to be developed for all the major cereals. The 'big biology' spawned by the genomics revolution has brought miniaturisation and automation to biological assays, so that levels of throughput relevant to the plant breeding process are becoming attainable. The issue that remains is the affordability of large-scale MAS. Because cereals are primarily broad-acre commodity products, their value is generally low, and this will slow the widespread adoption of MAS, except where F_1 hybrid seed is a viable proposition.

However, as economies of scale and improvements in technology drive down the assay price, the penetration of MAS into commercial cereal breeding will undoubtedly grow. For maize, this stage is already being reached. But for the 'red' crops wheat and barley, MAS use is likely to remain less central to the breeding process, and be deployed only for specific purposes. These include the accelerated selection of a few traits that are difficult to manage via CPS, for the maintenance of recessive alleles in backcrossing programmes, for the pyramiding of disease resistance genes, and for guiding the choice of parents to be used crossing programmes.

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