CHAPTER 19

Technical, economic and policy considerations on marker-assisted selection in crops: lessons from the experience at an international agricultural research centre

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SUMMARY

Molecular markers and related technologies have been used extensively in genetic characterization and identification of loci controlling traits of economic importance in many crop species. However, the application of such tools for crop improvement has not been extensive, at least in the public sector. Although there are clear advantages in using molecular markers as tools for indirect selection of traits of importance, available examples indicate that their use is restricted to traits with monogenic inheritance or when the inheritance is conditioned by a few genes with large effects. Another important limitation of large-scale marker applications is the cost involved in marker assays, which may be beyond the capacities of many public plant breeding enterprises. For an effective marker-assisted selection (MAS) activity to facilitate ongoing crop improvement programmes, especially in the context of the developing countries, laboratories with adequate capacity and adequately trained scientific personnel as well as operational resources are required. Although recent technological advances such as single nucleotide polymorphisms (SNPs) and associated assay protocols are likely to reduce assay costs significantly, for many of these operations, assay platforms with significant capital investments including computational capacity are required. Coupled with these limitations, private sector domination of biotechnology research with proprietary rights to important products and processes with immediate benefits to developing countries may further constrain the benefits these technologies may offer to resource-poor farmers. Policy-makers in different national programmes and international development and research agencies have a responsibility to sustain and augment the capacity of national public agricultural research organizations to ensure that biotechnology tools and processes are infused appropriately into national research efforts. They must also ensure that any biotechnology efforts undertaken are well integrated with national crop improvement activities.

INTRODUCTION

Due to their usefulness in characterizing and manipulating genetic factors responsible for qualitative as well as quantitative traits, molecular markers are considered to be valuable tools for crop improvement. These uses of molecular markers have been invaluable in helping researchers understand complex traits, dissect them into single Mendelian genetic factors, and establish their chromosomal locations via the use of linkage maps and/or cytogenetic stocks. Availability of well characterized genetic linkage maps is a prerequisite for tagging important agronomic or other traits with molecular markers, enabling their use in MAS related activities. To date, however, few practical applications have been published from these studies. This paucity of published studies may indicate the long-term nature of this research, or it might simply reflect the fact that marker technology has been applied to plant breeding efforts mostly by scientists working in the private sector (Hoisington and Melchinger, 2004).

Maize was one of the first crop species for which molecular linkage maps were developed, and Gardiner *et al.* (1993) consolidated several individual maps into a consensus map. Rice is another species for which high-density linkage maps have been developed (reviewed in Gowda *et al.*, 2003) while, due to its high ploidy level and large genome (21 linkage groups, as opposed to 10 in maize and 12 in rice), efforts to develop well characterized, saturated linkage maps with wheat have lagged behind. Other important cereals and legumes are at various stages of linkage map development. The availability of well-defined linkage maps and the extent of genetic studies conducted on them therefore vary among different crops, and this influences the feasibility of any MAS-related activity. Thus, while it is possible to carry out MAS to some degree in cereals such as rice, maize and wheat, and in legumes such as soybean, for species such as cassava and sweet potato, the so-called "orphan crops", genetic improvement with MAS may not yet be feasible. These crop species may benefit more readily from genetic modification arising from direct introduction of genes isolated from other species or organisms, which is not the focus of this chapter.

Citing practical lessons learned at the International Maize and Wheat Improvement Center (CIMMYT) as well as findings of studies conducted elsewhere, this chapter describes some actual and potential applications as well as the advantages and disadvantages of MAS, and outlines possible applications of MAS in developing country plant breeding programmes.

LESSONS LEARNED FROM CROPS

Numerous scientific reports describe molecular mapping and analysis of quantitative trait loci (QTL) for nearly every agronomic trait in a diverse array of crop species. The traits covered include many parameters associated with tolerance to drought and other abiotic stresses, maturity, plant height, quality parameters, qualitative and quantitative factors of disease and pest resistance, and numerous seed traits and yield. Although these efforts have resulted in a vast amount of knowledge and better understanding of the underlying genetic factors that control these traits, application of this knowledge to manipulate genes in an effective or simple manner for improving crop species has had limited success. The scientific community is faced with the challenges of accurate and precise QTL identification and application of the information derived to successful MAS efforts.

Scientific advances have been instrumental in increasing the power and accuracy of computational parameters as well as designing ways of combining the information generated from molecular genetics with traditional crop improvement efforts. Numerous simulation studies have been undertaken to evaluate the effectiveness of MAS, taking into account the influence of heritability, population size, linkage distance, etc. (Xie and Xu, 1998; Moreau *et al*., 1998; Ribaut, Jiang and Hoisington, 2002), and MAS procedures have been used to incorporate traits of interest from exotic species including wild relatives into elite cultivars through advanced backcross QTL analysis (Tanksley and Nelson, 1996; Fulton *et al*., 2000).

Manipulation of qualitative traits

Molecular markers that are tightly linked to genes having a strong effect on the expression of a trait can be used to introgress the genes (and thus the trait) into different backgrounds through backcross breeding schemes that rapidly and efficiently improve the recurrent parent for the target trait. In conventional backcross breeding schemes and line conversion activities, the donor parent containing the trait of interest is crossed with the recurrent parent, normally a well-adapted variety lacking the trait of interest. The resulting progeny are screened to identify the trait of interest, and individuals exhibiting the trait are crossed to the recurrent parent. The entire process is repeated several times. For traits that are conditioned by recessive gene action, a cycle of selfing is also required after each crossing cycle. After several cycles of backcrossing and a final self-pollination, plant breeders are often able to recover lines that are nearly identical to the recipient parent but also contain the trait of interest. Compared with traditional backcrossing, the use of DNA markers enables faster recovery of the recurrent parent genotype along with the introgressed target trait in line conversion activities. Ribaut and Hoisington (1998) reported that MAS should enable the recovery of the target genotype after three cycles of backcrossing, compared with a minimum of six cycles with traditional approaches (Tanksley *et al*., 1989).

CIMMYT has a long history of using molecular markers for certain traits in maize improvement. Although maize is widely used for both food and feed, maize kernels do not provide sufficient quantities of two essential amino acids, lysine and tryptophan. The *opaque2* mutation, identified at Purdue University (United States of America) in the mid-1950s, confers elevated levels of these two amino acids. Although initial efforts to introduce the *opaque2* mutation into breeding materials were not successful (Villegas, 1994), researchers eventually succeeded in producing nutritionally enhanced maize lines. These came to be known as quality protein maize (QPM). CIMMYT breeders have used traditional backcrossing to transfer the *opaque2* mutation and associated modifiers into elite lines. To perform phenotypic selection in segregating progenies for lines carrying the *opaque2* mutation, it is necessary either to wait until the plants produce mature ears, or to do random pollination on a large number of plants. Although reliable laboratory screening techniques are available, co-dominant microsatellite markers present within the *opaque2* mutation can be used earlier in the growing season. Using these markers in backcross progenies, plants heterozygous for the *opaque2* mutation can be selectively identified as a qualitative trait for use in the next crossing cycle. Markers

are not used to select for the background recurrent parent genotypes, but only to select lines carrying the *opaque2* mutation allele. Although CIMMYT uses markers for detecting the presence of the *opaque2* mutation, markers are not available to select for the modifiers, which are important in determining seed texture and quality and for which other traditional screening techniques are being used.

A well known example of marker-assisted backcrossing of a qualitative trait involves the introgression of the *Bt* transgene into different maize lines (Ragot *et al.,* 1994). Whenever plant transformation techniques are used to produce genetically modified organisms (GMOs), usually there are some cultivars that are more receptive to transformation procedures than others. When the cultivar with the best agronomic type is not the most receptive to transformation, it is often possible to transform another cultivar that is receptive and then use the diagnostic marker that detects the transgene to introgress it into more desirable backgrounds. This type of MAS-aided line conversion can be accomplished for any crop species. The presence of markers to detect the transgene enables the detection of converted progeny with a high degree of accuracy.

Another MAS-related CIMMYT experience involves the case of maize streak virus (MSV) resistance, for which a major QTL was identified on maize chromosome 1 that explains 50–70 percent of total phenotypic variation (Pernet *et al*., 1999a, b). As maize has a well-saturated molecular linkage map, several microsatellite markers associated with this QTL were identified in the specific chromosomal region. These markers were tested in three populations generated using three different MSV tolerant lines crossed with one susceptible line. After screening the F_2 progeny and F_3 families, lines identified by markers were sent to Africa, where MSV is prevalent. By phenotypic screening of the lines selected by MAS, it was established that MAS-selected lines were significantly more resistant to MSV (J-M. Ribaut, personal communication).

In legumes, resistance to soybean cyst nematode (SCN) is one example of an effective MAS approach. Routinely used phenotypic assays for SCN screening take approximately five weeks and extensive greenhouse space and labour. Successful identification of closely linked microsatellite markers has enabled transfer of the resistance gene *rhg1* with about 99 percent accuracy (Cregan *et al.,* 1999; Young 1999). Many public and commercial soybean cultivar improvement efforts use these markers to screen for SCN resistance (Young, 1999). Another example of successful MAS in common beans was reported by Yu, Park and Poysa (2000) who used markers associated with common bacterial blight. These markers identified a locus that explained about 62 percent of the phenotypic variation and have been used in MAS experiments.

As described earlier, linkage map construction in wheat is more challenging than in species such as rice or maize. The allohexaploid nature allows wheat to withstand chromosomal imbalances as the loss of one chromosome can be compensated by the presence of a homologous chromosome. As a result, wheat can be crossed with a range of wild relatives (both intergeneric and interspecific), enabling introgression of genetic material possessing resistances to different biotic and abiotic stresses. When translocations (especially intergeneric translocations) are present in wheat, markers can be readily developed

for the translocated chromosome segments. If a translocated segment carries a trait of importance, markers can then be used to transfer it into different wheats. Diagnostic or perfect markers (i.e. markers with complete linkage to the genes of interest with no possibility of recombination) have been developed for genes conferring resistance to different biotic stresses in wheat. CIMMYT's wheat improvement efforts use a set of markers routinely on a seasonal basis for introgression of a set of genes into high-yielding backgrounds. Examples of the perfect markers that are currently in use are:

- Cereal cyst nematode (CCN) resistance gene *Cre*1 (2BL), identified in wheat landrace AUS10894 and *Cre*3 (2DL), derived from *Triticum tauschii* (Lagudah, Moullet and Appels, 1997). These markers are used routinely in segregating populations to enable selective advancement of lines containing the *Cre* genes targeted to all environments, but particularly to marginal ones, where healthy root architecture is essential to allow plants to take advantage of minimal soil moisture. Phenotypic evaluation for CCN resistance is labour intensive as well as expensive. Given that it is impossible to screen for CCN resistance in Mexico (where CIMMYT headquarters are located) due to the lack of required screening facilities, the use of markers is essential for improving this trait.
- Barley yellow dwarf virus (BYDV) resistance, derived from a chromosome segment introgressed from *Thinopyrum intermedium*, on chromosome 7DL (Ayala *et al.*, 2001). BYDV is an important viral disease in certain wheat growing regions of the world. Environmental influence makes field screening less reliable. The diagnostic marker for the translocated chromosome segment allows the alien-derived resistance to be combined with the BYDV tolerance available in wheat.
- Marker for *Aegilops ventricosa*-derived resistance to stripe rust (*Yr17*), leaf rust (*Lr37*) and stem rust (*Sr38*) (O. Robert, personal communication). The translocation from *Ae. ventricosa* is present on chromosome 2AS. The diagnostic marker for the translocation is used mainly in bread wheat x durum wheat crosses, to identify the durum derivatives carrying the translocation.

In addition, CIMMYT uses a set of linked markers for transferring a locus with major effects for boron tolerance (*Bo-1*), crown rot resistance, scab resistance and stem rust resistance in its MAS efforts. These efforts with linked genes are conducted with the objective of increasing the allele frequency for desirable alleles in segregating populations (William, Trethowan and Crosby-Galvan, 2007).

Gene pyramiding/stacking

MAS lends itself well to gene pyramiding efforts for disease resistance. When a cultivar is protected by one gene with major effects against a specific disease, it is often not possible to introgress additional genes conferring resistance to the same disease because of the difficulty of phenotypic screening for the presence of additional genes (as the plant already shows resistance to the disease). However, if several genes can be tagged with closely linked molecular markers, MAS strategies can be used to develop lines with stacked genes, giving the cultivar more durable protection than that afforded by a single resistance gene.

Resistance to bacterial blight provides an excellent example of using MAS for gene pyramiding. Bacterial blight is caused by

Xanthomonas oryzae and is one of the most important diseases of rice. Several genes that confer resistance to bacterial blight have been tagged with molecular markers. Huang *et al.* (1997) and Hittalmani *et al.* (2000) developed strategies for combining four resistance genes, namely *Xa-4, Xa-5, Xa-13* and *Xa-21,* in a single cultivar using pairwise combinations of the genes. Due to the co-dominant nature of the markers used, the authors were able to select from F_2 generations without having to perform progeny testing. The derived lines containing pyramided genes showed higher level of resistance and/or a wide spectrum of resistance compared with the parental material. Another gene pyramiding example using MAS involves stacking of the resistance genes *rym4, rym5, rym9* and *rym11* for the barley yellow mosaic virus complex using molecular markers and doubled haploids (Werner, Friedt and Ordon, 2005). Other examples include pyramiding for barley stripe rust resistance (Castro *et al.*, 2003), and powdery mildew resistance in wheat (Liu *et al.,* 2000) and, in MAS applications at CIMMYT, crosses have been made to combine two genes for cereal cyst nematode resistance and three different genes for stem rust resistance (*Sr24, Sr26* and *Sr25*) in targeted wheat germplasm.

Manipulation of quantitative traits

Quantitatively inherited traits are genetically complex, are conditioned by a number of genes each having relatively small effects, and their expression often depends on interactions among different genetic components (epistasis). The environment also has a high degree of influence on the expression of the trait, which confounds the interpretation of QTL identification and often renders the results obtained from QTL studies cross-specific. When it is necessary to manipulate several genomic regions simultaneously, each having different effects on the same trait of interest, MAS-based approaches become more complicated and present formidable challenges. Mapping studies conducted at CIMMYT identified five genomic regions associated with the anthesis-silking interval which is a parameter associated with drought tolerance in maize (Ribaut *et al.*, 1996, 1997). The drought tolerant parent was used in MAS experiments as the donor parent to transfer the five QTL to CML 247, an elite inbred line with good combining ability that was drought-susceptible but high-yielding under favourable conditions. Markers were used to generate 70 BC_2F_3 lines containing the favourable alleles from the drought-resistant parent after two backcrosses and two self pollinations. These lines were crossed with two testers for field evaluation. Field tests indicated that under severe drought stress conditions, the 70 MAS-derived lines were significantly better yielding than the controls. The differences were less prominent under reduced drought stress (Ribaut and Ragot, 2007).

Other CIMMYT experiments aimed at comparing MAS with phenotypic selection have been conducted for stem borers in tropical maize (Willcox *et al.,* 2002). In the case of maize stem borer resistance, three QTL identified through mapping experiments were used in MAS. Three BC_2S_2 families that carried all three target QTL from the donor parent in homozygous state were developed. Comparative studies with MAS and traditional phenotypic selection did not establish a clear advantage for MAS, but both approaches yielded significant genetic gains in reducing leaf damage. MAS is not being used currently on a routine basis at CIMMYT for drought and stem borer resistance.

Other reports describing the manipulation of quantitatively inherited traits include those of Bouchez *et al.* (2002) for introgressing favourable alleles at three QTL for earliness and grain yield in maize, and by Yousef and Juvik (2001) who reported on MAS for seedling emergence and eating quality characters in sweet corn. Also, Han *et al.* (1997) attempted to select for barley malting traits using MAS. Additional scientific reports are available that describe MAS-related efforts for quantitatively inherited traits.

In general, manipulating several QTL associated with multiple genomic regions in segregating progenies is considerably more challenging. Often the success in genetic gains depends on the stability of these QTL as well as the cost efficiency of large-scale MAS applications.

Genetic diversity studies

In addition to being used in MAS activities, molecular markers have been used extensively for genetic diversity studies. Numerous scientific publications are available that describe the use of molecular markers in estimating the degree of relatedness of a set of cultivars in many cultivated crop species. In common with their use in trait manipulations, the practical outcomes of the numerous genetic diversity studies using molecular markers are not clear. Evaluation of genetic relatedness using molecular markers will have implications on understanding the genetic structure of existing populations, enabling the design of strategies for proper acquisition of germplasm for conservation purposes. The genetic uniqueness of accessions or populations in germplasm collections can be accurately estimated by the use of DNA profiling (Brown and Kresovich, 1996; Smith and Helentjaris, 1996). Molecular markers have also been used for identifying redundancies in existing germplasm collections in rice (Xu, Beachell and McCouch, 2004) and sorghum (Dean *et al.,* 1999). In cassava, Chavarriaga-Aquirre *et al.* (1999) used morphological traits, isozyme profiles and agronomic criteria to identify a core set of 630 accessions from a base collection of approximately 5 500 accessions.

Modern farming in advanced countries is based on high performing, genetically uniform new cultivars, which are generally derived from well adapted, genetically related parental material. Tanksley and McCouch (1997) have concluded that most modern soybean cultivars grown in the United States can be traced back to a very limited number of strains from a small area of northeastern China, while a majority of hard red winter wheats is derived from a few lines originated in Poland and the Russian Federation. The genetic basis of modern rice varieties grown in the United States is also considered narrow (Dilday, 1990).

Another application in the area of genetic diversity is the use of markers in identifying heterotic groups. Molecular markers have been used extensively in the construction of heterotic groups since the 1990s in many different crop species of economic importance. Heterotic groups are clusters of germplasm usually with similar characteristics and a high degree of relatedness that, when crossed with materials from another heterotic group, tend to give rise to progeny with high levels of heterosis. Although markers randomly distributed in the genome can be used to develop heterotic groups, their usefulness in determining hybrid performance is not clear. While it is reasonable to assume that heterosis depends on the interactions among favourable alleles belonging to the two parents, unless molecular markers that are known to be linked to

these favourable alleles are used in heterotic studies, the predictive power of markers in estimating heterosis for practical applications may not be very high.

At CIMMYT, large-scale, rapid characterization methods for inbred lines and populations have been optimized using up to 120 microsatellite markers spread throughout the maize genome. In the past, characterizing maize populations was costly and time-consuming, given that as many as 22 individuals had to be analysed individually to calculate allele frequencies for each marker. Currently, a bulking method in which 15 individuals from a population are amplified in the same polymerase chain reaction (PCR) and run on an automatic DNA sequencer, provides a reliable estimate of the allele frequencies within that particular population. Between one and two bulks can now be used to fingerprint populations with considerable savings in time and resources. Other studies of maize genetic diversity have been conducted for CIMMYT maize breeders as well as outside collaborators with objectives that include: determining how maize inbred lines from different national breeding programmes are related to each other (and to determine the possibility of sharing among regions or using lines from one region to expand diversity in another); establishing heterotic groups; determining levels of genetic diversity present in synthetic varieties; determining how landraces and farmers' varieties from different regions are related to each other; monitoring homozygosity levels in inbred lines; and tracking changes in lines that have been intensively selected for a given trait.

A core set of 100 microsatellite markers has been selected for wheat genetic diversity studies. Recent fingerprinting studies by CIMMYT and national programme scientists have been conducted to assist in regenerating gene bank accessions without losing genetic diversity, measuring the contribution of wild ancestors and exotic species in advanced backcross progenies of synthetic bread wheat, and to track the changes over time in diversity levels of CIMMYT wheat cultivars from the original Green Revolution varieties to modern breeding lines.

Marker implementation

To facilitate the use of MAS activities in wheat and maize improvement efforts, CIMMYT has recently established a marker implementation laboratory. This provides the facilities and technical expertise to provide CIMMYT wheat and maize breeders with access to biotechnology tools, including MAS. The laboratory carries out two main MAS-related activities, marker adoption and research support. The first includes constantly reviewing the literature to identify markers developed by third parties and verifying that these can be used to detect traits or genes of interest in CIMMYT germplasm improvement efforts, and developing efficient protocols for their in-house use. The second consists of a range of routine tasks that include growth and/or sampling of plant tissue, DNA extraction, marker detection, data analysis and dissemination of results to breeders.

Close cooperation between field and laboratory staff is important to be able to apply molecular markers in crop improvement efforts. Ideally, laboratory staff should have an understanding of field activities and field workers should have basic knowledge of different aspects of MAS-associated laboratory procedures. MAS is used when there is a high probability that markers will help plant breeders achieve genetic gains faster and more economically than field

or laboratory-based phenotypic selection methods. When perfect markers are available to screen for a particular trait, such markers are preferred. However, for traits that cannot be screened conveniently using traditional approaches and even when perfect markers are not available, if markers are available with close linkages to the trait(s) of interest, these can be used to increase the desirable allele frequency for the target gene. MAS-related activities in both wheat and maize at CIMMYT are conducted as collaborative projects involving both breeders and biotechnologists. The breeders use information coming from wheat MAS activities to define better their parental crossing block materials and to make selective crosses using parents identified by markers. Moreover, segregating early generation progenies in certain crosses are selected in the field based on whole plant phenotype, which are then further refined by sampling leaf tissue from field-tagged plants and processing for MAS assays in the laboratory. Only those entries that contain the target genes identified with MAS are advanced to the next generation. This enables breeders to reduce population sizes for the traits under evaluation and accumulate certain gene combinations in elite backgrounds. The material thus generated is advanced through several cycles of selfing and eventually used in field screening to identify the best performing lines.

ECONOMICS OF MAS

Establishing the capacity to conduct MAS

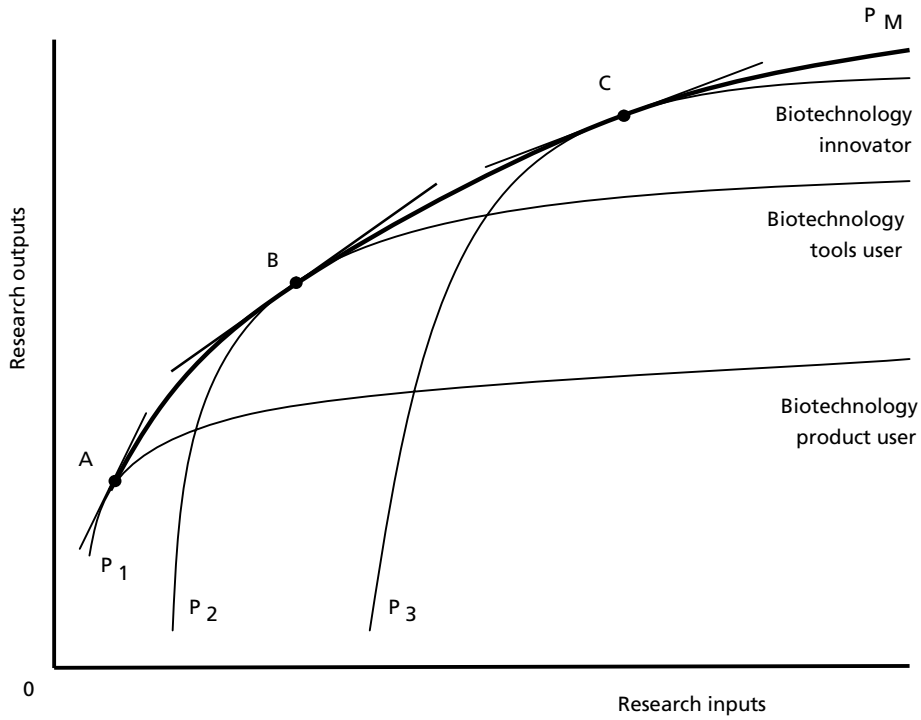
For MAS to be a viable option for a plant breeding programme, adequately equipped laboratory facilities must be in place and appropriately trained scientists must be available. Therefore, one of the first decisions facing research managers considering MAS is whether to invest in biotechnology research capacity.

Economic theory suggests that the most efficient level of research investment can be determined with the help of a research production function that relates research inputs to research outputs. At the national level, the research production function can be thought of as a meta-function encompassing the frontiers of many smaller functions, each representing a different level of research capacity distinguished by complexity and scope (Figure 1) (Brennan 1989; Byerlee and Traxler, 2001; Maredia, Byerlee and Maredia, 1999; Morris *et al.*, 2001). Movement outwards along the meta-function, accomplished by adding subprogrammes and thereby increasing the number of researchers and the extent of available research infrastructure, is associated with changes in focus and increases in the capacity of the national research programme.

For a plant breeding programme, adding new biotechnology-based subprogrammes is equivalent to taking a series of discrete steps involving increased complexity and cost. These steps have the effect of moving the programme from one level of research capacity to the next. These levels of research capacity can be broadly characterized as follows:

- *Biotechnology product user.* Here, the research programme imports germplasm products developed using biotechnology and incorporates them into its conventional crop improvement schemes, either by backcrossing them into local germplasm or by testing them for potential immediate release.
- *Biotechnology tools user* where the research programme imports biotechnology tools and uses them, if necessary, after adapting them to local

FIGURE 1
Biotechnology research production meta-function



Source: Morris *et al.* (2001).

circumstances, to improve current crop improvement practices.

- *Biotechnology methods innovator,* in which the research programme establishes the full capacity needed to develop innovative biotechnology tools and products.

Moving from one level of biotechnology research capacity to the next usually requires significant investments in laboratory facilities and staff training. The practical decision facing research managers is not to determine the optimal level of research investment, but rather to select from among the different levels of biotechnology research capacity characterized by increasing complexity and cost (A or B or C in Figure 1). The choice should be based on whether a given level of research capacity can be expected to generate enough additional benefits to justify the additional expenditure. For most plant breeding programmes, benefits consist of value added to crop production enterprises. Therefore, the incentive to invest in additional research capacity will tend to increase with the size of the area planted and/or the value of the crops expected to benefit from the research.

There are few published estimates of the cost of moving from one level of biotechnology research capacity to the next, and new estimates are not provided here. Empirical estimates would quickly be outdated, as the cost of biotechnology laboratory equipment and materials continues to change very rapidly. However,

for the purposes of this chapter it is important to point out that although establishing capacity to develop new molecular markers requires substantial investment, establishing the capacity to use freely available existing molecular markers requires only a modest investment.

Variable cost of MAS

At CIMMYT the capacity to carry out MAS on a reasonable scale has been developed, but the need now is to make the technology work on a high-throughput scale to reduce the cost per data point, while being able to handle large quantities of assays per growing season. In this regard, there are several challenges to consider as markers are not always cost-effective even when they improve the precision of selection. Depending on the nature of the target trait (quantitative or qualitative), the type of gene (major or minor), the form of gene action that controls expression of the trait (dominant or recessive effect), and the ease with which the trait can be measured (visually detected or more expensive field or laboratory analysis required), conventional selection may be cheaper than MAS. The desirability of using genetic markers therefore depends in part on the costs of genotypic versus phenotypic screening, which vary among applications.

Information about the cost of using MAS at CIMMYT for specific breeding projects is available from case studies. For example, Dreher *et al.* (2002, 2003) examined the costs and benefits of using MAS for a common application in maize breeding. This study generated three noteworthy conclusions.

First, for any given breeding project, detailed budget analysis is needed to determine the cost-effectiveness of MAS relative to conventional selection methods. Although the costs of field operations and laboratory procedures required for molecular marker analysis may remain relatively constant across applications, every breeding project is likely to involve unique phenotypic evaluation procedures whose costs will frequently differ.

Second, direct comparisons of unit costs for phenotypic and genotypic analysis provide useful information to research managers, but in many cases technology decisions are not made solely on the basis of cost. Factors other than cost often influence the choice of screening methods. Time considerations are often critical, as genotypic and phenotypic screening methods may differ in their time requirements. Even when labour requirements are similar, for applications in which phenotypic screening requires samples of mature grain, genotypic screening can often be completed much earlier in the plant growth cycle.

Third, conventional and MAS methods are not always direct substitutes. Using molecular markers, breeders may be able to obtain more information about what is going on at the genotypic level than they can obtain using phenotypic screening methods. For example, in conventional backcross breeding or line conversion projects (see section *Manipulation of qualitative traits*), background molecular markers can be used to identify those plants among a set of progeny that not only possess a desirable allele but also closely resemble the recurrent parent at the genetic level. Based on this additional information, breeders are often able to modify their entire breeding strategy, with potentially significant implications in terms of cost and/or time requirements (this issue is discussed in the next section).

The CIMMYT case study thus confirmed what many practising plant breeders

intuitively know: namely, the costs and benefits of MAS projects are likely to vary depending on the crop being improved, the breeding objective being pursued, the skill of the breeder, the capacity of the research organization, the location of the work being carried out, the cost of key inputs, and many other factors.

Economic trade-offs

While caution is required when extrapolating from the results of a case study, general conclusions regarding the cost-effectiveness of molecular markers in crop genetic improvement work can be drawn based on the findings of the CIMMYT study and a number of other studies carried out elsewhere. Broadly speaking, two types of benefits associated with MAS can be distinguished: cost savings and time savings.

Cost savings

For certain applications, MAS methods can substitute directly for conventional selection methods, and for these applications the relative cost-effectiveness of the two methods can easily be determined by comparing the screening cost per sample. Generally, as the cost of phenotypic screening rises, markers are more likely to represent a cost-effective alternative. For applications in which phenotypic screening is easy and cheap (e.g. visual scoring of plant colour), MAS will not offer any obvious advantages in terms of cost. However, for applications in which phenotypic screening is difficult or expensive (e.g. assessing root damage caused by nematodes or for a disease that is not present in the field site), MAS will often be preferable.

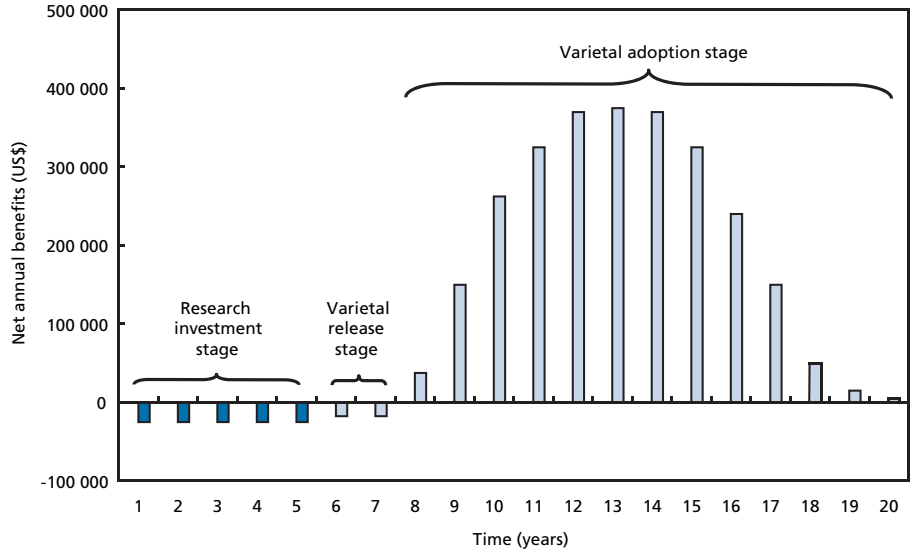
Time savings

Cost is an important factor affecting the choice of breeding technology, but it is not the only one. Plant breeders worry about controlling costs, but they also worry about getting products out quickly. Therefore, it is not sufficient to consider potential cost savings alone. The time requirements of alternative breeding strategies must also be taken into account, because even when MAS costs more than conventional selection (as it does in some, although not all, cases), breeders who use it may be able to generate a desired output quicker. Accelerated release of improved varieties can translate into large benefits, especially for the private seed industry, so time is an important consideration in addition to cost.

For breeding applications in which MAS offers cost and time savings, the advantages of MAS compared with conventional breeding are clear. More problematic, however, are the many applications in which MAS methods cost more to implement than conventional selection methods but also reduce the time needed to accomplish a breeding objective. This commonly happens, for example, with inbred line conversion schemes based on backcrossing procedures. In such schemes, MAS methods can often be used to derive converted inbred lines containing one or more incorporated genes in much less time than would be possible using conventional selection methods alone.

In applications that involve a trade-off between time and money, under what circumstances is the higher cost of MAS relative to conventional breeding justified? The choice of the plant breeding method can be viewed as an investment decision and evaluated using conventional investment criteria (Sanders and Lynam, 1982). Using data from the CIMMYT case study, Morris *et al.* (2003) explored the relationship between time and money as it relates to crop improvement research

FIGURE 2
Stylized economic model of a plant breeding programme



by developing a simple model of a plant breeding programme and using it to compare the returns with alternative inbred line conversion schemes based on conventional selection and MAS. Two measures of project worth were used: the net present value (NPV) of the discounted streams of costs and benefits, and the internal rate of return (IRR) to the investment.

Figure 2 depicts the stylized "variety life cycle" assumed by the model. The stream of costs and benefits associated with the development, release and adoption by farmers of an improved variety can be divided into three stages: a research stage during which the variety is developed; a release stage during which the variety is evaluated and registered for release, and commercial seed is produced; and an adoption stage during which the variety is taken up and grown by farmers. During the first two stages, net benefits are negative, because costs are incurred without any benefits being realized. During the third stage, net benefits turn positive as the variety is taken up and grown by farmers; they continue to increase until the peak adoption level is achieved and then decline when the variety is replaced by newer varieties.

The model was used to estimate the NPV and IRR of conventional and marker-assisted inbred line conversion schemes. Research cost data were taken from the CIMMYT case study. Plausible values were used for key parameters relating to the varietal release and adoption stages (for details, see Morris *et al.*, 2003). Figure 3 shows the streams of annual net benefits generated by each of the two breeding schemes. Annual net benefits are calculated as follows:

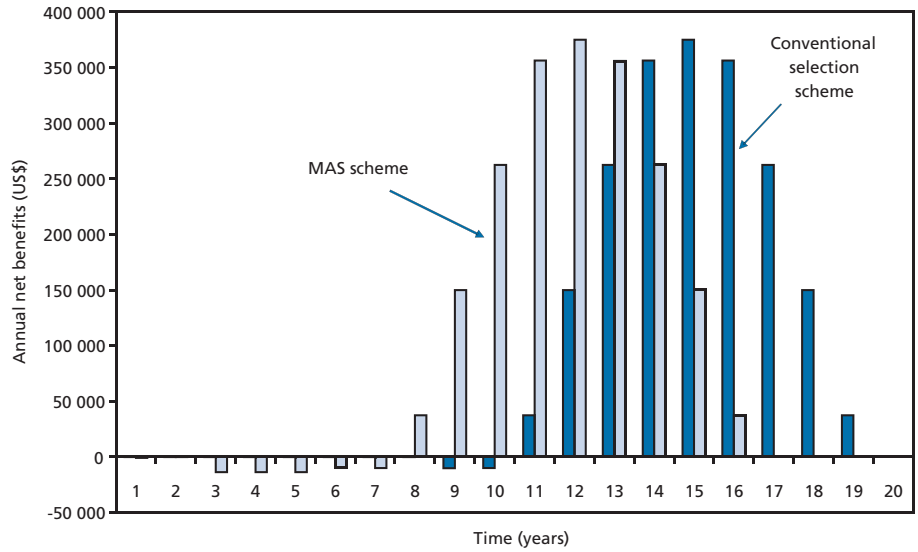
$$NB_t = (GB_t - VR_t - RC_t)$$

where:

NB = net benefits

GB = gross benefits (calculated as area planted to the variety x incremental benefits associated with adoption)

FIGURE 3
Annual net benefits, conventional vs. marker-assisted line conversion scheme



VR = varietal release expenses (cost of evaluation trials, registration procedures, seed multiplication, advertising and promotion, etc.)
RC = research investment costs
t = year (1…n)

NPVs were calculated by adding the discounted stream of net benefits associated with each breeding scheme over the life of the variety (n years):
where:

$$NPV = \sum_{t=1}^{n} (GB_t - VR_t - RC_t) / (1 + r)^t$$

NPV = net present value
r = discount rate

IRRs were calculated conventionally by solving the discount rate that drives the NPV to 0.

The profitability rankings of the two breeding schemes, MAS and conventional, were found to differ depending on the measure of project worth that was used. The MAS scheme generated the highest NPV, whereas the conventional breeding scheme generated the highest IRR on investment. These results, generated using a stylized model of a plant breeding programme and plausible values for varietal release and adoption parameters, provide an important insight into the relative cost-effectiveness of conventional selection methods and MAS in applications involving trade-offs between time and money. From an economic perspective, the relative attractiveness of conventional versus MAS methods will depend on the availability of research investment capital. If investment capital is abundant (meaning that the breeding programme can afford to absorb the higher up-front costs associated with MAS without curtailing other ongoing breeding projects), MAS may become a desirable option, because it generates the largest NPV. On the other hand, if investment capital is constrained (i.e. the breeding

programme cannot absorb the higher up-front costs associated with MAS, or that it can absorb them only by forgoing other potentially profitable breeding projects), it makes sense to choose conventional selection, because it generates the largest IRR.

IMPLICATIONS FOR DEVELOPING COUNTRIES

When discussing policy implications of MAS efforts in developing country scenarios, it is appropriate to consider the experience gained over the past several decades, mainly in industrialized countries. In advanced countries, the private sector has made significant investments in MAS efforts while there are a few publicly-funded research groups using MAS in breeding routinely and these are restricted to a few target crops (Eagles *et al.*, 2001; Dubcovsky, 2004; William, Trethowan and Crosby-Galvan, 2007). Information about the traits and the breeding strategies used in MAS applications in large agribusiness enterprises are not publicly available freely. To date, significant investments have been made in biotechnology applications only for widely grown crop species such as rice, maize, wheat, soybean, cotton and canola. While GM crops and their implications are not the focus of this chapter, it is reasonable to assume that technologies associated with GM crops offer significant potential for addressing biotic and abiotic stress tolerance in widely grown cereals and legumes as well as species that are important but thus far neglected such as tef, millets, yams and other tuber crops in the developing countries. For example, GM technologies that can make one crop species perform better are likely to be valuable with slight modifications to enhance the performance of a neglected crop species. When useful GM varieties of a particular crop are made available, they also become prime candidates to apply MAS-based introgression of the introduced gene construct/s to other well adapted cultivars in different agro-ecological regions.

Reports indicate that two rice varieties with improved bacterial blight resistance have been developed with MAS approaches and deployed in Indonesia (Toenniessen, O'Toole and DeVries, 2003). Moreover, rice varieties carrying multiple disease resistance genes are being developed by several national programmes with technical backstopping by the International Rice Research Institute (IRRI) (Hittalmani *et al.*, 2000). There are also reports describing the use of MAS in China for improving certain quality traits in rice (Zhou, P.H. *et al.*, 2003) and wheat (Zhou, W-C. *et al.*, 2003) and fibre related traits in cotton (Zhang *et al.*, 2003), but it is not clear whether these are one-time research efforts or there is continued activity using MAS.

Although it is not possible to obtain entirely reliable estimates of the costs, benefits and cost-effectiveness of MAS applications, the costs associated with MAS are frequently considered as the main constraint to their effective use by many plant breeders, especially in small- to medium-scale breeding enterprises. However, new marker technologies, especially those based on single nucleotide polymorphisms (SNPs) and associated ongoing large-scale genome sequencing projects, should enable the development and deployment of gene-based markers in the near future (Rafalski, 2002). SNPs are defined as single base differences within a defined segment of DNA at corresponding positions. These SNP-based polymorphisms are known to be abundantly present in human as well as in plant genomes. Consequently, the potential exists to develop SNP markers associated with many important traits in a diverse array of

economically important crop species. For species such as maize, rice and soybeans, robust SNP-based assay platforms already exist in the private sector as well as in some public sector enterprises. The added advantage of SNP-based marker systems is that they avoid gel-based allele separations for visualization and have the potential for automation in high-throughput assay platforms. These ongoing research efforts will inevitably lead to the development of more robust, high-throughput assays that are both simple and cost effective (Jenkins and Gibson, 2002).

When is it advantageous to use MAS?

In addition to the cost and time savings described above, for a number of breeding scenarios, MAS methods are likely to offer significant advantages compared with conventional selection methods. These scenarios assume the availability of markers for multiple traits and take into consideration the advantages of MAS under optimum situations (Dreher *et al.,* 2002; Dudley, 1993).

- *Gene stacking for a single trait.* MAS offers potential savings compared with conventional selection when it allows breeders to identify the presence of multiple genes/alleles related to a single trait, and the alleles do not exert individually detectable effects on the expression of the trait. For example, when one gene confers resistance to a specific disease or pest, breeders would be unable to use traditional phenotypic screening to add another gene to the same cultivar in order to increase the durability of resistance. In such cases, MAS would be the only feasible option, provided markers are available for such genes.
- *Early detection.* MAS offers potential savings compared with conventional selection when it allows alleles for desirable traits to be detected early, well before the trait is expressed and can be detected phenotypically. This benefit can be particularly important in species that grow slowly, for example, tree crops.
- *Recessive genes.* MAS offers potential savings compared with conventional selection when it allows breeders to identify heterozygous plants that carry a recessive allele of interest whose presence cannot be detected phenotypically. In traditional breeding approaches, an extra step of selfing is required to detect phenotypes associated with recessive genes.
- *Heritability of traits.* Up to a point, gains from MAS increase with decreasing heritability. However, due to the difficulties encountered in QTL detection, the gains are likely to decline beyond a certain threshold heritability estimate.
- *Seasonal considerations.* MAS offers potential savings compared with conventional selection when it is necessary to screen for traits whose expression depends on seasonal parameters. Using molecular markers, at any time of the year, breeders can screen for the presence of an allele (or alleles) associated with traits that are expressed only during certain growing seasons. For example, CIMMYT's wheat breeding station in northern Mexico is usually used for screening segregating germplasm for leaf rust resistance. However, expression of leaf rust is not uniform in all growing seasons. The same concept is true for field screening for drought tolerance. When there are seasons with low expression of leaf rust or less intense drought due to unexpected rainfall, markers, if available, can be a valuable alternative as a tool for screening.
- *Geographical considerations.* MAS offers potential savings when it is necessary

to screen for traits whose expression depends on geographical considerations. Using molecular markers, breeders in one location can screen for the presence of an allele (or alleles) associated with traits expressed only in other locations.

- *Multiple genes, multiple traits.* MAS offers potential savings when there is a need to select for multiple traits simultaneously. With conventional methods, it is often necessary to conduct separate trials to screen for individual traits.
- *Biological security considerations.* MAS offers potential advantages over selection based on the use of potentially harmful biological agents (e.g. artificial viral infections or artificial infestations with insect pests), which may require specific security measures.

In view of the above-mentioned factors, it is desirable to consider MAS approaches on a case-by-case basis, taking into account factors such as the importance of a trait in the overall breeding scheme, the amount of available resources in terms of both staff and operational expenditures, and the nature of the breeding materials. There are no "one size fits all" recommendations that can be made for MAS approaches. Usually, no breeding scheme focuses on improving just one trait. At current levels of capacity, MAS is likely to be used to achieve genetic gains for single traits such as host plant resistance to pests and/or diseases. Therefore, MAS activities should be integrated into an overall breeding programme.

Challenges for developing countries

The rapid expansion of agricultural biotechnology is generating a wide array of methodologies with potential applications, and therefore national programmes in developing countries face the difficult challenge of identifying priority areas for investment. To complicate matters further, the private sector dominates many fields of biotechnology research and therefore has proprietary rights to many technologies and products that have immediate applications in developing countries (e.g. transgenic technology). This is quite different from conventional plant breeding technologies, most of which were developed by publicly-funded research programmes and thus have remained more accessible.

There is no single answer to meeting these challenges, especially as developing countries are not uniform in their public agricultural research capacities. Broadly speaking, developing countries fall into the following categories:

- countries (a few) with strong public sector research infrastructure enabling biotechnology applications, as well as upstream research capability to develop tools for their own specific needs;
- countries with intermediate capacity in applied plant breeding, as well as in using biotechnology tools that are publicly available or can be acquired through bilateral partnerships with the private sector;
- countries (a considerable number) with moderate plant breeding capacity and practically no, or very little, capacity for biotechnology applications.

More advanced developing countries with major commercial farming sectors are more likely to succeed in adopting agricultural biotechnology. In addition, the presence of commercial opportunities will attract investment by private industry and thus allow the country to benefit from future advances in biotechnology. This is not always a positive outcome for the public sector because, as competition increases, it may be more difficult to justify large public investments in biotechnology. This

has occurred to some degree in maize biotechnology, even in the United States.

Developing countries, in which agriculture is still dominated by subsistence farming and where there is limited or no capacity for biotechnology research, are at an added disadvantage. Resource-poor farmers in such countries rarely offer adequate market incentives for the private industry that dominates biotechnology research. For example, the involvement of the private sector in research and development activities for root crops or grain legumes is doubtful as these crops are grown mainly by small-scale farmers in poorer regions of the world and there would be potentially low returns on investment. Therefore, it is important that international development agencies ensure that neither the "orphan commodities" yielding broad socio-economic benefits, nor the less advantaged and least developed countries, are left out from the prospect of harnessing potential benefits associated with biotechnology. In doing so, they must evaluate what biotechnology tools can be of immediate benefit to such crops and countries and then develop strategies leading to successful adoption by the target groups. This can only be accomplished if the efforts made are serious, long-term and sustainable. Many examples can be cited where international aid agencies have invested in purchasing equipment designed for biotechnology research in developing countries but, when the aid programmes terminate their short-term involvement, the capital investments either have not been optimally utilized or have remained idle.

Policy-makers in different national programmes must also bear in mind that sustained capacity in public agricultural research is a pre-requisite for successful application of biotechnology tools including MAS for crop improvement. Biotechnology tools can be used to enhance genetic gains for a few traits in a few crops, but their ultimate impact depends on how well they are adopted and integrated into existing plant breeding activities. This is a sobering thought, because in many developing countries public sector research capacity is being eroded and public sector extension services are being severely curtailed.

Other factors essential for the successful application of biotechnology tools are training and capacity building. Many biotechnology applications require learning new skills, some research infrastructure and effective operational capacity. It is especially important to train and nurture national scientists capable of using emerging technologies. In general, it may not be possible for older plant scientists to acquire the capacity for biotechnology applications. Therefore, policy-makers in developing countries have to consider long-term investments in training and nurturing a new generation of scientific talent. They also need to consider how to utilize this talent effectively by providing adequate resources and optimum work environments. Specialized technical training must in turn be underpinned by complementary government investments in basic education, e.g. by including biotechnology-related subjects in national university curricula.

Although it is widely assumed that enormous investments are needed to establish a capacity to carry out MAS, this is not always true. Certainly, a minimum level of investment is needed for laboratory facilities, equipment and trained staff. However, considering that most MAS work in developing countries is likely to be geared towards the use of existing markers rather than the development of new ones, investments in facilities and capital need not be

large. Developing countries are likely to have difficulty obtaining the required laboratory materials including consumables that are manufactured mostly in the industrialized world. Other factors such as local support for servicing and maintaining laboratory equipment and reliable basic services such as an uninterrupted power supply can also be challenging. In the less advanced developing countries, international research organizations and development assistance agencies will have a more significant role to play in ensuring the availability of the technology as well as the capacity to use it effectively, though on a limited scale.

Many developing countries are likely to use genetically modified cultivars with value added traits in the near future. Associated with transgenic technology are the complex, yet important, issues of biosafety and management of intellectual property. Policy-makers should therefore also consider ways of increasing the efficiency of publicly-funded research efforts, as well as finding opportunities and providing incentives for formulating productive public–private sector partnerships. As most tools of biotechnology that have potential practical applications are developed and patented by private industry, policy-makers have the challenges of addressing the need to forge research partnerships that allow the competitive private sector to maintain its interest in financial rewards while permitting technologies to be used by public sector researchers in relevant areas to serve farmers in species of importance that have so far been neglected. Coupled with these partnerships is the requirement to manage intellectual property issues.

In many situations, international development agencies are able to play a role in areas such as biotechnology priority-setting, raising funds for establishing the required biotechnology infrastructure and maintenance capacity, supporting public–private sector partnerships, and assisting in technology transfer and capacity building. International agricultural research institutes, which have had long-term involvement with national programmes in a large number of developing countries, should play a role in identifying key areas for contributing further in helping relevant national programmes identify, optimize and adopt MAS tools when it is feasible. International research centres can also play an active role in capacity building by identifying areas where it is needed and by providing necessary backstopping.

Novel marker systems based on SNP platforms are likely to bring the costs associated with MAS applications to an affordable level by many breeding programmes and it will be challenging to establish these technologies based on robotics and other automated, large-scale, screening platforms in many developing countries as the technology development and associated intellectual property rights remain in large private sector enterprises. This is an area where developing country policy-makers, together with international aid bodies and research organizations, should ideally work together to find partnerships with the private sector to devise ways of infusing these technological breakthroughs and associated benefits to the developing countries, at least on a limited scale.

In conclusion, MAS technologies have matured to the extent that they can be used for making genetic gains in certain traits and in some important crop species. National programmes in developing countries should evaluate the feasibility of applying MAS approaches for crop improvement as, despite the considerable limitations that exist in many developing

countries, the technology can be used at a relatively low operational cost. At least for major crops such as rice, maize, wheat and soybean, significant numbers of linked markers have been identified for genes of interest, and ongoing selection programmes have found them to be useful for making rapid genetic gains. Incorporating these tools into active breeding strategies will allow more rapid and efficient improvement of varieties for target traits.

As national programmes in developing countries vary in their capacities to absorb biotechnology tools, priority-setting and identification of MAS strategies should be done on a case-by-case basis, ideally supported by strong breeding programmes. Individual national programmes will have to be selective in their choice of technologies and markers to ensure that the level of investment is appropriate to justify the costs and produce the most rapid returns. This means that, while fully functioning biotechnology laboratories may not be feasible in all countries, initiating MAS is an important first step towards using modern biotechnology approaches in plant improvement. As the success of biotechnology applications depends on the existence of strong crop improvement programmes, policy-makers and international development agencies must ensure that the limited funds allocated to traditional agricultural research are not curtailed to support biotechnology activities. International aid agencies and agricultural research institutes should play a role in building research capacity within national programmes, encouraging public–private sector partnerships, and promoting technology transfer.

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CHAPTER 20

Impacts of intellectual property rights on marker-assisted selection research and application for agriculture in developing countries

Victoria Henson-Apollonio

SUMMARY

Although the impact of marker-assisted selection (MAS) in commercial and public sector breeding programmes in developing countries is to date limited to a few crops and traits, the potential benefits of using markers linked to genes of interest in breeding programmes for improving the productivity of crops, livestock, forest trees and farmed fish is substantial. While more recent methods associated with the use of MAS are technically demanding and often expensive, most applications of basic MAS were initially described in the literature, and thus will likely have very few intellectual property (IP) restrictions associated with their use, irrespective of the agricultural sector involved. For example, isolating DNA, amplifying specific gene sequences from that DNA (with most available primers), separating fragments using gel/polyacrylamide electrophoresis and imaging of fragments with standard techniques are likely to be available without restriction to scientists and breeders in the developing world, even as part of a commercial service. Problems arise when there is a need to use or develop high-throughput modes, which require more sophisticated technologies. For high-throughput use, a breeder will want to use the most efficient techniques that are currently available. This means that the more advanced processes/methods, reagents, software applications/simulations and equipment, which provide the most effective means to exploit MAS fully, are most likely covered by intellectual property rights (IPRs) such as patent rights, confidential information (trade secrets) and copyrights, both in industrialized countries and also in many developing countries such as Brazil, China and India. In situations where breeders wish to use cutting edge technologies and the most efficient markers, care must be taken to avoid activities that may infringe IPRs when using MAS methodologies.

INTRODUCTION

Other chapters in this book describe the usefulness and applicability of MAS for developing germplasm with superior qualities, in a timely manner. Markers have been developed and used by plant and animal breeders (Dekkers, 2004), for fish and shellfish (Consuegra and Johnston, 2006) and for forest trees (Kellison, McCord and Gartland, 2004; Lee, A'Hara and Cottrell, 2005). Introduction of MAS to developing country scientists has been taken up by a variety of projects such as the Generation Challenge Programme (cgiar.org/exco/exco8/exco8_generation_report), supported by the Consultative Group on International Agricultural Research (CGIAR) and MAS jamborees sponsored by the Syngenta Foundation for Sustainable Development (syngentafoundation.org/pdf/Report%20Nairobi%20meeting%20.pdf and T. St. Peter, personal communication). MAS is also being used by many of the centres belonging to the CGIAR, notable examples being the International Center for Tropical Agriculture (CIAT), the International Potato Centre (CIP), the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and the International Institute for Tropical Agriculture (IITA), as well as the International Maize and Wheat Improvement Centre (CIMMYT), in programmes such as the Asian Maize Biotechnology Network (AMBIONET), and the International Livestock Research Institute (ILRI) in the areas of livestock production and health through the Biosciences Facility for east and central Africa (BecA).

In this chapter, a brief review of general intellectual property law is used to introduce a variety of aspects regarding intellectual property potentially associated with the use of the techniques, reagents and equipment that are necessary for implementing MAS. This intellectual property "primer" is followed by a description of specific cases and some recommendations regarding steps that should be taken by scientists and breeders in developing countries who are contemplating using MAS in breeding programmes to avoid restrictions or incurring risks of infringing the intellectual property rights (IPRs) of others.

INTELLECTUAL PROPERTY RIGHTS AND PUBLIC ACCESS TO INNOVATION

IPRs are awarded on the basis of national laws. There are, however, a few examples of regional cooperation institutions granting IPRs on a regional basis, such as the African Regional Intellectual Property Organization (ARIPO), the Gulf Cooperation Council (GCC) and the European Patent Office. In addition, under the Patent Cooperation Treaty (PCT), an international agreement administered by the World Intellectual Property Organization (WIPO) that facilitates patent filing, a single international application can be filed in a national PCT-receiving office, which can then subsequently be submitted to all PCT member national patent offices.[1] In addition, an example situation is given in Box 1 that illustrates the, perhaps unexpected, "far reach" of national patent law.

IPRs comprise original and novel assets that involve the use of human intellect. The awarding of such rights is intended to balance the needs of society to access and use the products of human ingenuity, with rewards for the endeavours going to the individuals from whom these intellectual assets originated. Obviously, there is a certain amount of tension in this

[1] See www.wipo.int/pct/en/texts/pdf/pct_paris_wto.pdf for a list of countries that are members of important IP international treaties, including the PCT.

balance between private rights and the needs of society (Murchie *et al*., 2006). Society is presumed to benefit from public disclosure in the form of patent disclosure requirements and copyrights, which are awarded to creative works that have been fixed (made tangible).[2] In addition, through the combination of the requirement of full disclosure in the written description (manifested in a patent application), and the time limitation over patents rights, inventions are put into the public domain when the rights expire. The pharmaceutical industry's experience with the success of "generics" is a testament to the value of "expired" inventions (CBO, 1998). In some specific cases, however, such as patents on certain drugs, rights may be extended for a certain period of time upon request to compensate for long delays in obtaining authorization for drug commercialization, e.g. the "Hatch-Waxman" act in the United States of America. The filing, prosecuting and maintenance of patents are business decisions that are put into place as a part of the strategy for bringing products to the consumer. An additional part of such a strategy can also include a plan for maintaining profitability when patent rights expire (Smyth, 2006). For example, depending upon the creativity of inventors, it may be that improvements allow for the filing of additional patents to cover these improvements, thus having the effect of extending patent rights for additional terms. This is a fact-based process, in that the improvement must meet the requirements of invention.[3]

The balance between public and private rights is considered by some to be tilted in favour of private rights, leaving elements of some societies wondering if IPR systems work at all except to protect the monopolies that they award (Epp, 2004). A number of civil society organizations are monitoring the potential effect of changing India's patent law to include patents over pharmaceutical products and agricultural chemicals (Sreedharan, 2007).

INTELLECTUAL PROPERTY LAW AS IT RELATES TO MAS

The standard steps employed in MAS generally include: selecting individuals to be tested; harvesting material; extraction of DNA from the material; polymerase chain reaction (PCR) amplification of the DNA to enrich for gene sequences/fragments associated with a particular trait or phenotype; separation of these fragments; visualization/identification of DNA fragments; and interpretation and utilization of the information. Each of these stages involves certain methods and the use of particular reagents and/or equipment associated with the particular methodological steps. For the purposes of this chapter, a series of tables (numbers 1–3) has been prepared to exemplify the types of intellectual property and associated IPRs that exist for materials and/or processes within each of these seven steps.

There is a general set of categories of IPRs that are awarded in most countries/jurisdictions. These include industrial or utility patent rights, plant variety protection/plant breeders' rights, copyrights, rights of appellation/geographic indications, trademarks and secrecy rights (trade secrets) associated with undisclosed or confidential information. Other types of patent rights can be awarded in many jurisdictions.

[2] Not that efficient and sincere disclosure is not without problems – see Fromer (2007).

[3] Note that concerns regarding the abuse of the patenting of incremental changes versus incremental improvements are often raised by a practice of patent time extension called "evergreening". For further discussion of evergreening from the view of generic pharmaceutical manufacturers, see Hore (2004).

For example, in addition to utility patents, two others types of categories of patents are available to inventors in the United States: a design patent for a new original or ornamental design for an article of manufacture, granted to protect the external appearance rather then the function of a product, and plant patents, awarded for the invention or discovery of a cultivated plant variety that can be asexually reproduced, (except via tubers, but including grafts and spores). Other countries have additional categories regarding subject matter (e.g. designs, plants) and also with respect to examination rigour and length of the patent rights grant (e.g. "short-term" patents in Belgium and the Netherlands (see e.g. www.ipr-helpdesk.org/docs/docs.EN/invencionesTecnicasBP.pdf), and innovation patents in Australia (www.ipaustralia.gov.au/patents/what_innovation.shtml).

Patent rights are awarded to inventions on the basis of criteria associated with usefulness (industrial applicability), originality (newness or novelty), and an "inventive step" (non-obviousness to persons with technical skills in the particular field where the invention is applicable). There are also rules governing the subject matter of the invention for utility patent rights to be awarded. For example, all countries' patent rights prohibit the awarding of patent rights for elucidating the "laws of nature". Thus, the fact that scientists have described laws of chemistry and physics, natural selection, or other such natural laws, does not render them as products of a person's intellect in intellectual property law. However, an innovation that applies one of these laws may well qualify for protection. Similarly, in many countries a new plant variety, a variety, type or breed of livestock used for food production, or computer software cannot be the subject of patent rights. Japan and the United States are notable exceptions in this regard. While the European Union (EU) (Directive 98/44/EC of the European Parliament and of the Council, 1998 on the legal protection of biotechnological inventions) does not permit the patenting of plant and animal varieties, it does allow patents for inventions concerning animals or plants the feasibility of which is "not confined to a particular plant or animal variety". The fact that the term "variety" is not well defined in the context of animal breeding means that the scope of this exemption is far from clear.

Irrespective of whether one is dealing with patent rights, plant breeders' rights (PBRs), copyrights, trademarks, trade secrets, etc., the type of IPR sought or awarded varies with the type of intellectual asset over which protection is being sought. It is also possible for one asset to be protected by several types of rights, depending upon the law in the applicable territory. For example, it is not unusual to have "double protection", i.e. for an invention to be patented and the product resulting from that invention to be trademarked. The trademark for Aspirin® for the formerly patent-protected acetylsalicylic acid is such a case in many parts of the world. It is not uncommon for a process or a piece of machinery to be treated in a similar fashion. This situation pertains to IPRs associated with MAS, two notable examples being "Selective restriction fragment amplification: a general method for DNA fingerprinting", a patented process paired with rights associated with the AFLP® trademark or the "Methods for genotyping by hybridization analysis" patent and the associated DArT™ trademark.

PATENTS

Patent rights are awarded on the basis of claims based on the inventor's description

to explain the new, non-obvious patentable subject matter in a way that clearly distinguishes its novel characteristics from all other available solutions. This explanation is called a patent "claim", and using the words of the patent drafter a claim will describe the "metes and bounds" (Gallagher, 2002) of the invention. Patent drafters are usually licensed patent agents, patent attorneys, scientists working for legal firms in this capacity or, rarely, the inventors themselves. Drafting patent claims is an arcane art that requires detailed knowledge of the scientific and technical basis of the invention as well as a current understanding of the state-of-the-art, regarding the judicial interpretation of claims, in the context of national patent law.

One patent can have many claims. In fact, patent law requires that every patent must contain at least one claim. Each claim is "directed to" an invention, ranging from its broad use, to the most narrow use for which an inventor may wish to seek rights. For example, a broad claim could be for the use of an enzyme class to perform a type of function (where this combination is not found in nature). A narrow claim could then specify the particular enzyme, the quantity of enzyme and/or the specific function. A distinction should be made between a patent application (often numbered in a different style such as the "WO" designation for PCT-filed patent applications), and an issued patent (generally numbered with a country prefix, e.g. CA 2172863, a patent issued by the Canadian Patent Office) to avoid confusion.

Patent applications contain claims that are untested and unexamined and these claims are therefore often very broad. During the patent prosecution process, the patent examiner seeks to limit claims to the new invention held by the applicant at the time the patent was filed. The claims are accompanied by written descriptions that would allow someone else familiar with technology in the same general area ("person having ordinary skill in-the-art" or "PHOSITA"), to understand how to make and carry out or "work" the claimed innovation. This useful written description accompanying claims is directed by law to provide "enablement", and is a required part of a patent disclosure, in order to make the invention "available to the public" (this is part of the social contract to balance private rights and public good). The written descriptions can also be important for interpreting the exact limits of patent claims. Patent rights are given to inventions that cover the reduction of ideas and concepts to practical use, and these rights may also extend to other treatments/variations that are of a nature sufficiently similar to be equivalent to the patented innovation. Such a "doctrine of equivalents", as it is called in patent lingo, means that ideas/concepts that are the basis of the useful innovation are a part of the patent claim coverage. Therefore, it is often stated that patents cover conceptual ideas as well as the practical application of the idea (see www.dwalkerlaw.com/patent.asp). This means that it is often difficult to discern whether a party is committing infringement without the interpretation of a court. Literal infringement, whereby the invention is practised exactly as it is described in a claim, can usually be identified without a problem. Equivalent infringement is often used as a strategic business tool by either the patent rights holder and/or the infringer. This confusion over the exact limits of patent claims can often lead to company mergers or buy-outs, just to minimize the risk associated with the IPRs (Fulton and Giannakas, 2001; Kattan, 2002).

BOX 1

Territoriality of patent rights

Developing country scientists and breeders should be aware that patent rights are only enforceable within the jurisdiction of the country or countries where the patent rights have been awarded. The caveat to this is that patent laws in most countries cover material that is imported into a country when patent rights exist on that material in the country where the importation would take place. The language that is included in such patent laws contains the terms: "making", "selling" or "using" within a country's boundaries. For example, if patent rights over the formula for a particular herbicide had been awarded in Country AA, but no patent rights over this same herbicide composition had been awarded in Country BB, then the herbicide could be made in Country AA only with the permission of the patent rights holder. However, the herbicide could be made in Country BB without permission of the rights holder in Country AA; no infringement would be possible in Country BB. If someone wanted to import the herbicide that was made in Country BB into Country AA, then the importer in Country AA would need to obtain permission (a licence) from the rights holder in Country AA.

The situation for Argentinian soybean containing a transgene covered by patent rights issued to Monsanto in Europe is a good illustration of the territoriality of patent rights. Monsanto holds plant breeders' rights over the variety, but does not have patent protection for the gene in Argentina. Many farmers in Argentina are growing herbicide resistant soybeans developed by Monsanto, (often using seed multiplied by companies that do not have a licence from Monsanto). The company has taken the strategy of preventing the importation of Argentinian-grown soybeans or *products* made from Argentinian-grown soybean into any country where Monsanto has patent rights by informing potential buyers of Argentinian-grown soybeans that they will be infringing Monsanto's patent rights if they bring such material into a country such as the United States or an EU country, where Monsanto has patent rights over the technology embedded in the seed or over the seed itself (Balch, 2006), and therefore also present in the soybean imported grain. Monsanto's patent covers the final product, that is the gene, and extends its protection to the seed and the grain containing the gene sequence. The European Commission (EC), in fact, recognizes the right of Monsanto to prevent import of the soybean grain, but not the soybean flour, where the gene sequence can no longer be expressed.

What, however, is the relevance of such action to MAS, where there is no technology embedded in the seed, remaining in the seed itself? Patent law is usually interpreted to cover any material where a patented technology was used to produce a product, even though such a product does not literally contain the technology. This means that in most situations, if patent-protected techniques, methods, processes or products are used in a MAS scheme, the resulting products are covered by these patent rights. Of course, this type of infringement can be very difficult to prove and therefore is rarely the subject of a legal suit, but the risk is present and occasionally is enforced (AsiaLaw, 2004). However, for developing country farmers who are not going to be exporting a product to an industrialized country, in actuality, the risk of an infringement is minimal (Binnebaum *et al.,* 2003). Nevertheless, the situation of using a patented invention without permission of the patent rights holder is not straightforward and, if such a course involves public resources, it should only be embarked upon on the advice of an IP counsel or an IP lawyer.

TABLE 1
Examples of patents relevant to MAS

Technique		Selected patent examples[1]	Public domain equivalent	Status of selected patent example	Implications
Harvesting DNA	Use of silica particles	US 5 234 809	Many, e.g. Doyle and Doyle, 1987 and Saghai-Maroof *et al.* 1984	In effect in the United States; related patents in effect in: Austria, Australia, Canada, Denmark, Germany, EU, Greece, Japan, Republic of Korea, Netherlands, South Africa and Spain	Licence needed; often supplied with reagents, kits and/or equipment such as thermal cyclers
	Equipment "Matrix Mill"	US 6 063 616	Many, e.g. Edwards, Johnstone and Thompson, 1991. Combination with centrifuge tube	In effect in the United States	If specialized equipment is used, licence may be needed. Likelihood that coverage would extend to developing country areas
Amplification of specific DNA seqs	Reagents	US 4 683 195	None	Expired in all countries (therefore in public domain)	Advance or improvement likely will require licensing, many even in developing countries
	Primers/genes	Primers for identifying Soybean Sudden Death Resistance US 6 300 541	Many e.g., Röder *et al.*, 1998. *gwm493* in wheat	In effect in the United States	Sequence(s) to be used should be checked by a patent searcher such as Gene-IT.com if breeding product is valuable and would be grown for export
	Equipment	Applied Biosystems Thermal Cycler US 5 656 493	Other equipment is available; contentious legal issues associated with many	In effect is the United States and most other developed countries, and a few developing countries including Brazil, China, Republic of Korea, South Africa	
Identification of marker genes	Reagents	Agarose, no applicable patents found	Polyacrylamide	No patent rights on traditional gel/acrylamide media	
	Equipment	Charge-coupled device imaging apparatus US 5 672 881	Cameras	Many systems that are no longer under rights protection	
MAS methods, in general	Methods and compositions	Use of selective DNA fragment amplification products for hybridization-based genetic fingerprinting, MAS, and high-throughput screening. US 6 100 030 QTL mapping in plant breeding populations. US 6 399 855	Numerous	In effect in the United States	Likely defensive patents. Could be problematic with imports to the United States

[1] There will inevitably be innovative improvements or technological advancement associated with each of these methods and materials, many of which will have been awarded IPRs to the inventor and/or the inventor's company.

Examples of published patents where rights have been awarded in the area of MAS include the basic PCR amplification process patents in the United States, US Patent nos. 4 683 195, 4 683 202 and 4 965 188, originally issued to the Cetus Company and then assigned to Hoffman-Roche in 1992, on the use of DNA polymerase based on the Taq polymerase enzyme isolated from the organism *Thermus aquaticus.* As these amplification patents expired worldwide in March 2006, when only the basic techniques and reagents covered by these patents are used, one does not now have to be concerned with infringement of these patents anywhere. However, the equipment used to control the reaction conditions may also carry IPRs on its own and most PCR techniques currently used are patented as improvements to the basic technology. For example, Applied Biosystems' PCR and real-time instrument patents and other PCR-related patents such as US Patent no. 5 656 493, are still in effect. A licence to these instruments and other patents may be needed in the United States in order to use their thermal cyclers to carry out PCR, although this is normally granted as part of the purchase price of the equipment and reagent kits. Table 1 contains additional examples of selected patents that are associated with MAS.

Another strategy that should be pointed out is the concept of "defensive" patents. Patent rights may be awarded in most jurisdictions over processes (actions/processes), and machines, manufactures and compositions of matter (things). Enforcement of patent rights, e.g. bringing a lawsuit against a person or forcing a licensing situation when a person is practising your invention (infringing your rights) without permission is less equivocal when the infringement involves making, using, possessing or selling an object or composition. However, the detection of infringement of methods claims is often much less straightforward. A patent owner would need to have insight into or gain access to how something was made or formed by the other party (potential infringer), in order to know whether his/her patented process or method was being used. This means that it can be even more costly and time-consuming to pursue potential infringers of methods claims than lawsuits involving infringement of making, buying or selling a patent-protected material or composition. Thus, sometimes a company or institution will decide to file a patent application, seeking rights over a method where such a filing will simply represent an attempt to preclude a competitor from preventing the company from carrying out a method, without concerns of infringement. Such a method or process patent would likely never be enforced except in blatant infringement and is only sought to provide insurance for the filing organization to lower the risk that the organization will be sued by someone else. The distinction between a patent that is filed defensively and one that is filed to prevent someone from practising the claimed invention can be very subtle. A discussion of patenting strategies including defensive patents can be found at www.271patent.blogspot.com/2006/09/valuing-patents-and-patent-paradox-why.html. This is an area of patent law that is always in flux and enforcement can be very complicated and expensive.

COPYRIGHTS

These rights are awarded for creative innovations that are "fixed" in a printed, video, audiotape or other recorded form. Copyrights only cover the form of the fixation, and not the ideas or concepts

TABLE 2
Examples of copyrighted software relevant to MAS

Technique	Use	Selected software examples	Licensing conditions	Comments
Analysis of QTLs	For use primarily in analysing animal pedigree associations	Loki www.stat.washington.edu/thompson/Genepi/Loki.shtml	Very liberal, freeware-type of licence www.stat.washington.edu/thompson/Genepi/license.shtml	To be downloaded only if licence is accepted by user
	List of open source or freeware	www.stat.wisc.edu/~yandell/qtl/software/	Open source or as freeware	Source code provided.
Analysis of fragment patterns	For use with ABI electrophoresis equipment	Genotyper®	Usually licensed with ABI equipment purchase. (Applera Corporation). Additional individual personal copies cost ~ US$1 500. Stand-alone copy costs ~ US$5 000. Software manual is also licensed with software	Source code is not provided; explicit prohibition in licence
Creation of binary table of fragment patterns	For use with Genotyper®	PeakMatcher http://crop.scijournals.org/cgi/content/full/42/4/1361	Licensed under GNU-GPL v 2	Source code is provided
Analysis of fragment patterns	For use with electrophoresis with fluorescently labelled markers	Genographer http://hordeum.msu.montana.edu/genographer/	Licensed under GNU-GPL v 2	Source code is provided
Genotyping software for linkage mapping applications	For use with ABI electrophoresis equipment	GeneMapper®	Licensed by ABI (Applera corp.) with equipment. Manual is licensed with software. Manual has own independent copyright	Source code is not provided; explicit prohibition in licence
Simulation of biophysical processes in farming systems	Predictive software	ApSim	See, www.apsru.gov.au/apsru/Products/APSIM/Access%20and%20Pricing%20Policy.pdf Also an annual licence fee	Reduced licensing fee (on a case-by-case basis for NARS)
Simulation platform for quantitative analysis of genetic models	Predictive software	QuGene Original Reference:http://bioinformatics.oxfordjournals.org/cgi/reprint/14/7/632.pdf	Now only available under licence from University of Queensland/CSIRO	Reduced licensing fee (on a case-by-case basis for NARS)

associated with the innovation (as is the case with patents). Although articles written about MAS, drawings of breeding schemes and the like would be products for which copyrights are awarded, it would be quite rare for someone to be concerned about infringing copyright in carrying out MAS. However, most MAS as currently practised, especially at high-throughput levels, involves the use of computer software to analyse the often complex data that result from marker detection. While software applications can be patented in a few countries, most jurisdictions only allow software to be covered by copyrights. (In many jurisdictions, there is ongoing discussion regarding whether software code is an appropriate matter to be covered by copyrights. While in Europe, the EC Directive on the Protection of Computer Programs (91/250EEC) has clearly established that in the EU, computer programs are protected on the same basis as literary works, other countries have a more checkered history [Starkoff, 2001].) Such copyrights are used as the basis for "Open Source" licensing of software. Most software used in conjunction with MAS must be licensed before it can be utilized in MAS breeding schemes or analysis.

The ethical aspects of copyright should also be understood. For example, breeders need to be respectful and careful when giving talks or other presentations to ensure that the material they use is original, or that the owner of the copyright has given permission for its use. Just because there is no "©" sign on an article, drawing, slide, picture, etc. does not mean that the material has not been copyrighted. Copyright is attached to almost any fixation with immediate effect. There is no need for an author or creator (or employer of the creator), to apply for copyright in most countries because of the conditions set forward in the Berne Convention for the Protection of Literary and Artistic Works (1886), which requires its signatories to protect the copyright on works of authors from other signatory countries in the same way it protects the copyright of its own nationals. A main principle of the Berne Convention, and incorporated into the WTO's Agreement on Trade Related Aspects of Intellectual Property Rights (TRIPs), is the general principle of national treatment, "which requires each member state to accord to nationals of other member states the same level of copyright protection provided to its own citizens" (www.wipo.int/treaties/en/ip/berne/summary_berne.html). There are exceptions, e.g. publications that originate from the United States Federal Government cannot be covered by copyright, although sometimes copyright owners will register a copyrighted article with the government to take advantage of governmental assistance in infringement cases. Table 2 provides some examples of copyrighted materials that have relevance to the practice of MAS.

TRADEMARKS

These are registered marks given to an applicant as a result of a trademark application being made with a fee payment, and such an application withstanding a search by a trademark examiner for similar marks and use of marks (along with an opportunity for opposition to the awarding of the exclusive use by anyone in the public, based on use of the mark by someone else prior to the application to the trademark office). Trademark rights are different from patents, plant variety rights and copyrights, in that they are renewable, and thus, if national procedural rules are followed correctly, can likely last indefinitely. As

TABLE 3
Examples of trademarks relevant to MAS

Mark	Holder	Product	Use	Comments
AFLP®	KeyGene	Associated with the AFLP process/method and reagents	Creation of polymorphic markers based on difference in DNA sequence	Widely used system; developing country institutions often negotiate a low/no cost-licence on a case-by-case basis
DArT®	CAMBIA	Diversity array technology	Selection of markers based on variation from reference panels	Proprietary technology, often licensed under a BiOS licence
ABI®	Applera Corp.	Instruments such as capillary electrophoresis	Various electrophoresis equipment, sequencers, etc.	Widely used, associated with many patented technologies
Sybr®	Invitrogen	Fluorescent dyes	Visualization of DNA fragments	Widely used, patent on original dye in this series has expired in many jurisdictions
GeneChip®	Affymetrix	Microarray on glass substrate	Microarrays can be used for detection of nucleic acid sequences – DNA or RNA	Widely used methodology. Affymetrix one of the leaders in this field

mentioned earlier, "AFLP" is one example of a trademark. This means that in practice, when this method is referred to, the "®" symbol should accompany the term, i.e. the correct use of the term would be AFLP®. Another relevant example would be the Certified Angus Beef® protected by federal trademark law in the United States. In addition, the names of new markers, new varieties or types of crops, livestock, etc. would need to be checked by a professional trademark searcher if a breeder wished to be sure that no trademark infringement might occur by such naming. This is not precluding the fact that PBRs legislation requires the breeder to give its candidate variety a denomination that cannot be registered as a trademark, as it remains the generic denomination of the variety. Table 3 contains examples of trademarks that are often used as "brand" names, associated with products/processes used in MAS technologies. Commercial MAS practitioners need to be aware that use of a trademarked name in conjunction with a product requires the permission of the trademark holder.

TRADE SECRETS AND CONFIDENTIAL INFORMATION

These are not registered and, although considered to be non-statutory IPRs, they are protected by trade secret law in most countries. Crop breeders have used this approach for many years to protect the parent lines and information used to produce hybrid seeds for sale, and similar approaches are adopted in the poultry and pig industries. This type of IP is defined as commercially useful information that can be said to have the qualities of being any method, technique, process, formula, programme, design or other information that may be used in the course of production, sales or operations. It must also meet requirements such as not being known to persons generally involved in information of this type; having an actual or potential economic value due to its secretive and useful nature; and the owner has taken reasonable measures to maintain its secrecy. Infringement or non-authorized disclosure/use or misappropriation of a trade secret can result in criminal penalties. These rights might be of concern to scientists and breeders who are

working under conditions that require the use of confidentiality agreements or non-disclosure agreements (NDAs). Examples include MAS work being carried out by an employee of a company that requires employees to sign confidentiality agreements, or MAS carried out as part of joint work where breeders have been required to sign confidentiality agreements.

This is a very common type of protection used by commercial breeding companies involved in the development and use of markers and software in all sectors of agriculture. If a company becomes concerned that a trade secret risks being exposed, it may file a defensive patent application to ensure that a competitor will not obtain rights that would preclude use of its own trade secret. Obviously, when a patent application is filed on an invention that includes confidential information, the information will no longer be a trade secret. The applicant presumably would only resort to such a move if the possibility of "independent invention" were high, and thus the risk of disclosure in a patent application balances the risk of having the competition "know" of your trade secret. This will happen because of the way in which patent examiners normally decide if an invention is "new". Often such decisions are based upon the national IP law's definition of "new", as in the United States where there is a grace period of one year to file a patent application after an invention is made public and also where only use within the United States is considered to render an invention "not" new. A patent examiner cannot know that an invention has been used or described prior to the filing of a patent application if the invention is kept as confidential information. Therefore patent rights could be awarded to someone who actually copies a trade secret and companies must then consider filing for a patent or run the risk that a secret will be the subject of a competitor's patent.

Why would a company not simply file a patent application for each marker that it identifies? There are several strategic reasons. It is expensive to file for patent protection and, also, the applicant must disclose the invention and all of the specifics of the invention to satisfy the written description requirement of enablement. For a marker, this means that the applicant would need to disclose its nucleic acid sequence if it is known and, by wanting the rights over the use of the marker in MAS, also the trait(s) that is(are) associated with the presence (or absence) of detection of the marker, etc.

Obviously, it is impossible to list specific trade secrets that exist in MAS technology, although one indication of the existence of these can be a reference to a "personal communication" as, for example, in the case of the "15PICmarq" marker listed in Table 1 of the paper by Dekkers (2004). However, there are examples of information that is of the opposite nature, i.e. information that is publicly available and that can be used without permission because it is in the public domain such as information published by the United States Federal Government, or because no attempts are made to enforce rights. The company www.resgen.com, for example, sells kits comprising simple sequence repeat (SSR) primers mainly for use as MAS markers for many different species and based on sequences that have been published. These may therefore be covered by copyright, but these rights are not enforced.

CONTRACTUAL ARRANGEMENTS

An additional, "non-statutory" system of rights (Ricketson, 1984 as referenced

in Drahos, 2005), such as rights/requirements covered by conditions associated with a contract is often described as an IPR, although technically these types of rights or conditions are not the subject of IP law in most countries, but rather are a part of legal codes that deal with private rights. These requirements might be of concern to breeders working under conditions that require the use of contracts such as material transfer agreements (MTAs). Conditions that result from entering into agreements or contracts could carry a minimum level of awareness of the duties or responsibilities incurred by one agreeing to the terms. Other "non-statutory" rights could include contractual/legal terms, such as those included in a licence or a "Technology Use Agreement" (TUA). Enforcement and practice associated with contract law vary in all jurisdictions and can even vary at the local level. O'Connor (2006) has recently pointed out the degree to which MTAs are used to confer a licence to both patent rights and biological materials themselves. He refers to this arrangement as a "lease-licence" model wherein the IPRs and the physical property rights are "woven" together. Again, if the documents are read carefully, these conditions will not take anyone by surprise, in that they are a part of a contract or licence or other permission granted by an owner or provider of material. However, sometimes this permission may be agreed to in a manner that does not make a strong impression on a recipient. For example, the so-called "shrink-wrap" licence that accompanies software, or the "click-wrap" licence that covers software or other material downloaded from the Internet, may be too subtle for most people to be really aware that they have agreed to a licence. In agriculture, "bag-tag" or "seed wrap" licences exist that have the same sort of connotation (Kershen, 2004). Many courts have looked at the enforcement of these licensing/contract issues, with slightly varying results. The web site www.lex2k.org/shrinkwrap/shrinkwraprev.html describes individual cases and discusses these cases with regard to enforceability of "shrink-wrap" contracts in different jurisdictions and conditions.

EXAMPLES OF IPR PRACTICES ASSOCIATED WITH THE USE OF MAS AND RECOMMENDATIONS FOR SCIENTISTS AND BREEDERS

The type of formal IPRs most likely to cause a problem with the utilization of MAS are patent rights. Some examples of patents in this area are given in Table 1. Patents/patent applications are also listed in the paper by Concibido, Diers and Arelli (2004). Also, as mentioned in the preceding section, contractual arrangements/obligations may interfere with unfettered use of products and processes associated with MAS.

Patent rights have been awarded for most of the materials and methods that are involved in practising MAS within all fields of agricultural production. A careful researcher will choose methods and marker sequences that have been published and then carry out at least a cursory search of patent databases such as the European Patent Database (http://ep.espacenet.com) to make a first pass for determining the likelihood that the method and/or sequence(s) of choice are not covered by patent rights in the jurisdiction where they work. Depending upon the level of risk that one is willing to assume, for work that could result in a commercial product, more investigation is likely needed and perhaps the services of a patent information specialist (see www.piug.org/) or an IP lawyer will be required.

Most patents will be of concern primarily to those in developed countries, particularly the United States where many private companies have their base. For example, taking the company Pioneer, 209 US patents assigned to Pioneer are identified when the US Patent Database is searched for the terms "breeding" in the patent and "marker" in the claims. This is reduced to eight when the additional term "assisted" is searched in the claims of these 209. At the time of writing, Pioneer had 46 published US patent applications covering the "breeding"+"marker" category; reduced to one with the addition of "assisted". Monsanto, Bayer and Syngenta have utilized MAS practices for a number of years and accumulated patent portfolios and very likely many trade secrets in perfecting MAS techniques for their particular uses (Cahill and Schmidt, 2004). Monsanto announced in February 2007 that it would begin sharing its markers for soybean cyst nematode (SCN) resistance with academic and public institution researchers worldwide. According to the announcement, "Academic researchers and public institutions who request access will be given a royalty-free licence for using the rhg1 marker under a patent that was granted to Monsanto in December 2006 (US Patent no. 7 154 021)". It is of interest to note that the company, Genome and Agricultural Biotechnology, LLC, with five issued US patents and five US patent applications covering SCN inventions, has been sued for patent infringement in the use of SCN markers in conjunction with MAS (Genome and Agricultural Biotechnology had sought patent protection in order to establish "freedom-to-operate" testing services for material supplied by breeders who lack the facilities to perform MAS techniques for assessing the presence of particular disease-resistance alleles [www.siuc.edu/~psas/faculty/pubs/lightfoot_achv.htm]). As this situation indicates, persons wishing to establish their rights to use markers, by filing patent applications and even obtaining patent rights, need to understand that one cannot presume that an issued patent means that one then can practise the inventions, described in the claims, without concern that one may also be engaging in infringement of another patent or set of claims that have been allowed in other patents.

As of February 2007, a cursory search of the US Patent Database as an indicator of overall patenting activity related to MAS and plants revealed 372 issued patents and 112 published US patent applications. Of these 112 US patent applications, 79 were associated with plant breeding and 33 with animal MAS.

These numbers do not include most of the patents covering equipment, PCR and PCR-related technologies like AFLP®, such as US Patent no. 6 045994 that may be especially useful for generating markers. Also, analysis of the data indicates an increase in the number of applications submitted over the four years up to 2005, but most of these applications (58 percent) are for IPRs over specific plant varieties and sets of markers that allow identification of the germplasm variety. In recent years many patents have been granted that cover genes and markers associated with economically important traits in livestock species (Rothschild, Kim and Anderson, 2006; Barendse and Reverter-Gomez, 2007).

Potential commercialization of such inventions was predicted by Rothschild, Plastow and Newman (2004), as well as the associated development of inventions for methods covering breeding management

BOX 2

Representative claims that illustrate the breadth of patent claims over sequence information

US 6 235 972 , "Maize Rad23 genes and uses thereof" issued 22 May 2001

What is claimed is:

1. An isolated RAD23 polynucleotide comprising a member selected from the group consisting of:
   (a) a polynucleotide having at least 85 percent sequence identity to the polynucleotide of SEQ ID NO: 1; wherein the percent sequence identity is based on the entire region coding for SEQ ID NO: 2 and is calculated by the GAP algorithm under default parameters;
   (b) a polynucleotide encoding the polypeptide of SEQ ID NO: 2;
   (c) a polynucleotide encoding the polypeptide of SEO ID NO: 4;
   (d) a polynucleotide amplified from a Zea mays nucleic acid library using primers which selectively hybridize, under stringent hybridization conditions, to loci within the polynucleotide of SEQ ID NO: 1;
   (e) a polynucleotide which selectively hybridizes, under stringent hybridization conditions and a wash in 0.1.times.SSC at 60 degree C., to the polynucleotide of SEQ ID NO: 1;
   (f) the polynucleotide of SEQ ID NO: 1;
   (g) the polynucleotide of SEO ID NO: 3;
   (h) a polynucleotide which is complementary to a polynucleotide of (a), (b), (d), (e), or (f);
   (i) a polynucleotide which is complementary to a polynucleotide of (c) or (g); and
   (j) a polynucleotide comprising at least 75 contiguous nucleotides from a polynucleotide of (a), (b), (d), (e), (f), or (h); wherein the polynucleotides of parts (a), (d)-(e), (h)-(j) each encode monocot Rad23 polypeptides.

US 6 815 578 "Polynucleotide encoding MRE11 binding polypeptide and uses thereof" issued 9 November 2004

Claim 9. An isolated polynucleotide comprising of polynucleotide selected from the group consisting of:

(a) a polynucleotide encoding a polypeptide having at least 95 percent sequence identity over its entire length to SEQ ID NO: 2; as determined by the GAP program under default parameters, wherein the encoded polypeptide binds to a MRE11 polypeptide; and

(b) a polynucleotide which is fully complementary to the polynucleotide of (a).

and breeding–related computer applications (Schaeffer, 2002).

Previous search of patent literature

As mentioned earlier, and irrespective of the agricultural sector in which they are operating, breeders and scientists should adopt a habit of checking online patent databases such as the database of the European Patent Office and the US Patent Database (www.uspto.gov) for patents and patent applications that may cover information and/or innovations relevant to their area of breeding and research.

It can be quite difficult to search for sequences and combinations of SSRs that

might be covered in patents and patent applications. It is beyond the scope of the non-professional patent searcher to state definitively whether or not a particular sequence is covered by patent rights. Searching patents for specific DNA sequence coverage is not quite as easy as it may seem because of the peculiarity of the language used in drafting patent claims. A few example claims taken from two US Patents, numbers 6 235 972 and 6 815 578 are reproduced in Box 2 to illustrate the complexity of this type of claim language. However, there are companies, such as Gene-IT, that have developed software to search for all possible matches that might occur in any patent (available in electronic form), and where unlicensed use would be considered an infringement. A good patent drafter will attempt to cover as much ground as possible when writing a patent claim as the broader the claim, the larger its technical spread over the landscape of that particular area of science/technology. This results in claims to a sequence and its uses being written so that the inventor claims the sequence and any sequences that are closely similar. Just how broadly a claim is written is a matter of how much the patent drafter/prosecutor can get a patent examiner to accept. Without the assistance of sophisticated computer software, it can be difficult to determine whether the use of a particular genetic sequence would infringe existing patents. Fortunately, however, biotechnology patents are now examined by biologists and molecular geneticists, instead of, as in the "early days", by chemists.

Copyright aspects

Others have thought that copyrights would be of little concern to the breeder or scientist interested in using MAS, in that copyright infringement might only occur if a material such as text, a design, photograph or video was copied and re-used without permission, such as in a publication or video that was to be distributed widely or sold. However, most results of marker testing need to be analysed by a computer program for the breeder to obtain maximum value from such testing. Most software is covered by (at least) copyrights and therefore must be licensed from the rights holder. Even software that is distributed under an "Open Source" type of licence is indeed licensed, and the conditions of the licence must be adhered to when the product is used and/or improved.

In addition, care should be taken by persons creating training materials that will be distributed widely or sold as part of a workshop to either refrain from using materials written and created by others or to obtain permission before use, especially if such use might be part of a course where participants pay for instruction or must buy the training materials, or where materials might be distributed in an electronic format.

Trademarks aspects

In general, the same is true for trademarks as for copyright. A minor point would be to remind authors that terms such as AFLP® and "Breeding by Design™", both trademarks of Keygene, Inc., should carry the "®" or "™" designation. In this regard, breeders would be primarily concerned with the correct use of their own trademarks, both by themselves and others. When naming varieties, etc., care should be taken to ensure that the trademark of another entity is not being infringed. Those responsible for creating names should therefore check public trademark databases such as the UK Trademarks Database (www.patent.gov.uk/tm/dbase/),

and the services of a professional trademark searcher or attorney should be sought before proceeding with the registration of a "new" trademark.

Plant breeders' rights aspects

Breeders using basic MAS protocols with non-proprietary breeding materials (e.g. germplasm that does not qualify as an "Essentially Derived Variety" [Wendt and Izquierdo, 2001]) generally do not need to be concerned with using materials covered by PBRs for breeding purposes.

Contractual aspects

It is very important that licences, contracts and agreements are monitored for restrictions as these often contain provisions dealing with IPRs that last until a contract expires or is renegotiated. Permission to use equipment and associated reagents is normally granted as a licence granted as a part of the purchase price. However, this type of licence may often contain limitations on the use of equipment, reagents and kits for non-research applications. As an example, and as stated in its legal information Web page, Applied Biosystems has an exclusive licence with Roche/Hoffman-La Roche for its PCR patents: "Applied Biosystems is the exclusive licencee of Roche Molecular Systems, Inc., and F. Hoffmann-La Roche, owner of the basic PCR process and reagent patents, for the field of research and development, and for applied fields such as quality assurance and control, environmental testing, food testing, agricultural testing (including plant disease diagnostics), forensics and identity testing in humans (other than parentage testing), and animal identity and breeding applications." This means that when a researcher buys (or has legal access to) and uses an Applied Biosystems machine, the rights to use this machine for certain specified purposes (rarely commercial) are included in the purchase agreement. Note, however, that the use of kits or other products of Applied Biosystems that involve any processes or reagents licensed from Roche/Hoffman-La Roche to carry out MAS is not specifically mentioned as a "field of use" in the terms of this licence. While it could be assumed that use for MAS is possible under the Applied Biosystems licence, if it was considered necessary to have the lowest probable level of risk associated with the use of equipment/reagents for MAS, then legal advice in the jurisdiction of the user should be sought.

An equipment or reagent licence could also contain provisions for what are called "reach-through" rights. These arise when improvements are made to an existing technology. When such innovations come about through use of the existing technology the rights to them may have to go back to the owner of the original existing technology. Such a transfer of sharing of the rights is called "reach-through rights". Some argue, for example, that the requirement in some Open-Source licences for improvements going back to the original creator of the software for distribution are a form of "reach-through".

Agreements to purchase and "package insert" licences should therefore be routinely checked to ensure that these sorts of licence are avoided.

MTAs can also cause problems, depending upon the conditions that are set down in such agreements. Laboratory personnel need to make sure that MTAs are only signed by persons authorized to do so and that efforts are made to check MTA language for provisions that restrict or interfere with the intended use of the germplasm that is produced using MTA-

associated materials. A practical explanation of MTAs is available in COGR (2003).

Breeders and scientists need to keep a file and/or database of all licences, package inserts, purchase agreements and MTAs as part of their routine record keeping. They also need to learn to reject documents that contain provisions that indicate an assertion of rights or include a restriction, to negotiate for terms that they require, or source replacement brands/materials. Contracts can be enforced long after patent rights expire.

Of all the types of IPRs/proprietary restrictions that could affect scientists and breeders in developing countries, licences and agreements have the most potential to impede the use of MAS technologies, unless a sophisticated, high-throughput laboratory is sought. MAS has considerable potential and relevance to developing country breeding systems for capturing desirable characteristics from widely disparate germplasm. IPRs should not hold this back.

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