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GENETIC ENHANCEMENT OF SELECTED SPECIES

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Plant breeding is based around the identification and utilization of genetic variation. The breeder makes decisions at several key points in the process. First in deciding on the most appropriate parents to use for the initial cross or crosses and then in the selection strategy used in identifying the most desirable individuals amongst the progeny of the cross. The efficiency of the breeding and selection process can be assessed in many different ways including the ultimate success of the varieties released and the frequency with which new varieties are produced. A major cost and logistical issue in plant breeding are the actual number of lines that need to be carried through the evaluation and selection phases of a program. Large breeding programs for annual crops may carry hundreds of thousands of lines to produce a new variety only once every few years. Field trials can be expensive and evaluation of some traits, such as quality and yield stability can be technically and genetically complex requiring large batches of material. Molecular markers have proved to be a powerful tool in replacing bioassays and there are now many examples available to show the efficacy of such markers.

The use of molecular markers to track loci and genome regions in crop plants is now applied in many breeding programs. The location of major loci is known for many disease resistance genes, tolerances to abiotic stresses and quality traits. Improvements in marker screening techniques have also been important in facilitating the tracking of genes. For markers to be effective they must be closely linked to the target locus and be able to detect polymorphisms in material likely to be used in a breeding program. The prime applications of markers in most breeding programs has been in backcross breeding where loci are tracked to eliminate specific genetic defects in elite germplasm, for the introgression of recessive traits and in the selection of lines with a genome make-up close to the recurrent parent. In progeny breeding, markers have proved valuable in building crucial parents and in enriching F1s from complex crosses. Markers have also improved the strategies for gene deployment and enhanced the

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understanding of the genetic control of complex traits such as components of quality and broad adaptation.

Status

Recent developments that have occurred in molecular markers for many crop species have major implications for the future of the technology. There are three key components that are particularly significant. First, for many species we now have markers closely linked to many traits of importance in the breeding programs. Indeed for major crop species we have markers for more loci than can be screened in a conventional breeding program. Second, we have tools that allow marker scanning of the whole genome. Of particular importance has been the development of microsatellite or SSR markers that now form the base for analysis and allow highly multiplexed SSR screens. This trend will continue as newer, cheaper marker screening based on SNPs become available. The technological advances have improved our capacity for whole genome screens. Third, through association mapping projects we have or are in the process of developing, whole genome fingerprints for many key lines and varieties of importance in breeding programs. These studies are developing large databases of historic germplasm that should, over the next few years, start to reveal the ways in which breeding programs have selected for and against specific regions of the genome. We can see these developments particularly in crops such as maize and barley where markers for most of the major disease resistance clusters, for key components of feed or processing quality and for many loci conditioning tolerance to abiotic stresses are available.

The new marker systems have several important implications for the future of marker-assisted selection (MAS) and breeding strategies in general. Existing strategies for MAS were initiated with a view of markers as providing a rapid and cheap alternative to bioassays and they have largely been used in this role. While highly successful in the relatively small number of breeding programs that use markers, this strategy does not fully exploit the technology. There are two key limitations to the broad use of markers. The first is organisational or political and has resulted in the uneven application of molecular techniques with many programs in many species lagging well behind the more advanced programs. The second limitation is technological or conceptual and relates to an expansion of the scale and complexity of marker use due to the size of populations that would be required if one were to try and select for alleles at a large number of loci simultaneously. An important question here is how can we best take advantage of the genome information that has been generated for major crop species. We know, for example that chromosome 2H in barley and group 7 chromosomes of wheat, carry clusters of genes, often in repulsion, that we would like to break up. Again conventional use of markers has not been very effective in utilising such genome regions. Conversely, we know that there are some chromosomes where there is little allelic variation between lines and it is waste of effort to try and break these up in a breeding program.

The major challenge of new work is to investigate strategies for whole genome breeding: how we can use genome-wide information to design optimal breeding strategies that integrate as much of the available information as possible.

Limitations to adoption of MAS

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Extensive marker data has been generated for many crop species but detailed information is only available for a small number of species. Wheat and barley provide a good example of both the extent of marker development and the highly uneven application of the technology across breeding programs. Although the identification of marker trait associations is now rapid for simply inherited traits and for many QTL, adoption rates within breeding programs has been slow. In surveys of public sector breeders the key issues identified are:

- Lack of useful marker trait associations. Many breeders feel that the loci targeted for marker development have been selected by molecular biologists rather than the breeders.
- Poor polymorphism rates in "real" breeding material. Many marker development programs have been based around highly diverse germplasm, selected for ease of detecting polymorphism rather than value to breeding programs.
- Closeness of linkage. The closer the linkage the greater the reliability of the markers. Not surprisingly breeders would like diagnostic markers.
- Lack of resources for marker screening. Breeders are reluctant to redirect their current resources to marker screening and in many cases such redirection is not feasible. The most successful implementation programs have been funded separately from the core breeding programs. This applies to both public and private sector breeding operations.
- The long lead time for breeding means high risks to a breeding program if it modifies its breeding strategies. The real value of markers appears to lie in the development of new breeding strategies. The crosses, population size and screening methods using in traditional breeding and marker based breeding programs are not necessarily compatible. The resources need to make a change to a new system can be expensive and will lead to a significant hiatus in variety development.

In many organisations, such as large commercial breeding programs, marker screening is centralized. "Marker Labs" in such organisations have four roles;

1. Technology development. Identification of new marker trait associations and development of new marker systems
2. Resource development. Establishment of genotype databases, graphical genotypes and related resources.
3. Problem solving. For example, analysis of genetic background, retrospective analysis of breeding programs, studies of selective processes, tracking competitor programs.
4. Direct screening for the breeding programs. This would be as a replacement for bioassays or accelerated backcrossing.

The link between the marker labs and the breeders is crucial. The willingness of the breeders to embrace the technology is seen as a key factor in driving the success of marker application.

In the public sector, marker screening is being increasingly outsourced to large laboratories that offer cheap and high throughput screening. In medical research, nearly all marker screening is outsourced. For many breeders in the public sector, few resources and little time is available for developing and applying MAS. The training of the breeders and their access to marker labs has also been a major determinant of adoption.

Relevance of developments in cereals to other crops

Although the most detailed marker information is available for the major cereal crops, genetic linkage maps based around molecular markers are now available for most crop species. For some species, such as strong outbreeders, long generation perennials and clonally propagated species, it may be slow and complex to develop useful marker trait association. This is the case for most tree crops. However, even for these crops, many maps are available. Usually, the maps have been constructed from populations segregating for traits of relevance to improvement programs although the germplasm may not be the most recent. For example, molecular mapping studies in cassava have identified loci and associated markers for productivity, plant architecture, and resistance to cassava mosaic disease. Therefore, in many respects the technology is sufficiently well developed to be applicable to most crop improvement programs and the examples of using MAS in cereal breeding should have direct relevance to other breeding programs.

Although the breeding strategies may vary between crop species, there is sound reason to believe molecular markers could offer significant benefits to all breeding programs and could lead to increases in the speed of breeding and the sophistication of breeding programs. The examples we see in the major cereals demonstrates the gains that can be achieved but it is probable that the actual breeding strategies based around molecular information will be not only species specific but also program specific. Further, it is clear that broad application has not been achieved for most species and few farmers have access to germplasm that was developed through the use of molecular technologies. In this context it is important to remember that even in the cereals, marker adoption is far from universal. Indeed labs making extensive use of markers are in a minority even amongst well resourced breeding programs. Some of the major scientific and technical reasons have been identified above but additional political, management or social factors may also be important. These include access to facilities and resources, training in the use of the markers, time available to breeders to experiment with alternative strategies and priority within the crop improvement organisation.

Options for enhancing use of molecular technologies in national programs

The generally poor level of application of molecular technologies but their success in programs where they are used, provide support the concept that we should be looking to removing bottlenecks in the technology application. To see the technology used more extensively, in particular in national programs, there are a series of non-technical issues that need to be addressed. These include staff training, mechanisms for accessing the required infrastructure and a crop improvement environment that is receptive to new technologies.

The high demand for staff trained in these techniques has proved a major limitation in many countries. Job opportunities for skilled "molecular breeders" are extensive and many of these staff seek employment where they can maintain basic research activities rather than for technology application. Can we improve the opportunities for staff to remain active in molecular research while also encouraging them to work with breeders in applying the technologies? Research networks involving linkages between groups at the cutting edge of the technology development alongside researchers who are applying the technology may be a method for addressing this limitation. A key limitation in labs that have focused on the

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technology is now developing around the issue of accessing germplasm and phenotyping capabilities. Indeed in many labs this is the major limitation. National programs could offer these capabilities through the networks and collaborations. This mechanism would also help address the issue of technology and infrastructure access and would help ensure that germplasm used in the "research" based labs was relevant to the breeders and national objectives.

As demand for molecular screening work increases, we can expect to see commercial service providers proliferate. In the transition phase, centralized labs could be use to provide access. Large commercial breeding programs use in single centralized labs for almost all their molecular screening work even though the breeding programs are dispersed over the whole world. These commercial programs also use the concept of "technology champions" to stimulate technology adoption and reward those who focus their activities on technology delivery.

Coupled with these strategies, we should also consider revisiting the ways in which we train breeders and their staff in molecular technologies. Currently the main focus has been on the technologies rather than their application. This could be addressed by involving breeding staff from national programs in discussions focused on new breeding strategies and looking at ways in which they can modify their programs or their screening methods to take advantage of molecular technologies.

Access to expertise and technology is closely coupled to access to information and data on target species. The issue of information access has been growing as databases expand and the trait and genetic relationship between crop species becomes better understood. For example, cereal researchers make routine use of the rice genome sequence in their studies. Similarly, the soon to be completed sequences of Medicago and Lotus, will be a key resource of legume researchers. Bioinformatics platforms that offer ease of data access and analysis are becoming increasingly important but may exacerbate the distinction between the major groups of crop plants for which extensive data is available and the minor crops. However, the international trend towards the development of public databases and the sharing of the genetic and genomics information is encouraging. Current databases are focused on delivery molecular information and have often little relevance to plant breeders. The opportunity for groups to contribute phenotypic and diversity data will expand the utility of the databases.

Conclusions

Molecular markers are now well established as tools in plant breeding and genetics. They have also provided a major new impetus to plant breeding programs offering considerable improvements in the efficiency and sophistication of breeding. Their use as research tools is also well developed and they have played a key role in improving our understanding of genome organization, structure and behaviour for many of our major crops. However, the application of molecular markers in practical plant breeding has been patchy. Marker resources and capabilities for marker implementation are largely unavailable for minor crops. Even for some of the major crops, such as wheat and rice, markers are not widely used in public breeding programs with a few notable exceptions. Given their huge potential this slow acceptance and implementation is disappointing and is probably related to lack of flexibility by many public breeding programs to make the structural and strategic changes needed for effective marker implementation. It may also be partly due to the lack of active participation

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of breeders in the marker development programs in some countries. As the results of marker application become more apparent and move from theory to released varieties, this attitude may change.

The key developmental challenges for molecular markers now lies in developing new breeding strategies where the objectives will be increasing the germplasm base and increasing the number of traits that can be effectively selected simultaneously. The new marker technologies that offer greatly reduced costs in marker screening and high multiplexing capabilities will be central to these developments. Essentially we will move to whole genome based selection strategies where specific recombinational events are sought and changes will be assessed on a genome wide scale. In this way we can look to better manage chromosome regions that may come from wild relatives or land races, track several traits at once and keep the population sizes as small as possible.