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Research opportunities - molecular breeding perspectives

Bryan Whan

CRC for Molecular Plant Breeding, PMB 1, Glen Osmond, SA 5064

Background to CRC for Molecular Plant Breeding

The Cooperative Research Centre for Molecular Plant Breeding (CRCMPB) is a collaborative joint venture involving five core partners - University of Adelaide, Southern Cross University, South Australian Research and Development Institute (SARDI), Victorian Department of Natural Resources & Environment, and International Maize and Wheat Improvement Centre (CIMMYT). Eleven other research organisations, R&D Corporations and commercial companies are involved as Supporting Participants - Department of Agriculture WA, Murdoch University, University of WA, University of Queensland, University of Melbourne, Flinders University, GRDC, DRDC, SA Grains Industry Trust Fund, Advanta and ABB Grain Ltd.

The CRC participants have come together because their research capabilities are strengthened through the collaborative arrangements. The complementary expertise of joining with other participants adds value and produces synergies and more effective outcomes. The CRC provides access to an extensive national and international network that would not otherwise be available.

The overall aim of the CRC is to develop new technologies in molecular biology and implement effective strategies for their use in breeding programs for cereals and pasture grasses.

The CRC research and training programs

The four CRC research programs are based on the intended outcomes - markers and novel genes for cereal and pasture grass breeding.

- Molecular markers for cereal breeding
- Exploiting novel genes and technologies in cereal breeding
- Molecular markers for pasture grass breeding
- Exploiting novel genes and technologies in pasture grass breeding

The intended outputs for the **marker programs** in cereal and pasture grass breeding include genetic understanding, markers for disease resistance, quality and abiotic stresses, pathogen identification and quantification, germplasm and software for breeders. The outputs for the novel **gene programs** will include increased knowledge, novel genes, promoters, and transgenic cereals and pasture grasses.

The programs are designed to provide a continuous flow from basic research to implementation, actively involve breeders in implementation of the technology, and promote interactions between the cereal and pasture research.

The technologies developed by the Centre will be exploited and commercialised through Australian and international plant breeding programs, and this will ultimately benefit the cereal and pasture industries of Australia and developing nations.

There is also an important program on education and training in molecular plant breeding. The aim is to train new plant breeders and molecular biologists at undergraduate and post graduate levels, upgrade the skills of existing plant breeders, and improve community understanding of plant breeding and associated molecular technologies.

Research opportunities in molecular breeding

Research on molecular breeding will impact on future wheat breeding by providing the technologies and tools for breeders to utilise DNA markers in their selection strategies and to access new genetic diversity through transgenic barleys (Table 1). The importance of the basic research to provide a greater understanding of the genetic control of critical traits cannot be overemphasised, and this is a pre-requisite to the development of molecular tools. As well as leading to the development of markers, an understanding of the genetic controls can also help breeders design more effective, targeted breeding strategies.

The overall impact on breeding programs will be to change the focus from phenotypic breeding based on probabilities to genotypic breeding that will increase the certainty of successful outcomes. The vision of breeding programs in the future is one where markers will have a significant role in critical stages. This will include selection in early generations, managing linkage blocks, enrichment of F1s, monitoring of traits and recurrent genome in backcrossing, identification of parents and novel alleles, diversity analysis, and pure seed checks. However, it should be emphasised that traditional field and laboratory assessments will always be required for confirmation of phenotypes in late generations. To cater for changes associated with molecular techniques, the organisation and strategies used in breeding programs will be quite different. While utilising the advantages of molecular tools, breeders must also be aware of some limitations. Breeding strategies adopted should not be directed by the technology but rather the ability to maximize genetic gains, and breeders need to be confident that selection pressure of this nature will shift the populations in the desired direction. The impacts on the breeding programs will be

- a significant reduction in the time to develop a new variety,
- more effective and direct control of the alleles retained and eliminated,
- the possibility of improving traits that were not possible using traditional phenotypic screening,
- access to new genes that will provide greater diversity,
- the ability to manipulate the expression of existing genes,
- the opportunity to adopt more sophisticated, challenging breeding strategies.

A number of markers are already being utilised routinely, and some breeding programs are screening many thousands of lines each year in this way. Breeders have indicated that the use of markers will increase considerably as more robust markers for economically important traits become available. This is likely to be possible over the next couple of years as the results from the National Barley Molecular Marker Program come to fruition. A significant development in the next few years will be the use of markers to identify desirable and undesirable blocks of genes, or chromosome segments that have been conserved through generations of breeding and selection. Another use of markers will be in introgressing new genes from wild relatives and land races to improve new barley cultivars. Other new technologies that are likely to have a significant impact on marker utilisation in the future are Single Nucleotide Polymorphisms (SNPs), Diversity Array Technology (DARTs) and microarrays.

Opportunities exist to increase the use of markers in breeding programs by alleviating some major limitations. These include reducing the cost of DNA extraction and marker assay systems, developing systems to manage the large amount of molecular information, and developing the optimum breeding strategies for these technologies to be used most effectively.

Opportunities for improvement of barley through genetic engineering are limited only by the ideas for applications, however there are major challenges to overcome before transgenic barleys will become commonplace in breeding programs and the industry will see the benefits. Transformation of barley is now a relatively routine operation, although the efficiencies are significantly lower than for wheat. Different varieties vary in their ability to produce transgenic plants. In wheat, the CRCMPB effort at CIMMYT has developed new lines specifically for their ability to produce very high transformation efficiencies, and this has opened up new avenues for assessing new and novel genes.

Genes associated with disease resistance, quality and abiotic stresses have been isolated and cloned. Transgenic barleys incorporating these genes have been developed, and the effects of the genes demonstrated in the laboratory. Transgenic barleys are a reality from a technical point of view, but a number of challenges such as IP constraints and consumer acceptance need to be overcome before they will form a routine part of barley breeding programs. Breeders will also need to evaluate the role of transgenics in their breeding strategies. The barley industry is quite nervous about using barley varieties that have been improved using genetic engineering techniques due to the potential negative consumer attitude. While it is usually recognised that these perceptions are based on a lack of understanding and the potential risks are no different to conventionally bred barleys, the reality is that the market cannot risk a rejection by consumers. Education programs are gradually changing these attitudes, and recent surveys indicate that the public is less concerned about GMOs than other aspects such as pesticides. The public understanding and acceptance of GMOs is increasing. Developing a transgenic barley with clear benefits for the consumer will be significant in gaining acceptance of GMOs by the public.

The ultimate vision is to see breeders effectively managing molecular technologies through user-friendly computer software that will assist in short-term decisions on crossing and selection strategies and long-term strategic planning of breeding programs. This is one of the greatest opportunities to achieve the greatest impact and use of molecular technology in breeding programs. The CRC is developing software based on QU-GENE, and a module for predicting wheat quality based on glutenin alleles has been released recently. Similar modules will be developed for barley when appropriate data are available. One of the main limitations in managing the vast amount of molecular information is the lack of a user-friendly database that integrates all the phenotypic, genotypic and pedigree information.

Future breeders will require the skills to fully exploit molecular technologies in conjunction with traditional methods. Training future plant breeders is a high priority of the CRCMPB, but a prerequisite is to have access to high quality students interested in agriculture and plant breeding. The CRC therefore is involved in a number of initiatives to generate interest from potential students and encourage them into our discipline.

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Table 1: Opportunities for molecular breeding in the next decade

Opportunity	Objective	Progress	Limitations
Genetic understanding	• Genetic understanding of the control of target traits for improved quality, resistance to diseases and tolerance to abiotic stresses.	• Maps developed of critical populations. • Genetic control of many traits clarified.	• Full maps are expensive and time consuming
Marker Assisted Selection	• DNA markers for improved quality, disease resistance and tolerance to abiotic stresses developed, validated and used. • Molecular probes for identifying specific pathogens and potential disease problems. • Markers to identify desirable and undesirable blocks of genes or conserved chromosome segments identified and used. • User-friendly marker technology , e.g. SSRs, for use in Australia's cereal and pasture breeding programs. • Breeding strategies for using marker assisted selection most effectively. • Extensive and efficient use of molecular markers in Australia's cereal breeding programs.	• Markers available and used for resistances to scald, spot form of net blotch, net form of net blotch, CCN, powdery mildew, BYDV, rusts, Russian wheat aphid, Al toxicity, boron tolerance, beta-amylase, diastatic power, malt extract, Mn efficiency, Zn efficiency. • Markers for numerous traits developed and undergoing validation. • DNA probes to distinguish the net and spot forms of net blotch available.	• Lack of robust markers for critical traits. • Need for cheap, high throughput analysis systems. • Managing the large populations required. • Managing large amount of molecular data. • Identifying optimum breeding strategies for most effective use of molecular information. • Achieving relevant balance between central and

			regional involvement.
Transgenic barleys	• Increased knowledge on the genes and controlling mechanisms for a range of important genes in cereals. • Isolation and cloning of genes associated with disease resistance, quality, nutrient transfer, and abiotic stresses in cereals such as wheat and barley. • Routine regeneration and genetic transformation protocols for producing barley transgenic plants. • Transgenic barleys incorporating novel genes for quality, resistance to diseases and increased efficiency of nutrient utilisation.	• Routine transformation protocol developed for barley resulting in very high transformation efficiencies. • Genes associated with disease resistance, quality and abiotic stresses isolated and cloned. • Transgenic barleys incorporating genes for quality and disease resistance developed and effects demonstrated in the laboratory.	• Consumer perceptions and market attitudes against GMOs. • IP and Freedom-to-Operate positions. • Risk assessment needs to be undertaken. • Final field assessments and food safety analyses can be difficult and costly. • Breeder acceptance and understanding of role of transgenics.
Software	• Breeder-friendly computer software package for plant breeders to assist in short term decision making on crossing and selection strategies and long term strategic planning of breeding programs to manage and utilise molecular data most effectively.	• Software to simulate breeding strategies and strategic planning developed - QU-GENE. • Software to graphically present genetic and marker information being assessed. • Software module to predict wheat quality based on glutenin alleles developed and released.	• Lack of a user-friendly, integrated database to manage all phenotypic, genotypic and pedigree information. • Accessing, curating and managing large amounts of data. • Large resources required to develop effective software.
Education	• Highly skilled breeders and researchers able to exploit the new technologies of molecular biology in conjunction	• An undergraduate course in plant breeding implemented at University of Adelaide, and aspects incorporated into courses at	• A general negative public perception on agriculture

	with traditional breeding methods. • Improved community understanding of plant breeding and associated molecular technologies.	other Universities. • 42 postgraduates training in molecular biology and breeding • Workshops and additional training in molecular breeding provided for existing breeders. • An extensive program of school and general community education undertaken.	and plant breeding. • Lack of high quality students interested in plant breeding. • Minority groups promoting misconceptions about GMOs.