FINAL REPORT

GRDC
GRAINS RESEARCH & DEVELOPMENT CORPORATION

DAQ00047

Australian Winter Cereals Molecular Marker Program - Component: Implementation/Validation of Molecular Markers EGA - DPI Node

PROJECT DETAILS

PROJECT CODE:	DAQ00047
PROJECT TITLE:	AUSTRALIAN WINTER CEREALS MOLECULAR MARKER PROGRAM - COMPONENT: IMPLEMENTATION/VALIDATION OF MOLECULAR MARKERS EGA - DPI NODE
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Summary

Molecular markers for traits of high priority to the northern grains region are now becoming available for validation.These traits include resistance to black point, crown rot (CR) and pre-harvest sprouting (PHS). Markers for each of these traits have been validated to some degree in genetic material from the Enterprise Grains Australia (EGA) breeding program. Markers have been implemented for rust resistance (VPM resistance gene complex, *Yr17, Lr37 and Sr38*), resistance to black point, and resistance to barley yellow dwarf virus (BYDV), in a total of about 2,700 tests. Extensive planning has taken place within EGA to prioritise traits for marker development, validation and implementation.

Report Disclaimer

Conclusions

General

- Molecular markers are a valuable tool for genetic selection in wheat.
- Integration of marker-assisted selection (MAS) with the selection program as a whole is the key to accelerated genetic gain in wheat improvement.
- Marker implementation will increase dramatically over the next few years as markers for traits of high priority to EGA are validated.
- Validation is a complex process involving establishment of gene identity by descent, of surveying germplasm for polymorphism and of marker trait association in both structured and breeding populations.
- Validation is an ongoing process, requiring constant review as germplasm or selection pressures change.
- Most traits of importance are quantitatively inherited, so markers will be applied as a population enrichment strategy. This means that more lines can be produced in early generations, through more crosses or more progeny, but after enrichment a manageable number can be phenotyped. As a result, for the same number of lines phenotyped, more will be carrying the desirable gene combinations sought. The time for the process for breeding and selection from cross to release is not directly reduced by marker application, but the quality of the resulting new varieties will be improved.Thus genetic gain will be accelerated and better varieties will be delivered sooner.

Specific

- Black point is a complex trait influenced by large genotype xenvironment (GxE) effects.This project has validated two quantitative trait loci (QTL) for resistance to black point derived from Sunco. Simple sequence repeat (SSR) markers were found for these QTL, and they are likely to be useful either individually or in combination, for population enrichment, depending on the genetic material.The influence of a third loci is likely to be too small to be of practical use.
- The major QTL on 4A for pre-harvest sprouting (PHS) was confirmed, and markers more closely flanking the loci found. Based on available information, however, closer markers are required before MAS will be applied to this locus.
- Markers for the 2-49 source of crown rot (CR) resistance have recently become available.Polymorphism testing suggests that alternative markers may be required for the 1D region in a Hartog-type background, but the flanking markers for the 1A region are likely to be widely useful. Extra phenotyping is required for further validation of these markers.
- Markers for Lr47 are not effective in a Baxter background, but were effective in other genetic material tested.
- Resistance to the exotic pest Russian wheat aphid (RWA) seems to have a high GxE component under current testing conditions. So far, markers have not shown association with phenotype. This, combined with the fact that these

resistances have been overcome by a new RWA biotype in the United States (US), has meant that this work has been relegated to low priority.

Recommendations

On-going support is required to allow development of markers of greatest value to breeders, and for validation of these markers, so they can be applied to breeding and selection outcomes.

Outcomes

Enhanced capability in marker validation and implementation which will position the EGA breeding program with the capacity to efficiently implement useful markers, leading to more rapid development of improved varieties.

Economic outcomes

This project is contributing to improving the value of wheat crops by reducing grain defects and to reducing the risk of loss due to disease. This value will be realised in the contribution to the production of new wheat varieties, carrying robust, multiple disease resistances and resistance to grain defects while maintaining productivity and premium quality (e.g. CR is estimated to cause losses of $56 million annually in Australia, around $21 million in the northern grains region alone). Adoption of resistant varieties could potentially eliminate this loss. PHS has been estimated to cause losses of around $30 million annually.Marketing authorities have prescribed limits of less than 5% in Australia on the level of black pointed grains in deliveries to receival silos, and losses through downgrading have been as high as $50 million annually in Australia. Though markers are likely to contribute to the goal of protecting our industry from these losses, it only does so in combination with pathologists, quality specialists and other selection methodologies, and therefore the attribution of benefits of these technologies in isolation is not possible. Markers will allow the more efficient introduction of these traits into commercial wheat varieties, through application to parent selection, enrichment of early generation breeding material, and allowing the pyramiding of multiple sources of resistance to reduce the chances of the resistance being overcome by mutations in the pathogens. This benefit will be realised after the conclusion of the project, since it takes 10-14 years to develop a new wheat variety.

Environmental outcomes

Improved genetic disease resistances and improved yield stability will reduce pesticide usage, and alleviate environmental pressures to clear land to increase production area.

Social outcomes

The public benefits arising from the research findings in this proposal are a more profitable, sustainable wheat industry, which will provide employment opportunities and prosperity, particularly in rural areas. By producing more pest and disease resistant wheats, pesticide usage will be reduced, improving the environmental sustainability of the industry, and reducing risks to public health. Capacity building in biotechnology will benefit Australian research, particularly grains research.

Achievements/Benefits

Background

GRDC and its research partners have invested heavily over the past few years in developing marker technology in a range of crops, particularly in wheat. The delivery point of this research is the development of new varieties through the breeding program. Molecular markers are now available for many traits in wheat, including disease resistances, quality attributes and agronomic characters. Currently EGA is implementing molecular markers for a number of these characteristics in its breeding program. In the northern node these include VPM and Sr36 rust resistances, black point resistance and resistance to BYDV.

Markers for pre-harvest sprouting (PHS) are nearing validation. Many more markers for traits of high priority like crown rot (CR) and root lesion nematodes (RLN) are at an advanced stage of the discovery process and will be available for validation within the time-frame of this project.

The challenge is to access the molecular marker information in a timely fashion, and carry it forward to being actively applied

to selection. To achieve this, the strategic plan for integration of molecular markers in the EGA breeding programs developed during Phase 1 of the Australian winter cereal molecular marker program (AWCMMP) will be adopted in this project. The plan will allow the rapid adoption of marker assisted selection (MAS) in those areas where it will be of greatest benefit. The strategic implementation of molecular markers in the EGA breeding program will facilitate the breeding of superior grain varieties that offer a competitive edge to growers.

Marker Assisted Selection prioritisation

A desktop study was undertaken, prioritising traits for MAS for the northern region, based on importance of the trait to the industry, difficulty of current phenotyping methods, stage of the selection process at which the trait is currently assessed, and availability of validated polymerase chain reaction (PCR)-based markers.

The tasks identified were to:

1. Identify polymorphic probes across 95 key parent lines and apply to accelerated backcrossing.
2. Test breeding lines for presence of the VPM rust resistance marker.
3. Validate putative dormancy or PHS markers.
4. Validate putative black point resistance markers in Cook$^{(\dagger)}$ derived breeding material.
5. Validate and implement 2-49 CR resistance markers.
6. Develop improved DNA preparation and throughput.
7. Validate putative late maturity alpha amylase (LMA) markers and implement.
8. Test RWA markers on local sources of resistance and recurrent parents and implement.
9. Validate and implement *Pratylenchus thornei* (RLN) marker for the GS50A resistance source.
10. Validate *Lr19* rust resistance marker and implement.
11. Validate *Lr47* marker and implement.
12. Test selected lines with BYDV marker.
13. Validate *Rht8*, *Rht1* and *Rht2* dwarfing gene markers, and
14. Validate *Pratylenchus neglectus* (RLN) marker.

All of these goals could not be achieved in the current two year project due to constraints of budget and marker availability. If achievement of a particular goal was required to be postponed, resources were moved further down the list.

Parent evaluation

A set of 95 lines was assembled to represent parents contributing to the current northern breeding population. Fifty three trait-associated markers, associated with 28 different traits have been assessed for polymorphism in this set. Traits for which associated markers have been tested include resistance to black point, PHS, RLN, CR, cereal cyst nematode (CCN), yellow spot, rusts (*Sr2, Sr30,Sr36, Lr1*), boron toxicity, LMA, granule bound starch synthase (GBSS), as well as hardness, dwarfing and glutenins. This set of data is used to determine which markers can be used in any particular parent combination. This list has now been expanded to 190 lines including new advanced lines, and new parental material entering the program.

Implementation of VPM marker and BYDV marker

Fewer breeding lines than expected were available for testing for VPM rust resistance complex. The importance of VPM has diminished due to the breakdown of resistances in Australia. 1,482 lines were tested from June 2002-June 2004. Future testing is likely to be at a similar level or diminish. VPM is likely to remain important, but will need to be used in combination with other rust resistances.

In 2003/04 nearly 300 lines were also assessed for resistance to BYDV using markers.

Validation of markers for black point resistance

Three genomic regions were validated for their association with resistance to black point in Cook-derived lines. Markers used for validation differed from the original study (Lehmensiek et al 2004). It was desirable to use SSRs so that they can be applied in a high through-put situation, and these were required to be polymorphic between the validation parents. Markers were validated in a Batavia x PelsartF_1 derived doubled haploid (DH) population. Using a marker for locus 1 alone, a 1.41 fold enrichment of the population could be achieved, but 11.76% of resistant lines were not selected. Using a marker for locus 2, a 1.55 fold enrichment was achieved, but 23.53% of resistant lines were not selected. The resistance allele at locus 3 was found

to be derived from the susceptible parent Pelsart, but is likely only to be useful in combination with locus 1 and 2. By applying markers for locus 1 and 2 in combination, a 1.83 fold enrichment of the population was achieved and 35.29% of resistant lines were not selected. For all three loci, a 2.47 fold enrichment was achieved, but 52.94% of resistant lines were not selected. It is likely then that loci 1 and 2 will be used independently or in combination for selection. Locus 1 is associated with the translocation from *T. timopheevi* associated with the rust resistance gene *Sr36*. This translocation was found to be preferentially transmitted. In cases where alternative rust resistances carried on 2B (e.g. *Lr35/Sr39*) are being selected, locus 2 will be selected. This information has been posted on the AWCMMP blackboard.

A further two populations of over 200 lines segregating for the Sunco-source of black point resistance has since been investigated, and associations between markers and traits were less significant. Very large GxE effects make black point resistance a difficult trait to select regardless of the methodology.

Nine hundred and twenty two breeding lines have been screened using one or two black point markers. The core 95 parental lines have been tested for polymorphism with these and five alternate markers for locus 2 and 3. Since locus 1 is contained within an alien translocation, any marker associated with that segment will be for practical purposes a 'prefect' marker. Markers for QTL associated with black point resistance identified in Cascades were tested in a Hartog x Genaro DH population, but no association was found, indicating that these lines carry different sources of resistance. Further sources of black point resistance are being investigated, and QTL locations are likely to be available within the life of the next AWCMMP.

Validation of markers for crown rot resistance

The flanking markers from 1D and 1A identified at the University of Southern Queensland (USQ) as associated with resistance to CR have been assessed on the core 190 lines.Alternative markers will be required for some parental combinations, particularly Hartog, due to lack of marker polymorphism, for 1D markers. Breeding material will be used for marker validation, but phenotyping of early generation material in addition to routine screening will be required. Further validation is required before these markers will be implemented. QTL will be identified for two or three further sources of CR resistance within the life of AWCMMPII. These will also require validation. Given the high cost of accurate CR screening, it is planned to use breeding material for this purpose wherever possible.

Validation of markers for dormancy

Two markers have been reported as associated with one of the major loci determining resistance to dormancy (Mares and Mrva, 2002). These markers were applied to four validation populations, and a strong QTL indication associated with one marker. Six other markers thought to be located in that region were tested for technical robustness, polymorphism in the core 95 parents and trait association. Two of these were found to more closely flank the QTL than those previously reported. Analysis of one years results, however, suggest the association is not sufficiently strong for routine application of these markers.Population enrichment that could be achieved was around 1.33, 1.50, 1.70, 1.58 fold for each population, but numbers of resistant lines discarded on this basis was considered by the breeders to be high at 32%, 22%, 50% and 38%, respectively. The degree of enrichment required and the percentage of resistant lines discarded that is acceptable varies with the trait, depending on factors like cost and accuracy of phenotypic screening. More markers in this region are being sought and a further year of phenotypic results is being collected.

Validation of marker for *Lr 47*

Published marker systems have been unsuccessful in resolving the presence of *Lr47* in a Baxter background. In addition, a number of SSRs located in the region have been applied to these lines. Lines with *Lr 47* in non-Baxter backgrounds were tested and all markers gave expected results. The PCR products were sequenced and showed that Lr47 is present in some lines. A series of new primer pairs was designed from published sequences. Some primer pairs appeared to produce a wheat specific product, but no *Lr47* specific products. It has been difficult to confirm this result, and therefore to use these markers as a dominant wheat specific marker, because no rust strain is available to distinguish *Lr47* in a Baxter background (i.e. with *Lr17*). In all, about 900 lines have been screened with various *Lr47* markers. *Lr 47* is currently being crossed into non-Baxter backgrounds.

Validation of markers for Russian wheat aphid

Six markers, associated with eight different RWA resistance genes were tested for association with phenotypic scores from Colorado State University of over 100 potentially resistant breeding lines. No marker-trait correlations were found, but large

genotype by year effects were found. All of these genes for which marker associations have been published have now been overcome by a new biotype of RWA in the US, so for the moment this work has been relegated to 'low priority' with regard to markers.

Marker implementation plan for EGA beyond 2004

A list of traits of interest for molecular marker application has been prepared and prioritised as a group by the EGA partners. A report has been prepared describing the current situation with marker implementation and validation within EGA. Also discussed are the requirements for phenotyping and a three year plan is described. High priority traits are listed and indications given of marker information available for these traits, and plans for validation and implementation work. Planning for further integration of breeding and selection with molecular marker-aided selection is extensively described. Plans are also discussed on the investigation of whole genome approaches, of new technologies and system integration, and communication and intellectual property (IP) issues. The report concludes with a description of what EGA biotech hopes to achieve and the roles it would like to take by 2007 and beyond.

Benefits to industry

Molecular markers are relatively new tools to help plant breeders select improved wheat varieties. By aiding in parent selection, in early generation population enrichment and in accelerated recurrent parent recovery, markers can allow breeders to accelerate the rate of genetic gain. The outcome is the production of varieties with the desirable combination of robust, multiple disease resistances, excellent agronomic performance and premium quality in the shortest possible time.

Other research

The work started in this project will be on-going. As breeding populations become enriched for desirable traits, markers for these traits would be expected to be used less frequently, though they will not cease from use altogether, since new germplasm is constantly being introduced to any breeding program, bringing new traits. Pests and diseases are constantly evolving, so new resistances will continually need to be found even to maintain production.It is hoped that with the aid of markers these genes can be more successfully pyramided, therefore extending their useful life to a very great extent. Pre-emptive breeding is also required for potential new introductions of pests and diseases from overseas, and for currently minor problems becoming more important due to changes in farming practices or environmental conditions, particularly climate. Such changes may also necessitate adaptation to different agronomic conditions, such as less water or more saline soils. As new markets for wheat are sought and as consumer demands change, so do the qualities required in our products. Markers associated with all of these traits will aid their deployment.

In the shorter term, traits of importance to EGA, for which markers are available, or are expected to be in the next three years, for validation include PHS resistance, resistance to CR in bread and durum wheat, septoria blotch resistance, salinity tolerance in durum, LMA resistance (some sources), resistance to RLN, tolerance of boron toxicity, black pointresistance in bread and durum wheat, rust resistances (*Lr34*, *Lr46*, *Lr24/Sr24* and *Lr35/Sr39*), resistance to the exotic fungal disease Karnal bunt in bread and durum wheat, milling yield and water absorption. Some of these only require polymorphism studies in relevant genetic backgrounds, others need extensive testing for marker-trait association.

New technologies, like diversity arrays technology (DArT), also offer the promise of being able to apply a whole-genome approach to molecular MAS. The Validation/Implementation projects should play a significant role in interfacing between the technology development and application to selection by breeders of this and other new technologies.

A Project Specification for a joint EGA project for validation and implementation of molecular markers in wheat has been submitted to GRDC.

Intellectual property summary

Outputs of this project are ultimately realised in the release of new EGA wheat varieties. Intellectual property (IP) concerning these is dictated by plant breeders' rights (PBR) legislation and the EGA partner agreements.

Additional information

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Projects DAN00072, UQ00026 and DAQ00077 follow on from this project.