FINAL REPORT

GRDC Grains Research & Development Corporation

Molecular biology support for barley improvement - North

DAQ00078

Project Details

- **Project Code:** DAQ00078
- **Project Title:** Molecular biology support for barley improvement - North
- **Start Date:** 31.12.2004 **End Date:** 30.06.2007
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Summary

The principal aim of this project was to improve the rate of genetic gain and selection efficiency in developing barley varieties for Queensland (QLD) and New South Wales (NSW). The use of molecular markers in the Barley Breeding Australia – Northern Node (BBA-North), which has expanded significantly over the course of the project, has successfully contributed to improvements in the adaptive breeding of elite malting and feed barley germplasm; the deployment of genetic resistance to diseases including leaf rust, net and spot form of net blotch (NFNB, SFNB), stem rust and Russian wheat aphid (RWA); the rate of development of varieties with multiple disease resistance; the rate of genetic gain for grain yield by fixing other important traits; and in selection for animal feed quality traits.

Conclusions

The benefits of marker deployment to plant breeding are now starting to be realised. The uptake of marker assisted selection (MAS) and marker assisted recurrent parent recovery (MARPR) within BBA-North has successfully contributed to improvements in the adaptive breeding of elite malting and feed barley germplasm; the deployment of genetic resistance to diseases including leaf rust, NFNB, SFNB, stem rust and RWA; the rate of development of varieties with multiple disease resistance; the rate of genetic gain for grain yield by fixing other important traits; and in selection for animal feed quality traits.

This project focused on the use of molecular markers identified as identical by descent (IBD) through a known pedigree hierarchy, rather than identical by state (IBS) alone as inferred through identical marker alleles, in order to maximise the likelihood of maintaining the linkage disequilibrium between the marker(s) and quantitative trait loci (QTL) or gene of interest. The integration of the molecular breeding component of BBA-North with the breeding, pathology and quality components has been pivotal to the success of this project and has also allowed us to undertake marker-trait validation work where appropriate, particularly in situations when a new source of the favourable QTL allele was being used.

In addition to the use of polymerase chain reaction (PCR)-based molecular markers flanking QTL for targeted traits, we have also used diversity arraystechnology (DArT) markers for the cost effective development of whole genome scans for over 150 key germplasm lines in the BBA-North gene-pool, in addition to targeting three back-cross breeding populations for MARPR. The generation of whole genome scans has been complemented by the marker inference functionality in the pedigree-based m*arker assisted*selection system (PBMASS), which can infer large portions of the genome from genotyping a carefully selected subset of markers and genotypes,therefore reducing genotyping costs significantly.

The development of the software resources, particularly PBMASS but also laboratory information management systems (LIMS), for sample tracking within our laboratory, provides a platform for future molecular breeding strategies in the Northern Region. Additionally, the enhanced sample through-put capacities in the Biotechnology Laboratory at the Hermitage Research Station (HRS), which occurred during the course of the project, have positioned the project to maximise the impact of molecular markers implemented in BBA-North.

The successful implementation of the molecular breeding strategy for BBA-North has depended on its seamless integration with the breeding, pathology and quality components of the breeding program. Additionally, this project's activities have been integrated with other GRDC-funded projects in the Northern Region: the pedigree-based genome mapping project (PBGMP) (DAQ00077), the Northern Region marker discovery project (USQ0007) and the Department of Primary Industries and Fisheries (DPI&F) molecular marker component of the EGA wheat breeding program. It has also strengthened established collaboration with other Australian Winter Cereal Molecular Marker Program (AWCMMP) research groups. The marker data resource, validation information, technical experience gained, and new breeding lines generated from this project, will ensure good progress in the barley breeding program in the Northern Region for many years to come.

Recommendations

Continued investment in the application of molecular marker technology to address high priority research and development (R&D) needs for barley improvement activities in the Northern Region is required and marker validation activities in barley should be a high priority. Integrating marker validation and breeding programs - as has been adopted in the current Northern Region breeding program - will be the most effective and efficient means for future marker validation activities.

The current project activities have been successfully integrated with those of the PBGMP for marker assisted selection and recurrent parent recovery in wheat and barley project (DAQ00077) and the marker discovery project for significant traits in the Northern Region (USQ00007). This integration will further develop and implement systems for maximising the potential of marker-assisted selection in breeding programs, through generating information about the frequency, distribution and ancestral origin of QTL, and information about historical selection for specific genomic regions within BBA-North.

It is recommended that the advances and knowledge gained in the current suite of projects are further developed to enhance the capacity of BBA-North to improve the rate of genetic gain, in order to more rapidly deliver improved barley varieties to support the state, national and international competitiveness of the Australian grains industry.

In particular, further investment for the identification and implementation of QTL for new sources of resistance to NFNB, SFNB, crown rot, spot blotch and common root rot (CRR) is recommended, due to the importance and prevalence of these diseases and current lack of barley varieties with durable resistance. Pyramiding multiple sources of disease resistance must be a high priority for future breeding efforts. In light of predicted impacts of climate change on agriculture, and in particular the anticipated increased variability in weather patterns, including more widespread and frequent droughts and higher temperatures, the effectiveness of current breeding strategies may be compromised in the absence of an expansion in research efforts to include new patterns of pathogen spread and infection, in addition to further research focusing on yield stability in harsher environments. Additionally, continued investment in the pre-emptive breeding strategies already underway in DPI&F for resistance to RWA is recommended, as this pest poses a potentially significant threat to the Australian wheat and barley industries.

The continued improvement of existing marker technologies is required, in order to apply them to key traits of interest to breeders, in the most efficient and effective manner possible. Utility of DArT technology in the Northern Region has been validated for many applications and using DArT markers in the breeding program will continue. There are exciting opportunities for complementing continued support in the DArT technology with enhancements to PBMASS involving marker inference functionalities. This will predict optimal sets of markers for maximum benefit through flanking marker inference. Real opportunities exist for reducing genotyping costs considerably through allowing large portions of the genome to be inferred from genotyping a carefully selected subset of markers and genotypes.

Outcomes

The overall aim of DAQ00078 was for an improved rate of genetic gain within the northern barley improvement program (NBIP) (now BBA-North) through the continued development and efficient implementation of molecular marker technologies in the early generation material, together with integration with disease screening and near-infrared (NIR) selection strategies, enhancing our capacity to release new and improved varieties in a timely manner. The principal benefit to the barley industry will be faster delivery of robust varieties with high yield, disease resistance and quality requirements that meet market demand.

Economic outcomes

The main financial benefits to the grains industry of the routine implementation of molecular markers for selection decision within BBA-North will be derived from 1) the increased on-farm productivity, and 2) the improved crop protection expected from the accelerated release of new, disease resistant, elite barley varieties. These are a consequence of the marker technology enabling the breeders to make selection decisions with more confidence and earlier in the plant breeding process. The increase in selection efficiency also impacts on the development cycles for individual varieties, which can be reduced, therefore making them available to industry earlier than by conventional means. It also impacts on new varieties having improved combinations of essential traits, for example disease resistances. A longer-term, significant impact will be that the BBA-North's gene pool will be improved, which will in turn lead to an increased overall rate of genetic gain as better parents are incorporated into each cycle of the BBA-North crossing program. This increased genetic gain will result in better varieties, faster.

Environmental outcomes

The deployment of genetically resistant barley varieties will positively impact on the decreased use of chemical protectants in the grains industry, therefore providing a significant benefit to the Northern Region environment. These resistant varieties will also better fit into crop rotation and stubble retention systems, e.g. through the incorporation of genetic resistance into the stubble borne diseases such as NFNB and SFNB. By encouraging stubble retention through facilitating the release of disease resistant varieties, the project will contribute to soil and moisture conservation practices.

Social outcomes

A healthy regional grains industry creates strong farming businesses, income for farming families and jobs for people in associated industries, such as transport, farm input suppliers and grain handling authorities. Stable supplies of regional sources of barley grain also contribute significantly to stability in the downstream malting, brewing and intensive livestock industries, which rely on the crop as a critical input commodity.

Achievement/Benefit

The contribution of this project to the overall objective of AWCMMP was to improve the rate of genetic gain and selection efficiency in developing barley varieties for QLD and NSW. The use of molecular markers in the BBA-North initiative, which has expanded significantly over the course of this project, has enhanced the capacity to speed up delivery of robust varieties with high yield, disease resistance and market quality requirements to the grains industry.

During the course of this project, the strategy has been to deploy MAS, in combination with other cost-effective high-throughput selection tools such as NIR, seedling screening and hillplots using the most cost-effective option in each case. The researchers have pursued an integrated approach to breeding and selection, using the best combination of techniques to narrow the pool of candidate lines into a focussed set of material with a high probability of producing elite varieties. The new, elite varieties developed, led by DAQ0110 (BBA-North), contribute to overall market stability through both productivity increases and risk reduction. Recent shifts in market forces in the Northern Region have resulted in a shift in the principal focus of the breeding program, which occurred during the course of this project and which is now focussing more on meeting increasing demand for feed grain.

In order to keep up with the increasing molecular marker screening requirements of BBA-North, DPI&F has significantly enhanced its capacity for molecular marker throughput in its well-equipped laboratory at the HRS, with the purchase, during the course of this project, of a Beckman Coutler capillary electrophoresis machine (CEQ8800), with a sample throughput of approximately 10,000 samples per week. The facilities are currently shared with the DPI&F sorghum breeding program. Resources at the HRS have been very well integrated to provide a strong winter and summer crop marker support capacity and make the most efficient use of the facilities and personnel. Additionally, during the course of this project, the laboratory information management system (LIMS) together with very effective use of eLabBooks, has stream-lined many activities and provided an excellent means of sample tracking, data and protocol management, ensuring data integrity, and prioritising and viewing work.

The implementation of MAS in BBA-North has focused on the use of markers linked to QTL for key traits that are IBD to the donor germplasm. When the selection of targeted genomic regions is based on markers that are IBD from the same ancestral source, the likelihood of maintaining the same non-random association between marker alleles and a particular QTL is greatly enhanced (Jordan et al., 2004). Alleles based on IBD will have greater utility because the linkage phase is more likely to be conserved in regions linked to markers with the same ancestral identity. In contrast, relying on markers that are just IBS, as inferred through identical marker alleles,with no common pedigree to the donor germplasm, there is a risk of losing the marker-trait association and therefore having to re-validate prior to MAS implementation in new germplasm. The ability to trace alleles IBD through pedigree hierarchies in the BBA-North gene pool has been greatly facilitated through the development of the software PBMASS through the associated GRDC-funded project DAQ00077. PBMASS has been developed as a user friendly desktop tool to integrate pedigree, molecular marker and phenotypic data and to assist plant breeders in the selection of breeding lines. The application of key functionalities developed for PBMASS, including graphical genotype displays based on IBD, marker inference functionalities and pedigree tree displays, has greatly enhanced the ability to maximise efficiencies and effectiveness of MAS for BBA-North.

Selected traits for inclusion in the molecular breeding strategy, during the course of this project, included foliar disease resistances for NFNB (*Pyrenophora teres* f. *teres*), SFNB (*P. teres* f. *maculata*), leaf rust (*Puccinia hordei*), and stem rust (*Puccinia graminis* f. *tritici*). Additionally, grain quality traits were targeted including kernel discoloration, malt extract and resistance to pre-harvest sprouting (PHS). After early generation selection for multiple target loci of F2 and F3 breeding populations, 11-46% of breeding lines were retained. Markers assisted in the selection of homozygous lines which enabled important loci to be 'fixed'. Another important benefit to breeding is that the total number of lines that need to be tested can be reduced, permitting more efficient use of glasshouse and field facilities.

PCR-based molecular markers flanking QTL for these traits were carefully investigated prior to implementation. This involved using the recently developed and highly informative high density barley consensus map resources (Wenzl et

al., 2006; Varshney et al., 2007) in order to identify the highest number of suitable PCR-based markers in each target QTL region, together with marker protocol optimisation to maximise multiplexing capacity, followed by assessing each marker's polymorphism level in selected BBA-North breeding populations. Particular attention was given to whether the marker(s), or haplotype blocks, could be identified as IBD through a known pedigree hierarchy, rather than just IBS as inferred through identical marker alleles, in order to maximise likelihood of maintaining the linkage disequilibrium between the marker(s) and QTL or gene of interest. In the absence of information on the source of the favourable QTL allele, validation of the association between the marker and QTL in new germplasm is essential. The project researchers believe that there has been an underestimation of the effort needed to validate or commission specific markers prior to implementation. During the course of this project, markers linked for both NFNB and leaf rust (Rph3) have been validated.

An important component of this project was pre-emptive breeding for RWA (*Diuraphis anoxia*). The strategy adopted used previously published markers associated with RWA resistance loci on 1H and 2H to develop new breeding lines. These backcross derived families were subsequently phenotyped by overseas collaborator Do Mornhinweg (United States Department of Agriculture, Agricultural Research Service (USDA-ARS). Results indicated that markers for each locus were associated with resistance in two out of three families. Therefore the markers and breeding material should be useful for pre-emptive breeding for RWA in the future.

In addition to the use of limited sets of PCR-based markers flanking key QTL, DArT markers have also been used for the cost effective development of whole genome scans. DArT markers offer breeding programs an alternative approach to whole-genome profiling, through its high multiplexing level, being able to simultaneously type several thousand loci per assay, while being independent of sequence information. During the course of this project, whole genome profiles have been generated for over 150 key germplasm lines in the BBA-North gene-pool in addition to the targeting of three backcross breeding populations for MARPR. Grimmest[A], Binalong[A] and MacKay[A] were used as recurrent parents (RP). DArT markers provided good genome coverage and provided insight into chromosome recombination and lengths of donor segments containing selected target genes (e.g. in the breeding population C05.030, the donor segment containing the Rpg1 donor segment ranged from 39-112cM). Data also indicated that DArTs have good potential for accelerating the RP genome during backcrossing. This would reduce the time required for a backcross program.

DArT markers were also evaluated for whole genotype selection in two F2 populations arising from complex F1s. Difficulties were encountered in data analysis for DArT markers in complex F2 populations since dominant markers cannot discriminate between heterozygous and homozygous genotypes. However, DArT data could still be used by breeders to select lines with a desirable genomic constitution for further generation advance and specific regions could be screened using single sequence repeat (SSR) markers to confirm genotype.

The generation of whole genome scans is complemented by the marker inference functionality in PBMASS, which can infer large portions of the genome from genotyping a carefully selected subset of markers and genotypes, and thus reducing genotyping costs significantly. During the course of this project, the inference of missing data functionality in PBMASS, together with the cost effective medium density whole genome scans generated by DArT markers, greatly enhanced the capacity to generate high density whole genome scans and to identify IBD for marker alleles and allow these alleles to be traced through pedigrees and provide more information for breeders. The whole genome mapping approach, which uses the pedigree information also stored in PBMASS, has been applied to the molecular breeding strategy for BBA-North to combine marker and trait data when selecting parents and elite breeding lines. This information has also been used to identify regions under selection, validate putatively identified QTL, and identify pedigrees in which particular markers will or will not be useful for MAS (e.g. haplotype blocks of QTL regions associated with feed quality traits, identified from an associated GRDC-funded AWCMMP project, (USQ0007) have been traced through known pedigrees in BBA-North and have identified potentially promising genotypes for further feed quality evaluation).

During the course of this project, a variation request was made for output 3, relating to doubled-haploid population development and impacting on milestones 10 and 11 in DAQ00078. This request was sought to reflect strategic changes to the BBA-North program in keeping with the national barley breeding negotiations. The strategy to outsource doubled haploid development has been adopted as being more cost effective. Additionally, the recent investment by DPI&F in a capillary electrophoresis machine, as mentioned previously, and a liquid handling robotics system for the Biotechnology Laboratory at HRS has changed the emphasis of work undertaken in this laboratory and has allowed for a more focused development of the capacity to implement molecular marker technology to support

the barley breeding program. A number of methods to reduce the BBA-North breeding cycle are being evaluated. During the course of this project, the use of single seed descent (SSD) for rapid population advancement was implemented for five selected breeding populations, resulting in the generation of a mean recombinant inbred line (RIL) population size of 249 individuals within two years. These populations have also been targeted for MAS in later generations, specifically focusing on Rph3 (7HL) and mla (1HS) loci.

In summary, the potential for molecular markers to assist industry development in the north and to contribute to BBA-North's lead in developing feed barleys for Australia is significant. Specifically, in the Northern Region, successful implementation of MAS has contributed to improvements in:

- adaptive breeding of elite malting and feed barley germplasm
- deployment of genetic resistance to diseases including net blotch, crown rot and stem rust
- the rate of development of varieties with multiple disease resistance
- the rate of genetic gain for grain yield by fixing other important traits, andselection for animal feed quality.

The seamless integration of the molecular breeding component of BBA-North with the breeding, pathology and quality components has been pivotal to the success of this project. In keeping with this philosophy, the marker validation and implementation activities from this project have also been successfully integrated with the Northern Region PBGMP(DAQ00077), the Northern Region marker discovery project (USQ0007) and the DPI&F molecular marker component of the EGA wheat breeding program. During the course of this project, established collaboration with other AWCMMP research groups has been strengthened, particularly through the use of, and contribution to, the intellectual property (IP) register, the marker-trait register and attendance of the annual AWCMMP planning meetings.

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Other Research

In the course of this project, a number of additional research and development opportunities have been identified.

Through continued collaboration with the PBGMP, opportunities have arisen to identify genomic regions under strong selection pressure within the BBA-North gene pool (DAQ00110) and to validate markers for QTL of relevant traits previously identified in diverse germplasm backgrounds prior to implementation. In particular, markers linked to QTL for NFNB, SFNB, powdery mildew and leaf rust have been validated using a set of diverse breeding lines, in addition to including breeding material itself in QTL detection, so covering multiple generations and linking many crosses through their common ancestors in the pedigree. Markers linked to QTL associated with traits for enhanced feed quality, contributing to net energy and average daily gain, have been identified in the associated GRDC-funded project USQ0007. The project researchers have traced the favourable alleles through pedigree hierarchies in BBA-North in order to identify genomic regions IBD to the donor genotype in selected progeny from the BBA-North gene pool, to determine the frequency and distribution of potential QTL, and to identify potentially promising genotypes for further feed quality evaluation. Markers linked to resistance to the other traits of importance including the physiological black-point condition and CRR have also been identified through USQ0007 and are being validated on breeding populations in BBA-North.

Additionally, collaboration with the PBGMP (DAQ00077) has allowed the effective implementation of the PBMASS software in both DAQ00078 and DAQ00110, which has enhanced the capacity to identify appropriate combinations

of molecular markers and germplasm for MAS, through the use of graphical genotype displays based on IBD, the marker inference functionality, and the pedigree tree display options. The combination of the integration of PBMASS together with the whole-genome scans generated using DArT markers, from Triticarte, has enabled the effective implementation of molecular breeding strategies in the Northern Region. This will impact industry development in the north and contribute to BBA-North's lead in developing feed barleys for Australia.

Intellectual Property Summary

Information generated during the course of this project has been communicated to other AWCMMP participants through the quarterly IP register and annual AWCMMP meetings. The majority of the IP generated from this project was considered unencumbered. Where the IP was related to genetic material developed through BBA-North (DAQ00110), the information is available to other Australian and international barley researchers and breeders under the control of legally binding Material Transfer Agreements (MTAs).

Collaboration Organisations

During the course of this project, there were collaborations with two international partners.

The first relates to the pre-emptive breeding work undertaken for RWA, potentially a significant threat to the Australian wheat and barley industries. The pest does not currently exist in Australia, but the potential for introduction is high, and it is likely that damage to crops would be severe. In barley several widely-used sources of resistance, STARS9301B and PI 366444/PI 366453 from Afghanistan, have been well characterised. Due to the importance of RWA overseas to wheat and barley production, numerous QTL mapping studies have been undertaken in the past 15 years. PCR-based sequence tagged site (STS) markers have been identified for two resistance loci derived from PI366444/PI366453 barley sources on chromosomes 1H and 2H (Nieto-Lopez and Blake, 1994). During the course of this project, the breeding line 94-05073*11-1 was developed from the PI366444 source and the STS markers were utilised for simultaneously validating and pre-emptive breeding of new germplasm. A critical partnership between HRS and one of the leading RWA screening research groups in the United States (US) (Do Mornhinweg from USDA, ARS, SPA Stillwater OK 74075, US) was established during the course of this project, toundertake the phenotyping of the material derived from the 94-05073*11-1 resistance sources. Barley germplasm was shared under an MTA with USDA. The phenotyping work undertaken allowed the researchers to identify that the molecular markers used (1H & 2H) are useful in predicting resistance.

The second relates to feed quality assessment work undertaken by Jan Bowman from Montana State University (MSU) (Animal Range Sciences, Bozeman, Montana, US), also in conjunction with an associated GRDC funded project, USQ0007. These analyses have been provided at no charge to the project, on the understanding that Dr Bowman and her team can use the data in their research into barley (cattle) feed quality. The material is sent to Dr Bowman in the US under appropriate MTAs which protect the ownership rights of the project stakeholders.
Feed quality data including acid detergent fibre (ADF), hardness, starch, in sacco dry matter digestibility and average daily gain were generated for two RIL populations:Tallon^{A}/Scarlett (n=140) and Patty/ Tallon (n=95). These populations, comprising 235 and 240 markers respectively, were combined with the phenotypic data generated by MSU, together with NIR predictions generated from DPI&F and QTL analyses performed. Within each mapping population, numerous QTL overlapped for multiple traits. The inclusion of DArT and SSR markers permitted direct comparisons between maps and with the DArT consensus map (Wenzl et al. 2006), and the development of a consensus map for feed quality traits is currently on-going.

Collaboration Details

In both cases, the nature of the international collaboration was that of phenotyping work for specific traits carried out overseas.

Additional Information

A list of relevant publications to which project team members and/or work have contributed during the course of DAQ00078.

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