FINAL REPORT

GRDC
GRAINS RESEARCH & DEVELOPMENT CORPORATION

DAQ00046

AWCMMP Component: Molecular biology support for barley improvement - Northern Region

PROJECT DETAILS

PROJECT CODE:	DAQ00046
PROJECT TITLE:	AWCMMP COMPONENT: MOLECULAR BIOLOGY SUPPORT FOR BARLEY IMPROVEMENT - NORTHERN REGION
START DATE:	01.07.2002
END DATE:	31.12.2004
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Summary

The principal aim of this project was for the Northern Barley Improvement Program (NBIP) to develop the capability to efficiently use molecular marker technologies. During the two-year time frame of this project, the routine implementation of marker technology has increased significantly from eight populations targeted for two traits in 2003, resulting in approximately 60% of lines (150 lines) assayed being positively selected, to 19 populations targeted for seven traits in addition to marker-assisted recurrent parent recovery (MARPR) in 2004, resulting in approximately 55% of lines (1,551 lines) assayed being positively selected on the basis of the marker data. This project was closely integrated with the Pedigree Mapping (PM) project (ET8, UQ00026).

Conclusions

This project is strongly linked with the NBIP. It was designed with the intention of achieving effective implementation of marker technology within a two-year time frame.

Within the time frame of this project, the Queensland Department of Primary Industries and Fisheries (QDPI&F) has developed a capacity for a moderate level of molecular marker throughput in its well equipped laboratory at the Hermitage Research Station (HRS), Warwick, developed with funding from QDPI&F, GRDC, Grainco and the Cooperative Research Centre (CRC) for Tropical Plant Pathology. Through the optimisation of a 96-well plate DNA extraction protocol, its throughput capacity has increased fourfold, from 250 samples/week to 1,000 samples/week.

This has contributed to the significant increase in marker assays conducted in the final year of this project and also to achieving outcomes greater than those contracted in the Project Specification. Marker-assisted selection (MAS) has been implemented on targeted breeding populations in order to enhance our capacity to make selection decisions in conjunction with pathology and quality selection screens, such as near-infrared (NIR), seedling screening and hill plots, in order to identify the most cost-effective option in each case.

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 is being pursued.

The generation of a genotypic data set for 93 selected barley lines has proved to be a very valuable resource, in terms of whole genome descriptions for key barley lines and varieties facilitating the identification of significant genomic regions, and offering new opportunities for the validation of selected markers in ‘northern’ barley germplasm, in addition to the identification of polymorphic markers for marker-accelerated breeding (incl. backcrossing), enriching and designing crosses and for quality assurance.

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 grains region is required.

The current project worked together with the pedigree-based genome mapping project for marker-assisted selection and recurrent parent recovery in wheat and barley (ET8) to implement and further develop systems for maximising the potential of marker-assisted selection in breeding programs, through generating information about the frequency, distribution and ancestral origin of known quantitative trait loci (QTL), and information about historical selection for specific genomic regions within the NBIP.

It is recommended that the advances and knowledge gained in the current project are further developed to enhance the capacity of the NBIP to improve the rate of genetic gain through increasing the number of assays possible in early generations in the most cost-effective manner, in combination with other selection strategies such as disease screening and NIR screening for quality assessment.

Currently, the NBIP is implementing MAS in early generation populations for 11 disease resistance and quality traits, with a throughput of approximately 20,000 polymerase chain reactions (PCRs) per season, allowing for positive selection of the top 25-50% for population advancement.

Continued investment in this important research area will offer the opportunity to build on and enhance the selection filters applied by molecular markers in order to triple the total number of samples screened in the breeding program, and hence significantly enhancethe capacity to release new varieties in a timely manner.

Investment in a robotic liquid handling system for the HRS would make considerable savings in time and consumables, improve the capacity of the laboratory and provide improved assay reliability. A proposal for this investment was submitted through the Australian Winter Cereals Molecular Marker Program (AWCMMP) in 2004. As the investment would have considerable benefits to marker implementation in the northern region barley and sorghum breeding programs, it may be worth further consideration once the National Barley Breeding Strategy has been resolved.

Outcomes

The principal aim of this project was for the NBIP to develop the capability to efficiently use molecular marker technologies and achieve the specified project outcome:to improve the rate of genetic gain and more precise selection in developing barley varieties for Queensland (QLD) and New South Wales (NSW). This has been achieved through the routine implementation of markers for selection decisions within the NBIP. 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 decisions within the NBIP will be derived from:

1. increased on-farm productivity;
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, hence 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 NBIP'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 NBIP 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, hence 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 to stubble borne diseases such as net form and spot form net blotches (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 down-stream malting, brewing and intensive livestock industries, which rely on the crop as a critical input commodity.

Achievements/Benefits

The NBIP began to conduct marker assays for resistances to leaf rust, SFNB and Russian wheat aphid (RWA) in 2000/2001. Markers were also used for 'fingerprinting' parents and putative F1s for doubled haploid production quality assurance, identification of barley lines and a spot blotch pathotype diversity study. However, the resignation of Dr Merrill Fordyce in December 2001 left the NBIP without a molecular biologist, and a chain of events left the position vacant until Dr Emma Mace commenced duty in late September 2003. This meant that the NBIP did not have the capacity to conduct marker work during that period. GRDC granted a six month extension to the current project, its completion date, outputs and milestones. Because of these delays, it became essential for the NBIP to fast-track the validation and implementation of marker technology. A whole genome PM approach was selected as the best way to achieve this goal, as it would facilitate the validation of a suite of useful markers across a range of NBIP germplasm, while providing information which could be used to analyse the genetics of variety adaptation, develop new marker application strategies and provide data points which could be used for practical applications such as marker-assisted backcrossing.

As the PM concept can be generically applied, the northern PM project (ET8) was developed as a pilot study using both the northern barley and wheat breeding programs. This approach was designed to create efficiencies in the capture, storage and analysis of mapping data. It has been the role of the current barley project to generate the genotypic marker data, provide the data for PM analysis and then use the results to develop and implement new, efficient marker-based selection strategies. Marker validation and capture of data points for relatedness studies, backcrossing, etc., were additional benefits from the application of the PM concept. Staffing issues were addressed by funding both the molecular biologist and laboratory technician positions across two projects: DAQ00046/UQ00026 and DAQ00046/DAQ00038, respectively.

Marker data were generated initially from 65 barley genotypes, a genetically linked set of 29 varieties and 36 breeding lines, all relevant to the NBIP and all connected through lineage from either Triumph or Koru and later expanded to include an additional 28 lines, representing material currently used by the NBIP, either as parents or in populations.

The genotypes were sown in the NBIP glasshouse for DNA extraction, with many of the same plants being used as parents in the 2003 crossing program. Seed harvested from these plants has been stored for reference and future use. By using the same plants for extraction and crossing, the data analysis should be of greater use to the NBIP as it reduces the possibility for complications caused by parental heterogeneity or mistaken identity.

Marker assessment of the genotypes commenced in the second half of 2003, with the commissioning of Dr Harsh Raman (NSW Agriculture) to conduct assays of the full genotype set with 20 microsatellite or simple sequence repeat (SSR) markers. Most of the SSRs for this work were selected on the basis of being linked to important traits for barley in QLD and NSW. Some markers linked to 'southern' traits were included to give better coverage of the genome. Following the appointment of Emma Mace in September 2003, PCR assays were also undertaken at HRS. In total, the selected barley lines were genotyped with 56 markers, identified from National Barley Molecular Marker Program NBMMP/AWCMMP data, published literature and international collaborators. The linked traits included disease resistances (NFNB (*Pyrenophora teres* f. teres), SFNB, leaf rust, stem rust, powdery mildew, barley yellow dwarf virus (BYDV), scald), malting quality traits (hot water extract, diastatic power, beta-amylase, protein, kernel discolouration, plump grain, pre-harvest sprouting) and agronomic and environmental tolerance traits (aluminium and manganese tolerance, flowering, dwarfing).

During the course of this project, diversity arrays technology (DArT) has become available through Triticarte Pty Ltd. The 93 barley genotypes were also submitted to Triticarte for DArT analysis in two separate batches. The first batch, with results delivered in October 2003, consisted of 297 polymorphic DArT markers across 58 genotypes. The second batch, with results delivered in January 2005, consisted of 409 polymorphic DArT markers across 35 genotypes. 120 DArT markers were found to be common between the two data sets. It is anticipated that 50–75% of these markers will be mapped in the near future and as the DArT map becomes available and marker/trait linkages become established, this data set will become a highly valuable resource. The addition of the DArT work means that the marker data for the genotype set will be more comprehensive than originally thought. This will greatly enhance its value for both PM and practical marker applications in the NBIP.

An additional advantage for running this project in conjunction with the PM project (ET8) was the opportunity to validate a substantial number of trait-linked SSR markers across a broad selection of genotypes from the NBIP. The first step in this process has been conducted through SSR assays of 93 barley genotypes, as described above. By screening these markers across the genotype set, a clear picture of the range of NBIP populations with which each marker may be useful for application of MAS emerges. The data set was then used to develop integrated selection strategies that combine conventional and marker technologies. As this project is directly linked to the NBIP project (DAQ00038), the final step of validation overlapped with implementation as markers have been applied within appropriate breeding populations.

In 2003, the NBIP concentrated on applying MAS against a QTL inherited from Canadian barleys, such as Harrington and Metcalfe, which confers extreme susceptibility to pre-harvest sprouting (PHS). Northern region crops are particularly vulnerable to PHS because of the predominantly spring and summer rainfall patterns. This has meant that elite, but PHS susceptible, Canadian varieties have seen little use in the NBIP, since recovery of useful breeding lines has been low and resources have been better allocated to other populations. This is unfortunate, as Canadian germplasm is likely to be a good source of malting quality alleles and resistances to net blotches, spot blotch, stem rust and the smuts. However, the use of the PHS marker/trait association means that breeding populations can be pre-screened for the trait, so that susceptible lines are avoided and recovery rates of useful genotypes are increased. Tissue from 170 plants from six breeding populations was sampled in August-September 2003 and 340 assays were conducted using two microsatellite markers linked to the PHS locus on 5H (GMS01 and HVM6) prior to harvest in December 2003. The work was conducted as a cross/family evaluation strategy and two of the crosses were discarded on the basis of the marker data and five families were further discarded from the remaining four crosses.

In addition, in 2003, three markers (ABG8 on 2H, Kv1/Kv9 and D14 on 2H) linked to RWA resistance were implemented for the selection of resistant genotypes. The use of markers for selection of RWA resistance will be particularly useful as a pre-emptive breeding strategy, as bioassays are impractical in Australia. Leaf tissue was sampled from 80 plants from two breeding populations and in total, 240 assays were performed resulting in 37.5% of the lines being discarded based on the marker data. Physical screening to validate the selection for resistance is currently being organised in collaboration with David Moody of the Department of Primary Industries Victoria (DPI VIC).

In 2004, the number of marker assays conducted significantly increased, with more populations being targeted than in 2003 and more markers being implemented. In total, 2,784 individuals were assayed from 19 breeding populations and were targeted for seven foreground traits, resistance to five diseases(RWA (on 1H and 2H), leaf rust (Rph2 on 5H, Rph3 on 7H and Rph7 on 3H), stem rust (Rpg1 on 7H) NFNB (APR on 4H and 6H) and SFNB (7H)) and two quality traits (PHS (on 5H) and kernel discolouration (on 2H)), in addition to marker-assisted recurrent parent recovery on five breeding populations. Overall, 27,82 assays were performed, 8650 of which were conducted at HRSand the remaining outsourced to Triticarte for DArT genotyping.

The opportunity of using MAS to pyramid resistance genes for NFNB and leaf rust was also explored, in addition to pyramiding leaf rust resistance genes through integrating the marker and pathology selection strategies. The former pyramiding opportunity was explored using the Harrington/Tallon/Arapiles/Scarlett breeding population, and the latter explored using CMO/APO-31-04/Amulet F2 breeding population which was initially screened in the glasshouse for seedling resistance to leaf rust. The resistant genotypes identified were also assayed with Rph2-linked markers.

The use of different markers to assess the most effective means for marker-assisted recurrent parent recovery was also initiated this year. Five BC1F3 breeding populations were selected and in total 227 lines were assayed using either microsatellite markers selected from each chromosome arm or DArT markers. In total, 19,612 assays were performed and from these 129 lines from the five populations were selected for progression to the next generation, based on their similarity to the recurrent parent.

Overall, 1,551 lines (55.7%) assayed were selected for progression to the next generation.

Other research

Throughout the course of this project, a number of additional R&D opportunities have been identified.

Through continued collaboration with the pedigree-based genome mapping project, opportunities exist to identify genomic regions under strong selection pressure within the NBIP, and to validate markers for QTL of relevant traits previously identified

in diverse germplasm backgrounds prior to implementation. In particular, the identification of a core panel of validated molecular markers offers opportunities to fast-track their implementation within NBIP for the selection of key QTL and for application in MARPR.

In addition, marker validation will be undertaken for newly identified trait-linked simple sequence repeats (SSRs) applicable to NBIP. It is anticipated that markers for feed quality will be developed in the AWCMMP GA project 'Molecular markers for high priority traits in winter cereals for the northern region', together with disease resistance markers for adult plant resistance (APR) to NFNBto supplement seedling resistance markers previously identified on 2H, 3HL, 4HS and 6H. Markers for black point and stripe rust may become available through other collaborative projects and will immediately be validated on NBIP germplasm.

Opportunities exist to continue and enhance marker implementation for routine screening procedures for resistance to pathogens, for pyramiding disease resistance genes, for selection of key quality traits, to accelerate retrieval of recurrent parents, and through co-operation with existing projects, to validate markers for desirable quality and agronomic traits,therefore improvingthe understanding of the genetic control and genetic variation that exists for certain traits. Molecular marker implementation in the early generation material will be carefully integrated with the other selection filters available to the NBIP, specifically pathology and quality screening. Wherever possible, integrated strategies will be implemented to maximise efficiencies and enhance the potential genetic gain in the progression of the early generation material.

In 2004, marker screening was implemented for 19 populations, totalling approximately 3,000 samples screened for a total of seven traits, in addition to MARPR implementation, resulting in a total of approximately 28,000 assays undertaken. It is anticipated that this will increase every year to meet the increased sample throughput requirements of NBIP. This will require the optimisation and refinement of the high throughput protocols in place in the biotechnology laboratory at HRS to maximise efficiencies and minimise unit costs for DNA extractions, DNA dilutions, PCR assays and electrophoresis.

Another research opportunity exists in the routine implementation of the doubled haploid technology applied to selected populations within the NBIP to accelerate the development of fixed lines. Specifically, this approach should be carefully integrated with molecular marker technology for *in vitro* screening of doubled haploid lines for target traits prior to transplantation, therefore maximising the potential for identification of superior genotypes in Stage III and IV trials.

Intellectual property summary

Background intellectual property (IP) (i.e. barley germplasm and breeding lines) and variety commercialisation are managed through the contracted arrangements for the NBIP project (DAQ00038). AWCMMP related IP is managed through the AWCMMP Participation Agreement.

Additional information

Dr Poulsen presented a seminar at Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, in which data from this project were presented and the integration of conventional, high throughput and molecular selection strategies by the NBIP were discussed.