FINAL REPORT

GRDC Grains Research & Development Corporation

Barley Breeding Australia - Northern Node

DAQ00110

Project Details

- **Project Code:** DAQ00110
- **Project Title:** Barley Breeding Australia - Northern Node
- **Start Date:** 01.07.2006 **End Date:** 30.06.2011
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Summary

This project covered the 2006-2011 operations of the Northern Node of Barley Breeding Australia (BBA-North). BBA-North collaborated with the Southern and Western nodes and all BBA participants to deliver improved barley varieties to the Australian grains industry. BBA-North focused on the northern region and was the national leader in breeding high yielding, disease resistant barleys with grain quality that enhanced the crop's status as a preferred feed grain. Development of varieties for the malting and brewing industries was also targeted. This project incorporated coordination, breeding, regional evaluation, foliar and soil-borne disease tests, molecular marker screens and grain and malt quality analyses.

Conclusions

The DAQ00110 project supported the 2006-2011 operations of BBA-North. BBA-North collaborated with the Southern and Western breeding nodes and all BBA participants to deliver improved barley varieties and support the international competitiveness of the Australian grains industry. BBA-North focused on the GRDC northern region (NR) and led nationally in breeding high yielding, disease resistant barleys with grain quality that enhanced the crop's status as a preferred feed grain.

Neither Australian barley varieties nor introductions are well-suited for production in the NR. Some attributes favouring local adaption are: early maturity, resistance to foliar pathogens, grain plumpness, lodging resistance, heat stress tolerance, high water use efficiency and high yield. Barley varieties previously released for the NR are high yielding, but lack many of the other desirable traits. Thus, the base germplasm for the NR and breeding procedures were modified to address these breeding objectives.

To achieve the BBA-North targets, Australian varieties were crossed with introductions and the progenies were selected to increase desirable gene frequencies in elite breeding materials. The time required to develop breeding lines was shortened by establishing off-season nurseries for rapid generation advance. The effectiveness of foliar disease screens was enhanced by the establishment of new protocols for seedling tests and of isolated disease screening nurseries for specific diseases to conduct adult plant screens. The use of non-destructive quality evaluations based on near infra-red (NIR) reflectance for evaluation of feed and malt quality parameters was expanded. Breeding lines and mapping populations were phenotyped for agronomic traits, grain quality and disease reactions, and genotyped using whole genome scans with DArT molecular markers.

Shepherd[A] was released in 2008 to fill the immediate need for a high yielding, disease resistant variety. Although selecting good lines from the new crosses proved difficult, two lines were judged worthy of seed increase for possible commercialisation in 2012 as non-malting barleys.

The major achievement of DAQ00110 was building a program capable of rapidly producing barley germplasm better adapted to the NR and its diverse environments. New genetic resources were identified and breeding procedures were modified to enable utilisation of the new genetic resources and molecular markers as a breeding tool. Traits introduced with North Dakota (ND) accessions include tolerance to heat stress, improved malt quality, new disease resistances, earlier maturity, better lodging resistance, increased kernel size and new semi-dwarfing genes. Genetic factors for resistance to exotic pests were mapped and are available for evaluation in elite breeding material. The new breeding opportunities were based on an expanded ability to combine phenotypic and genotypic data. Two components of plant breeding, better trait expression and combinations of traits, contributed to better utilisation of genetic resources. An opportunity to market a new type of barley for the NR - dual purpose fodder varieties - was exploited in the breeding program.

Recommendations

Market forces will determine the future of barley production in the NR. On-farm prices and crop reliability will have a major influence on future production. The availability of high quality varieties can influence profitability and the market utilisation of barley compared with other crops. Each year, early harvest of barley in the NR provides an indicator of national supplies, which play a role in determining domestic barley prices. Barley is still a preferred feedgrain in the expanding livestock industry. Barley varieties well-adapted for production in the region are currently demanded by growers, livestock feeders, maltsters and brewers. Since the NR has unique environmental constraints, maintenance of an effective barley improvement program is strongly recommended.

Good malting quality is a key to the success of barley varieties in the NR. Growers often choose to plant varieties that might reap the higher prices offered for malting barley. Yet, some NR livestock feeders contract barley at malting prices to have an assured supply of good quality grain. Thus, new varieties must ideally have traits that make them competitive as feed barleys. Their potential success will be determined during the two to four years of production prior to final accreditation as malting barley by the Malting and Brewing Industry Barley Technical Committee (MBIBTC). Performance in the NR, therefore, becomes a critical breeding objective that drives the design and goals of breeding programs. It is recommended that malt quality be maintained in elite breeding materials as other desirable traits are accumulated.

Early maturing varieties with heat stress tolerance, measured by grain plumpness and yield stability over stressed environments, will be favoured by growers. Such varieties will also minimise production costs, both actual and perceived. Growers do find attractive varieties that are easy to manage, produce vigorous seedlings, have multiple disease resistances, do not lodge, cost less to harvest, and produce plump bright grain with high test weights. It is recommended that barley researchers target many agronomic traits and disease resistances besides malt quality.

Targeting the large number of desirable traits is a challenge, which is made more difficult by the fact that barley has seven chromosomes or linkage groups. Since many of the desirable traits are neither simply inherited nor fully expressed, linkage drag is an inevitable problem. For example, stable resistance to the net form of net blotch may require a certain combination of four or more genomic regions. Similarly, the development of high quality lines and adequate dormancy will likely involve three regions of chromosome 5H. Traditional approaches to barley breeding have produced many good varieties, but future efforts would benefit greatly from using both high quality phenotypic data and genotypic data from whole genome scans with molecular markers. Regrettably, current Australian barley programs are just building the resources to effectively apply this technology.

Initial steps in the development of dual purpose barleys for use as hay and silage in domestic and export markets have been taken. The quality of barley hay is equal to or better than that produced by other cereal crops if the awns are removed. Barleys without barbed awns would be competitive in markets currently dominated by oaten hay. Germplasm low in lignin content would enhance this advantage. Therefore, continued development of barley as a fodder crop is recommended.

Outcomes

Economic Outcomes

GRDC and several Australian research agencies agreed to form a collaborative national barley breeding program, to be known as Barley Breeding Australia (BBA). BBA featured three breeding nodes (North, South and West) and a germplasm development coordinator. The overall aim of the DAQ00110 project for BBA-North was to build grower and industry confidence in the NR barley industry through greater productivity, stability and market demand for barley. The prime objectives were the delivery of reliable feed and malting varieties and information for use in decision making throughout the value chain. Secondary objectives were the generation of information and genetic resources that permit continuation of the long-term process of barley improvement.

Annual barley production in the NR ranges from 0.9 to 2.4 million tonnes. At current prices, this has an estimated farm gate value of $270 to 480 million. The NR barley value chain is worth much more, as barley is one of the three feed grains that underpin the more than $2 billion feedlot cattle industry, and is also a critical ingredient of the region’s more than $2 billion brewing industry. In addition, barley fits well into many crop rotation systems. Flexible cropping options enable growers to take advantage of planting opportunities and have a significant impact on farm viability.

While the dollar contributions of a barley variety are difficult to assess, new varieties increase productivity and support overall industry stability. Although barley can be sourced from other parts of Australia, both the regional malting and brewing and livestock industries prefer to use local grain supplies. This minimises transport costs, which must ultimately be passed on to the consumer. Regionally stable and reliable supplies of high quality grain protect markets for Australian barley. Due to the earlier NR harvest and the demand for feed grains, industry sources say that the NR crop has a major effect on barley prices across Australia. Individual grain growers benefit from new varieties that deliver increased on-farm productivity, more marketable grain and protection against losses caused by biotic and abiotic stresses.

Environmental Outcomes

The use of barley in crop rotations contributes to soil health, topsoil retention and efficient water use and helps to avoid the many problems associated with monocultures. These attributes have long-term benefits to the environmental sustainability of the northern agricultural industries. Incorporation of genetic resistances to biotic agents (fungi, insects) helps to minimise the application of fungicides and pesticides and may provide benefits to human and environmental health.

Social Outcomes

Barley contributes as one of the three main winter cropping options to the on-farm productivity of winter/summer crop rotations. Successful operation of grain farms can be vital to the health of rural communities in Queensland (QLD) and northern New South Wales (NSW).

Achievement/Benefit

a) Introduction

The DAQ00110 project was funded in 2006 to continue the improvement of the two-rowed, spring growth habit barley for the GRDC NR as BBA-North. The prior project, DAQ00038, was developed to transition the Northern Barley Improvement Program (NBIP) from a malting barley focus and to emphasise high yielding, disease resistant varieties. This change was based on an expanding demand for feed grains in the NR and a need to address feed quality issues. For the DAQ00110 project, the primary regional goal was well-adapted, high performance barleys that would address the consumers' need for both feed and malting barley varieties.

The northern region barley (NRB) breeding lines developed by the DAQ00038 project yielded competitively with introductions from other parts of Australia and Europe and had better resistance to foliar diseases. However, the NRB lines are still susceptible to other diseases and did not alleviate weather related issues such as drought, post-anthesis heat stress, grain size and lodging. Changes in the breeding objectives and procedures were made in DAQ00110 to facilitate meeting the demand for locally adapted barleys. In making these changes, the key genetic resource was two-rowed accessions from the barley program at North Dakota (ND) State University, Fargo, USA.

The first new crosses were made in 2007 to produce lines having adequate multiple resistances to foliar diseases, good agronomic traits, high yield and improved grain quality. Breeding lines from previous crosses were evaluated extensively and compared to the lines selected from the new crosses. Capturing all the desirable traits of the ND lines proved impossible during the first cycle of breeding. Intercrosses among promising lines were initiated in 2010 to accumulate more of the desirable attributes. Second cycle parents were selected based on phenotyping in yield trials, disease nurseries and grain quality tests and genotyping with whole genome scans based on Diversity Arrays Technology (DArT) molecular markers.

Changing the breeding materials precluded the release of new varieties during the term of DAQ00110. Lines selected from prior breeding materials lacked sufficient attributes to make them competitive as varieties. The homogeneity of lines from the 2007 crosses was not adequate to recommend their release. However, the application of new breeding tools has generated much improved breeding materials and the genetic information needed for the next step in developing regional adapted barley varieties.

b) Varieties released

Shepherd[A], released in 2008, was the only variety released. It was introduced as a breeding line from Western Australia but Shepherd was found to be a reselection from the European variety Baronesse during the release process. Shepherd yielded as well as Grout[A], released in 2005, but it matured slightly later. Shepherd has improved disease resistance, which was valuable during the leaf rust epidemic of 2010. Over the past few years, Grout and Shepherd have dominated barley production in the NR even though neither was accredited as malting barley.

The best lines from the first cycle crosses are reselections of NRB091124. The original line yielded similar to Shepherd, but matured later. The reselections have a semi-dwarf growth habit (the *sdw1* and *sdw4* genes) plus much better lodging resistance. They are resistant to four foliar diseases (powdery mildew, net and spot forms of net blotch and leaf rust) and have at least two genes associated with tolerance to heat stress, triple awn (*trp1*) and small lateral spikelets (*sls1*). The malt quality of NRB091124 was adequate to recommend continued evaluations of the reselects.

c) Core breeding and biotechnology

Core breeding activities centred on the generation of elite germplasm and the time required to select promising lines. The time factor was changed by growing more generations per year and reducing the degree of homozygosity required before lines are evaluated in yield trials or used as parents. Since a large number of desirable traits are in-

volved, early generation tests rapidly identified breeding lines for further evaluation, which have 'good' trait combinations.

Using off-season nurseries in glasshouses at Hermitage Research Facility (HRF) and in field nurseries at Glen Innes and Applethorpe permitted growing two generations per year. By transplanting seedlings after foliar disease screens, space-planting F2 progenies and establishing single hills from F2 spikes, the time from crossing to initial evaluations was reduced by two years. Screening of S1 trial entries for adult-plant reactions to four foliar diseases helped to rapidly identify promising lines. Molecular marker profiling of elite lines (S2 entries) provided confirmation of the phenotypic data, identified new gene-molecular marker linkages, and determined the presence of genes for which phenotyping is expensive.

The core activities each year included: making more than 200 crosses, producing F2 seed of over 150 crosses, growing about 100 space-planted F2 progenies and establishing bulk plots for 100 F2 progenies in off-season nurseries. Some 8,000 to 10,000 single hills from individual F3 spikes were sown each year. During the last three years of the project, 10 doubled-haploid populations were generated and evaluated.

d) Trial program

Changes initiated under DAQ00038 were continued and a few additional modifications were made. The number of trial sites remained at 16 for S3/4 trials across northern NSW and QLD and the number of entries in S3/4 trials was fixed at 49. Biplot analyses were conducted over sites and years to determine trends in entry performance over sites and years. Even though the planting dates varied by as much as two months, differences among varieties in yield ranks over locations were relatively small during wet years, while major changes in rank were observed during dry years.

The number of trial stages remained at three, with S1 being the preliminary yield assessment with approximately 1,200 entries, S2 being intermediate trials with about 400 entries and S3/4 being advance trials with 49 entries. The cross evaluation trial (CET) for testing the yield potential populations under DAQ00038 was not retained. The number of sites where S2 trials were grown remained about eight and S1 trials were established at two sites. To reduce the number of plots grown at each site, partial replication statistical models were employed using spatial design techniques and ASReml data analysis software across site x year analyses. These systems increased precision and improved the selection power, while keeping plot numbers at manageable levels.

Pedigrees and a line naming system were standardised to facilitate continued utilisation of bar coded electronic data capture systems. This increased nursery harvest efficiency, while the bar coded identifiers were used to trace each grain sample through the weighing, cleaning and quality assessments. The data management system also made possible linkage of the S2 trials system to molecular data collected under project DAQ00132 ('Integrating new technologies to enhance genetic gain in barley and sorghum breeding programs'). Quantitative trait loci (QTL) for numerous traits and associated molecular haplotypes were identified.

Seed increases were grown at HRF for all entries in yield trials (S1 entries in paired 6-m rows and S2 and S3/4 in paired 10-m rows) to produce seed supplies for trials grown the following year.

e) Foliar pathology

Control of foliar pathogens in the NR is best accomplished by accumulating genetic resistances. Routine screening of breeding materials for six foliar diseases (net and spot form of net blotch, leaf and stem rust, spot blotch and powdery mildew) was conducted by the foliar pathology group in both field nurseries and glasshouse experiments. Isolated field nurseries were established to screen for adult plant resistance (APR) in advanced material, S1 entries and crossing parents. Glasshouse tests were used to assess the seedling reactions of entries in S2 and S3/4 trials. Over 12,000 individual disease assays were conducted each year. The pathology team applied a high-throughput single plant screening system to selected F2 progenies to identify resistant seedlings from among the over 35,000 tested each year. The number of disease reactions for which seedlings were assessed was increased for three to six by using combined inoculations of three net form of net blotch isolates, spot form of net blotch and spot blotch. Surviving plants (about 100/cross for 20 crosses) were transplanted into a birdcage facility. This resulted in more lines with 'good' disease resistance and ones with better resistance to specific pathogens.

Adult-plants and seedling screens of lines in two doubled-haploid populations were conducted to map disease resistance genes. Resistance alleles were mapped and associated with specific DArT marker profiles. The loci identified

controlled reactions to leaf rust, stem rust, powdery mildew, net and spot forms of net blotch, spot blotch and scald. A relatively stable source of adult plant resistance to leaf rust, named *Rph20*, was found in many breeding lines, based on the presence of molecular marker bPb-0837. Combining *Rph20* with genes for seedling resistance could lead to the long-term control of losses caused by leaf rust.

f) Soilborne pathology

Breeding lines and varieties in S3/4 trials were screened for reactions to the crown rot, common root rot, covered smut pathogens and to root-lesion nematodes. The differences observed were not consistent over experiments, except for covered smut. Resistances previously identified in several *Hordeum vulgare* subsp.*spontaneum* backcross-derived lines did not appear to provide high levels of stable resistance to crown rot. Association mapping studies helped identify, without conducting screening experiments, breeding lines resistant to covered smut, barley stripe mosaic virus and cyst nematodes.

g) Quality assessment

The substantial restructuring of quality assessment during DAQ00038 has been maintained. The laboratory is still capable of processing over 15,000 whole grain samples and 3,000 malt samples per year. The BBA-North laboratory was the first Australian cereal chemistry laboratory to be certified with ISO9001 quality assurance accreditation (led through the Australian Malting Barley Centre project). Consequently, the breeding program has had access to a world class Quality Assurance (QA) system for all of its grain and malt quality samples and data.

Near infra-red (NIR) reflectance was used to analyse grain and malt quality traits throughout the breeding pipeline. Utilisation of the NIR data enabled earlier selection for grain and malt quality. This reduced the need to conduct resource intensive malting assays. Wet chemistry quality tests were necessary on only a select subset of advanced lines. The calibration equations for NIR provided by the *Premium Grains for Livestock Program* (PGLP) through the Pork Cooperative Research Centre (CRC) permitted evaluation of breeding materials and introductions for feed quality attributes. Fistulated cattle were used in one study of grain quality. The introductions from ND had some of the highest values for digestible energy and lowest for acid detergent fibre content.

Inheritance patterns of feed and/or malt quality parameters were studied in four mapping populations based on NIR data and calibrations for pigs and cattle. A QTL for starch content was found on chromosome 3HL in all populations. Kernel plumpness was associated with 6HL and 1HL. Protein content was associated with 2HS, hectolitre weight with 4HS and net energy for cattle with 5HS.

h) Industry development

The Barley Industry Development Officer (BIDO) played a leading role in the communication of project findings and outputs in the NR to all sectors of the value chain. These activities were funded by a separate GRDC project to promote barley cropping and optimise production. Under the DAQ00110 project, data was supplied for Annual Information Packages and Barley Planting Guides covering variety selection, targeting nitrogen (N) levels, plant populations, storing malt barley, planting time recommendations and herbicide sensitivity. These documents were made available as hard copy and were posted on the Department of Employment, Economic Development and Innovation (DEEDI) (now Department of Agriculture, Fisheries and Forestry (DAFF)) website. Data and information from trial results in northern NSW were supplied to the NSW Department of Primary Industries (NSWDPI). The BIDO also coordinated the Northern Region Barley Advisory Committee (NRBAC), a key industry consultative process, which assembled biannually.

i) Dual purpose barleys

In the NR, feed barley is grown as either a grain crop or as fodder in the form of direct grazing, hay or silage. Since lodging is a major production problem, growers decide at planting whether to target fodder or grain production based on water availability and N fertilization levels of individual paddocks. Shepherd is now the preferred variety for this use. The quality of fodder can be improved by awn removal using the hooded (*Kap1*) or awnless (*Lks1*) genes. The orange lemma (*rob1*) gene would reduce the amount of non-digestible lignin. These genes in combination should improve fodder quality and increase consumption. The *Kap1* and *rob1* genes have been incorporated into breeding lines with good foliar disease resistance and high yield potential.

j) Co-operative research

DAQ00110 involved cooperation activities for several related projects including the Australian Wheat and Barley Molecular Marker Program (AWBMMP) designed to study attributes needed for the future improvement of barley. Doubled-haploid populations were developed and scored for DArT molecular markers to map genes and QTL for resistance to leaf rust, stem rust, powdery mildew, net and spot forms of net blotch, spot blotch and covered smut. Some of this work involved international collaboration. Materials and support were provided to projects on exotic pests (Russian wheat aphid, barley stripe rust and UG99 race of stem rust), kernel defects (black point, dormancy and kernel discolouration), grain quality factors and morphological traits.

Other Research

Our knowledge of barley genetics has improved considerably and some traits have been associated with specific genomic regions, yet our understanding of genes and their interactions is still rudimentary. The challenge to barley breeders is expanding and translating our current knowledge of genes and traits into new varieties to which improved production practices can be applied. Plant breeding involves the placement of new genes and gene combinations in what Tom Ramage called a 'happy home' or a well-balanced biochemical system. Efficient genetic systems can better handle the range of climatic stresses encountered in target production areas. New genes must be integrated into genetic backgrounds specifically modified to enhance their expression. This concept opens many research opportunities.

The knowledge accumulated by the DAQ00110 project is an initial step in managing traits based on marker haplotypes. Traits that show complex inheritance patterns or have expensive evaluation protocols can be followed based on plant genotypes. Even though molecular markers offer a less expensive means of selecting for desirable genes, gene interactions and linkage drag have a large effect on the phenotypic expression of desirable traits. Germplasm resources and genetic knowledge do exist to undertake more in depth studies of genes and their interactions. Research on tolerance to heat stress should be a priority because heat stress limits barley production in the NR more than any other single factor except water supply.

Initial attempts to incorporate a new semi-dwarf gene (*sdw4*) from China into material adapted to the NR have produced promising results. Even though maturity is delayed, lodging is greatly reduced. The lodging potential of barley currently plays a large role in how the crop is managed and used in crop rotations. To further exploit the *sdw4* gene, it must be combined with earlier maturity and plumper kernels. A number of genes for early heading under short-day conditions have been identified, yet their interactions are poorly understood. The stay-green trait found in heat stress tolerant introductions could contribute to the lengthening of the grain fill period and plumper kernels.

If heat stress tolerance is improved, barley production in subtropical areas such as coastal and northern QLD should be revisited. Barley could be very useful as a cereal in soybean rotations. The two most important diseases in warmer and more humid production areas are spot blotch and Fusarium head blight or scab. Good resistance to spot blotch and partial resistance to scab exist in introductions used in the DAQ00110 project. Incorporating these resistances into heat stress tolerant germplasm would benefit barley crops in Australia.

Little progress has been made in developing material resistant to crown rot and common root rot because current screening techniques cannot handle large volumes of material. As lines better adapted to the NR are generated, the importance of these root diseases will increase. Research on more effective screening techniques and sources of resistance will be necessary before genetic resistances to soil-borne pathogens are accumulated.

Intellectual Property Summary

Intellectual property (IP) will be managed as per the BBA Cooperation Agreement, Commercialisation Policy and other policies as determined by the BBA Board. A legal agreement has been signed between DEEDI (as a component of BBA) and Pork CRC to protect the IP of all parties of BBA.

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Collaboration Details

Collaboration with the Nordic Genetic Resources Center facilitated maintenance of the 'Bowman' genetic stocks. Differential sets for resistance to net blotch and spot blotch were established by exchanging data and accessions. Data was collected in Uruguay on leaf rust and powdery mildew reactions for DH lines from HRF. Breeding lines were exchanged with researchers in Canada, South Africa, Syria (ICARDA), Uruguay and the USA. Material was sent to Kenya and Mexico via Prof. Colin Wellings for Ug99 stem rust and barley stripe rust screens, respectively. Screening for Russian wheat aphid resistance occurred in the USA. J Franckowiak was an IAEA consultant on fodder crops for developing countries.

Additional Information

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