MPBCRC: New markers, genetic mapping and QTL analysis (3.1.03c)

PROJECT DETAILS

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PROJECT TITLE: MPBCRC: NEW MARKERS, GENETIC MAPPING AND QTL ANALYSIS (3.1.03C)

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Summary

New molecular markers are constantly being developed by international and local research programs. In this project, 945 new markers have been added, producing updated, highly curated wheat and barley genetic maps, suitable for use in national breeding and germplasm development programs. The accurate localisation of these markers in Australian germplasm has provided breeders with greater ability to select genes during marker assisted breeding. Important marker-trait associations have been determined via genetic mapping and the control of key agronomic, disease and abiotic stress traits have been elucidated.

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Conclusions

Australia leads the world in molecular breeding of cereals in publicly funded programs. The advantages over classical breeding have been proven by the accelerated release of the first varieties developed using marker-assisted selection (MAS). To maintain this advantage, it is vital that breeders have the most up-to-date genetic knowledge and user-friendly marker systems at their disposal. This genetic knowledge and improved markers have been delivered by this project. For the first time, they have been delivered in real time via an internet-based server, in conjunction with the Molecular Plant Breeding Cooperative Research Centre (MPBCRC) bioinformatics group.

Barley:
To enhance existing genetic maps of barley previously used for identifying markers useable in molecular breeding, a new set of simple sequence repeat (SSR) and indel markers was added to the maps. These markers were developed through (i) database mining of barley expressed sequence tag (EST) sequences, (ii) comparative barley and rice genome analysis, and (iii) screening of a genomic library with SSR probes.

In the three years of this project, 254 new markers were added to seven barley maps that were created in the preceding six years, an overall increase in mapped markers of 13%. Importantly, 34 of these markers are closely linked to key genes and quantitative trait loci (QTL) identified for resistance to cereal cyst nematode (CCN), spot form of net blotch, leaf scald and improved malt quality. These are readily useable for marker-assisted barley breeding.

Wheat:
Two new maps of wheat have been constructed in approx. 18 months, compared to 3-5 years in the original National Wheat Molecular Marker Program (NWMMMP). More than 460 SSR and gene-based markers have been used to generate the new maps using traditional PAGE and emerging MultiPlex Ready Technology. At least 100 SSR markers have been added to existing Australian wheat maps and 328 Diversity Arrays Technology (DArT) markers have been incorporated into the Berkut *Krichauff map.

Improving the end use quality of wheat is a key target for many breeding programs. With the exception of the relationship between glutenin alleles and some dough rheological characters, knowledge concerning the genetic control of wheat quality traits is somewhat limited. The new maps generated from this project have allowed breeders to further dissect the end-use quality of wheat through the discovery of new QTL that will assist in the release of improved varieties faster. In conjunction with ACT, 14 QTL have been identified in the Trident* Molineux population. Six of these have not been detected before and will prove important for MAS based on CCN resistance, photoperiod sensitivity and improved bread making quality. Also of importance in ever changing climatic conditions are the markers for vernalisation genes, which have been mapped and utilised in wheat breeding programs for the first time in Australia. All the wheat maps are available to breeders and the use of markers is unencumbered.

Recommendations
To successfully implement molecular plant breeding, breeders require a continually improving understanding of crop
molecular genetics, and the appropriate tools to access and adopt this knowledge.

This project has continuously updated molecular genetics knowledge through the improvement of genetic maps, identification of new genes, and web-based provision of this information.

These activities must be continued if Australia is to maintain its world leading position in cereal molecular breeding.

Outcomes

Economic Outcomes:
Barley and wheat are the two most important cereal crops in Australia. Improved varieties will be delivered to the primary producer in a more timely fashion through the use of marker-assisted breeding. This will be more effective because of the greater number of informative, inexpensive markers linked to genes controlling important agronomic traits. Newly developed markers will be used to create new maps and improve the accuracy and reliability of existing genetic maps, to enable the discovery of critical marker-trait associations of use to researchers and breeders throughout the cereal community.

Environmental outcomes:
Varieties developed using MAS that target plant growth under stresses such as soil nutrient deficiencies and toxicity in addition to disease and water stresses will be better adapted to Australian conditions. This will lead to improved farming practices through the reduction of unnecessary seed and soil treatments and agronomic practices.

Achievements/Benefits

Existing maps:
Gaining an understanding of the genetic control of crop growth and yield can be achieved by mapping the genes involved in segregating populations using molecular markers. This project is adding to the pool of breeding-adapted molecular markers available for genetic analysis through mapping newly developed EST-derived and genomic-derived SSR markers. The primer set selected for this study comprised of 517 EST-SSR (eSSR) and 25 genomic SSR (gSSR) markers which were screened for polymorphism on seven doubled haploid (DH) or recombinant inbred line (RIL) populations. A total of 250 SSR (eSSR and gSSR) markers have been added to Australian barley maps, of which 195 are unique and nine of these were also mapped in wheat. Seventy three new EST-SSRs have been added to wheat maps increasing the density and availability of inexpensive selectable markers. A number of these new markers are linked to genes for resistance to diseases such as CCN, net blotch and scald and are ready for immediate implementation. These markers have been integrated into Australian genetic maps and the improved maps have been made available to barley breeders via a secure MPBCRC web page. These maps have already been used to identify new genes and QTL for a number of MPBCRC projects.

The RAC875-2*Cascades population continues to be an important resource for genetic analysis and has been improved in this project by the addition of extra markers. The RAC875-2*Cascades population was screened for septoria tritici and septoria nodorum blotch resistance in associated projects at a number of sites around Australia, as well as leaf, stripe and stem rust. Important resistance loci were identified for these diseases, including an unexpected major stem rust gene. Data were corrected for height and maturity effects where these were expected to contribute to the observed phenotype. These markers are available for immediate implementation by breeders.

Zinc (Zn) mapping in RAC875-2*Cascades revealed the following marker-trait associations:

- Field screening in Turkey revealed QTL for Zn content on chromosomes 1B, 5A and 6B.
- Field screening in Turkey and growth room seedling screening in Adelaide revealed conserved QTL on chromosomes 2B, 6A and 7B affecting Zn efficiency, visual scores, dry matter production and grain Zn content. The QTL analysis of Zn was confounded in this population by the segregation of the dwarfing genes on chromosomes 4B and 4D, which affected many of the measurements of other traits.

New maps:
In addition to adding new markers to existing maps, this project also created new maps and performed genetic analysis to determine the location of genes of value to MPBCRC-associated breeding programs. This year, theTrident* Molineux map has been completed, with all chromosomes covered by 271 markers. Most of these markers are SSRs and as such will be MAS user-friendly if associated with significant and reproducible QTL. Glutelin and vernalisation gene-based markers have been...
mapped and the vernalisation markers have proven to be useful in explaining peculiar growth habits with relation to extended photoperiod exposure during summer trials. In addition, the mapping of vernalisation markers in both Trident* Molineux and Berkut* Krichauff has led to an honours project that characterises Vrn genes in elite wheat germplasm. Phenotypic data were collected over four years and up to five sites for seven traits aiming to dissect the basis of Trident’s superior agronomic performance and Molineux’s superior quality characteristics. QTL analysis for CCN resistance confirmed the Cre8 CCN resistance and tolerance gene on chromosome 6B, but also a significant CCN resistance locus has been detected on chromosome 1B for the first time. This map has also been used to identify loci that contribute to septoria tritici blotch resistance, although the effect of plant maturity has confounded the analysis. Several new QTL have been found relating to wheat grain end-use such as dough strength, loaf volume, crumb quality and flour protein content which may provide fresh opportunities for the application of MAS to improve bread making quality. This map has been supplied to breeders and used to map a number of new genes for disease resistance, maturity, quality and yield components.

Mapping of the Berkut* Krichauff DH population has also been completed with 210 SSR and gene-based markers and 328 DArT markers positioned on the map. Approx. 130 of these SSRs are from the Multiplex Ready Marker platform. This population is being phenotyped and evaluated for yield in multiple environments in Australia, Mexico and other countries from 2006 as part of MPBCRC project 3.1.03b. Links to important traits such as drought and salt tolerance, and pratylenchus resistance are characteristics of this cross that will be further understood through QTL mapping. QTL analysis has evaluated fructan content, stripe rust, canopy temperature, height, maturity under drought and irrigated conditions and flag leaf erectness. Significant QTL were detected for fructan content on 7A, 6D, 4A and 2B, as well as for stripe rust on 2B. It is anticipated further screening of the population’s response to drought and irrigated conditions will yield more informative data.

Map curation:
In addition to adding new SSR markers to existing Australian wheat and barley maps, the project was also involved in the quality control of these maps in conjunction with Paul Eckermann of Biometrics SA. Many of the maps have areas of poor resolution, low statistical significance and conflicts with other published maps. As new markers are added, the marker order is re-assessed to try and increase the accuracy and reliability of the maps. There is also involvement in the curation and presentation of wheat and barley maps as part of a GRDC project led by Rudi Appels. This work aims to develop a consistent nomenclature and excellent quality control for maps. A number of issues have been identified, particularly in regard to alternative names for markers, correct labelling of maps and version control. These need to be addressed before all updated maps are placed into the public domain using the map viewer Cmap. This will allow fast and equal access to the most accurate and up to date wheat and barley maps for all MPBCRC participants, whether researchers or breeders.

Other research
The genetic maps, localised genes and QTL identified in this study can be used as a starting point for functional analysis and modification of genes to further improve wheat and barley crops. For example, in this project markers have been added to the Clipper*Sahara barley map that is being used to clone a boron tolerance gene in the Australian Centre for Plant Functional Genomics (ACPFG). This could be replicated for other genes identified in this project.

Intellectual property summary
NWMMP and National Barley Molecular Marker Program (NBMMP) maps are published and freely available. Markers obtained will either be unencumbered or will be subject to a Material Transfer Agreement (MTA) permitting research or commercial use.

Intellectual Property (IP) generated from QTL mapping will be of considerable value to both Australian and international groups.

Additional information