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## Construction of a genetic map and identification of markers for quantitative traits in barley (*Hordeum vulgare* L.)

Mehmet Cakir<sup>1</sup>, Nick Galwey<sup>1</sup>, David Poulsen<sup>2</sup>, Reg Lance<sup>3</sup>, Garry Ablett<sup>4</sup>, Joe Panozzo<sup>5</sup>, Barbara Read<sup>6</sup>, David Moody<sup>5</sup>, Andy Barr<sup>7</sup>, Paul Johnston<sup>2</sup>, Rodger Boyd<sup>1</sup> and Peter Langridge<sup>7</sup>

<sup>1</sup>Plant Sciences, Faculty of Agriculture, University of Western Australia, Nedlands WA 6907

<sup>2</sup>Queensland Department of Primary Industries, Hermitage Research Station, Warwick QLD 4370

<sup>3</sup>Agriculture Western Australia, South Perth WA 6151

<sup>4</sup>Centre for Plant Conservation Genetics, Southern Cross University, Lismore NSW 2480

<sup>5</sup>VIDA Private Bag 260 Horsham VIC 3401

<sup>6</sup>NSW Dept. of Agriculture Wagga Wagga NSW 2650

<sup>7</sup>Department of Plant Science, University of Adelaide, Glen Osmond SA 5064

## Abstract

A genetic map of barley with 195 AFLP and 37 SSR markers was constructed using a dihaploid mapping population between parental varieties Tallon and Kaputar. Linkage analysis of the markers revealed seven large linkage groups. These linkage groups were assigned to individual barley chromosomes with reference to the published map locations of the SSR markers.

This genetic map was used to identify markers that are linked to agronomic and quality traits in barley. The population, which comprised 65 lines, was tested in a range of environments across Australia. Quantitative trait loci analyses were performed using Mapmanager and Qgene software. Significant associations with markers were found for a number of traits. In particular grain yield, over a range of sites throughout Australia, showed significant associations on chromosomes 5H and 6H, and also with a marker not yet allocated to a chromosome ( $R^2 = 36\%$ ). Regions on chromosomes 2H and 5H explained 42% of variation in lodging. Among quality traits, diastatic power was associated with regions on chromosomes 3H and 5H ( $R^2 = 37\%$ ). Both hot water extract and alpha amylase activity were associated with a region on chromosome 2H with  $R^2$  values of 50% and 30%, respectively. The markers identified here present an opportunity for marker assisted selection of these traits in barley breeding programs.

## Introduction

Genetic maps have been used to identify markers for single-gene and complex traits that are otherwise difficult and expensive to select for in plant breeding programs (Philips and Vasil, 1999). Numerous studies have been conducted in barley to identify genetic markers for novel traits (Kretschmer et al., 1997; Zhu et al., 1998; Marquez-Cedillo et al., 2000). The ultimate aim of these studies is to improve simultaneously the yield potential and quality characteristics of barley.

The National Barley Molecular Marker Program has focused on the QTL analysis of malting quality, agronomic and disease resistance traits in a range of populations representing germplasm used across Australia. This study presents the results obtained from genetic map construction and QTL analysis of agronomic and quality traits from the Tallon/Kaputar population.

## **Materials and Methods**

### ***Plant Material***

The barley parent varieties Tallon and Kaputar were used to construct a DH population using the anther culture technique. The population comprises 65 lines. Tallon is a malting barley, bred in and adapted to the Northern Region of Australia. Kaputar is a feed barley, bred by CIMMYT and released for the Northern Region.

### ***Field Trials and Statistical Analysis of Phenotypic Data***

Agronomic and yield trials of this population were conducted by the barley breeding programmes throughout Australia in 1998 and 1999. Quality traits were measured in VIDA, Victoria on samples taken from 1998 harvest from five locations (Warwick, QLD, Charlick, SA, Wagga Wagga, NSW, Wongan Hills and Katanning, WA). Numerous traits were scored but the following only are included in this study: yield, lodging, broken straw, Zadok value, malt extract, diastatic power, protein content, hot water extract and alpha amylase activity

Phenotypic data for every trait studied were collated and checked. Biometrical analysis was then performed (including spatial analysis and multi-environment analysis in some cases) to maximise the precision with which genetic effects were estimated.

### ***Molecular Analysis***

DNA was isolated from 10-day-old plant tissues. Sixty-five lines were characterised genotypically with AFLP and SSR markers to construct a skeletal linkage map. Twenty-four primer combinations (MseI and PstI) were used for AFLP analysis (Vos et al., 1995), which included digestion, pre-amplification and selective amplification of DNA. SSR analysis was conducted by PCR amplification of the DNA with primers known to bracket SSR regions. SSR markers were selected from current published barley maps (Ramsaya et al., 2000; Karakousis et al., 2001) based on their distribution along the barley chromosomes

All marker loci were subjected to a chi-square goodness-of-fit test for segregation analysis using the software Qgene (Nelson, 1997) to determine whether the alleles occurred in the expected 1:1 segregation ratio. Linkage analysis of the markers was conducted using the software Mapmaker/exp (Lander et al., 1987) and Mapmanager (Manly, 2001). Linkages are established with a minimum LOD score of 3.0.

The genetic map with 232 DNA markers was used to identify QTLs associated with the traits. QTL analyses were performed using Mapmanager and Qgene software. A threshold LOD (logarithm of odds ratio) score of 3.0 was chosen for declaring the existence of a QTL. Wherever appropriate simple regression and interval mapping analysis were used to find the associations. Separate analysis for each site and a joint analysis over all sites were performed for each trait.

## Results and Discussion

### *Linkage map construction*

AFLP analysis revealed 211 loci polymorphic between the parental lines. From SSR analysis, 37 markers were found to be polymorphic between the parents. Sixteen out of a total of 248 polymorphic loci were found to have considerable distortion from the expected ratios and were excluded from the linkage analysis. SSR and AFLP markers were collated for linkage analysis. Analysis revealed 7 large and 7 smaller linkage groups (not shown). By reference to the location of the SSR markers the large groups were assigned to the seven barley chromosomes. These groups accounted for 175 out of 220 loci. Thirty-three loci were found to be unlinked to any of the linkage groups. In total the map covered 1300 cM, which is about the expected size of the barley genome.

### *QTL discovery*

About a third of the variation in grain yield, over a range of sites throughout Australia, was found to be associated with regions on chromosomes 5H and 6H and also with a marker not yet allocated to a chromosome. Regions on chromosomes 2H and 6H explained nearly half of the variation in lodging (Table 1). Among the quality traits studied, several showed significant associations with marker loci. In particular, diastatic power was associated with regions on chromosomes 3H and 5H. Malt yield showed significant associations on chromosomes 2H and 6H. Both hot water extract and alpha amylase activity were associated with a region on chromosome 2H (Table 1).

**Table 1. Chromosomal locations of, and % variation accounted for by, major QTLs for agronomic and quality traits in the barley doubled haploid population Tallon/Kaputar**

Trait	Chromosome	% variation (overall $R^2$ )
Grain yield	5H, 6H	36

Lodging	2H, 6H	42
Broken straw	2H, 5H	40
Zadok score	5H	45
Diastatic Power	3H, 5H	37
Diastatic Power (Katanning)	5H	50
Hot water extract	2H, 6H	30
Alpha amylase activity	2H, 7H	30
Protein content	3H,5H	52

Loci were also identified that were associated with the pattern of genotype  $\times$  environment interaction for these traits. For example, a principal component analysis of diastatic power identified a second component that contrasted Katanning with the other sites studied (Charlick, Blighty, Wagga Wagga and Wongan Hills), and the score on this component was associated with a region on chromosome 5H ( $R^2 = 50\%$ ). That is, an allele can be selected in this region of the genome that will confer specific adaptation to environments similar to Katanning. A similar pattern was found for hot water extract.

## Conclusions

DNA markers are being used as tools in marker-assisted selection of barley in breeding programs throughout Australia (Barr et al. 2000). The current project has identified a number of markers that are associated with important quantitative traits. In addition it was shown that SSR markers can be used to assign AFLP linkage groups to the individual chromosomes of barley. The regions of the chromosomes in which significant markers are located will be focal points of further research for validation and implementation of the markers for routine selection in breeding programs. The markers located in these regions could also be used in pedigree-based association mapping studies using diverse barley genetic resources. This process will allow the identification of markers associated with these traits in different genetic backgrounds

## Acknowledgements

Funding for this research is provided by GRDC through the National Barley Molecular Marker Programme. We are grateful to the Western Australian State Agricultural Biotechnology Center for providing laboratory facilities and the Scottish Crop Research Institute for providing the SSR markers.

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