

Mapping and QTL analysis of the barley population Tallon × Kaputar

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Abstract. A genetic map of barley with 224 AFLP and 39 simple sequence repeat (SSR) markers was constructed using a doubled haploid (DH) mapping population from a cross between the varieties Tallon and Kaputar. Linkage groups were assigned to individual barley chromosomes using the published map locations of the SSR markers as reference points. This genetic map was used to identify markers with linkage to agronomic, disease, and quality traits in barley. The population, which comprised 65 lines, was tested in a range of environments across Australia. Quantitative trait loci (QTLs) analyses were performed using software packages MapMaker, MapManager, and Qgene. Significant associations with markers were found for several traits. Grain yield showed significant association with regions on chromosomes 2H, 3H, and 5H over a range of sites throughout Australia. Regions on chromosomes 2H and 3H explained 30% and 26% of variation in lodging, respectively. Among quality traits, diastatic power was associated with regions on chromosomes 1H, 2H, and 5H ($R^2 = 37\%$). Hot water extract was associated with a region on chromosome 6H and a marker not assigned to a chromosome ($R^2 = 45\%$). There were also environment-specific QTLs for the traits analysed. The markers identified here present an opportunity for marker assisted selection of lines for these traits in barley breeding programs.

Additional keywords: SSR, AFLP, genetic mapping, quantitative trait loci.

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 (Thomas *et al.* 1995; Kretschmer *et al.* 1997; Zhu *et al.* 1998; Marquez-Cedillo *et al.* 2000). The ultimate aim of these studies is to simultaneously improve the yield potential, quality characteristics, and disease traits of barley.

The National Barley Molecular Marker Program has focused on the quantitative trait locus (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, disease, and quality traits from the Tallon × Kaputar population.

Materials and methods

Plant material

The barley varieties Tallon and Kaputar were used to construct a doubled haploid (DH) population using the technique of anther culture. The population comprises 65 lines. Tallon (Triumph/Grimmett) is a

Table 1. Pre-selective and selective primers, and their universal codes and sequences, used in the mapping of the Tallon × Kaputar population

Primer	Universal code	Primer sequence
<i>Pre-selective primer</i>		
<i>Pst</i> I-A	P01	5'-GACTGCGTACATGCAG A-3'
<i>Mse</i> I-C	M02	5'-GATGAGTCCTGAGTAA C-3'
<i>Selective primer</i>		
<i>Pst</i> I-AA	P11	5'-GACTGCGTACATGCAG AA-3'
<i>Pst</i> I-AC	P12	5'-GACTGCGTACATGCAG AC-3'
<i>Pst</i> I-AG	P13	5'-GACTGCGTACATGCAG AG-3'
<i>Pst</i> I-AT	P14	5'-GACTGCGTACATGCAG AT-3'
<i>Mse</i> I-CAA	M47	5'-GATGAGTCCTGAGTAA CAA-3'
<i>Mse</i> I-CAC	M48	5'-GATGAGTCCTGAGTAA CAC-3'
<i>Mse</i> I-CAG	M49	5'-GATGAGTCCTGAGTAA CAG-3'
<i>Mse</i> I-CAT	M50	5'-GATGAGTCCTGAGTAA CAT-3'
<i>Mse</i> I-CCT	M54	5'-GATGAGTCCTGAGTAA CCT-3'
<i>Mse</i> I-CGA	M55	5'-GATGAGTCCTGAGTAA CGA-3'
<i>Mse</i> I-CTG	M61	5'-GATGAGTCCTGAGTAA CTG-3'
<i>Mse</i> I-CTT	M62	5'-GATGAGTCCTGAGTAA CTT-3'

malting barley, bred in and adapted to the north-eastern region of Australia. Kaputar (5604/1025/3/Emir/Shabet//CM67/4 F₃ Bulk Hip) is a feed barley, bred by CIMMYT and released for the north-eastern region of Australia.

Field trials and statistical analysis of phenotypic data

Agronomic and yield trials of this population were conducted by barley breeding programs throughout Australia in 1998 and 1999. Quality traits were measured at VIDA (the Victorian Institute of Dryland Agriculture), Victoria, on samples taken from the 1998 harvest of trials at 5 locations (Warwick, Qld; Charlick, SA; Wagga Wagga, NSW; Wongan Hills and Katanning, WA). Assessment protocols were applied according to the Royal Australian Chemical Institute (2000). Numerous traits were scored, of which the following are reported here: yield, lodging, broken straw, basic vegetative period, maturity, Zadok value, net type net blotch, stripe rust, leaf rust, diastatic power, protein content, hot water extract, and α -amylase activity.

Phenotypic data for every trait studied were collated and checked using GENSTAT software (Genstat 5 Committee 1993). Statistical analysis was then performed (including spatial analysis and multi-environment analysis in some cases) to maximise the precision of estimating genetic effects.

Molecular analysis

DNA extraction

DNA was essentially carried out according to Rogowsky *et al.* (1991). Approximately 2 g of fresh leaf tissues from 10-day-old seedlings was ground in liquid nitrogen and incubated in 4 mL of Sarcosyl-based extraction buffer for 10 min. A volume of 4 mL phenol-chloroform-isoamyl (25:24:1) was added and then samples were homogenised for 10 min using a rotary centrifuge and then centrifuged for 10 min at 4000 rpm. After centrifugation, the aqueous phase was transferred to a clean 10-mL tube and subjected to a second phenol-chloroform extraction, as above. The aqueous phase was transferred to a clean tube and 4 mL of cold isopropanol was added to precipitate the DNA. Tubes were inverted by hand ~10 times and precipitated DNA was either spooled out or spun down and afterwards washed with 70% warm EtOH. After air-drying the DNA pellet, 400 μ L of TE buffer containing RNase (40 μ g/mL) was added to each sample to resuspend the DNA overnight.

AFLP analysis of genomic DNA

Amplified fragment length polymorphism (AFLP) analysis was carried out essentially according to the procedure of Vos *et al.* (1995) with minor modifications. Adapters were as described by Vos *et al.* (1995). The list of pre-selective and selective primers used in this study is given in Table 1. Eight hundred nanograms of genomic DNA were double-digested with the restriction enzymes *Mse*I and *Pst*I and ligated with the corresponding adapters. Total volume of the reaction was 60 μ L including 6 μ L of 10 \times restriction-ligation buffer (100 mM TRIS-HCl, pH 7.5; 100 mM magnesium acetate; 500 mM potassium acetate; 50 mM DDT), 1 μ L of each adaptor (5 μ M and 50 μ M for *Pst*I and *Mse*I adaptor, respectively), 5 U of each enzyme, 1.2 μ L of 10 mM ATP, and 0.2 μ L of T4 DNA ligase (5 U/ μ L). Samples were incubated at 37°C for 3 h using a PTC-100 Programmable Thermal Controller (MJ Research, Inc., Watertown, MA 02172, USA).

Pre-selective amplification reactions were performed in a 20- μ L volume, including 4 μ L of restricted-ligated template DNA, 1 μ L of each of the *Mse*I-C and *Pst*I-A pre-selective primers (75 ng/ μ L each), 5 μ L of 5 \times polymerisation buffer (Fisher Biotech International, Perth, W. Aust.) containing 67 mM TRIS-HCl (pH 8.8), 16 mM [NH₄]₂SO₄, 0.45% Triton X-100, 0.2 mg/mL of gelatin, 0.2 mM dNTPs, 1.2 units of *Taq* DNA polymerase, and 1.5 μ L of MgCl₂ (25 mM). PCR amplifications were carried out in the thermocycler as mentioned above, with 20 cycles of 94°C for 30 s (denaturation), 56°C for 1 min (annealing), and 72°C for 1 min (extension). Pre-selective polymerase chain reaction (PCR) products were diluted 7 times and subjected to electrophoresis on 1.5% agarose gel for checking the amplification quality.

Selective amplification reactions were carried out in a 20- μ L reaction mixture containing 5 μ L of pre-selective product as template DNA, 4 μ L of 5 \times polymerisation buffer, 1.2 μ L of MgCl₂ (25 mM), 0.5 U of *Taq* DNA polymerase, 0.15 μ L of fluorescent-labelled *Pst*I-XX primer (50 ng/ μ L), 0.45 μ L of unlabelled *Pst*I-XX (50 ng/ μ L), and 0.6 μ L of *Mse*I-XXX primer (50 ng/ μ L). For this amplification, a 'touch-down' cycle profile was used as 94°C for 30 s, 65°C for 30 s, and 72°C for 1 min, reducing the annealing temperature by 1°C per cycle for 9 cycles to 56°C. Once 56°C annealing temperature was reached, another 25 cycles were carried out to complete the selective amplification.

*Pst*I selective primers were labelled with FAM, TET, and HEX fluorocolours. This allowed us to assay 3 *Pst*I selective primers for each

*Mse*I selective primer on the same gel by mixing 1 µL from a reaction using each colour. The mixed PCR products were air-dried overnight at room temperature. The remainder of each PCR product was kept at 4°C.

AFLP gel analysis

AFLP marker products were separated by an ABI Prism 373 DNA sequencer (PE/Applied Biosystems) using 4.5% polyacrylamide gels containing 40 g urea, 9.5 mL 40% 19 : 1 acrylamide/bis, and 20.5 mL dH₂O. The urea was dissolved in warm water. One teaspoonful of resin was added to the urea solution and it was stirred for 5 min. The solution was mixed with 8 mL of 10 × TBE (pH 8.3) and 14 mL dH₂O, and degassed for 10 min. After adding and mixing thoroughly with the 400 µL of 10% ammonium persulfate and 45 µL TEMED, the gel solution was poured between glass plates and allowed to set for 2 h. While the gels were setting, the air-dried PCR samples were resuspended with 2 µL of loading buffer (1.1 µL formamide, 0.5 µL loading dye, 0.4 µL GeneScan 500 Tamra size marker). Following denaturation at 90°C for 3 min, samples were immediately chilled on ice. Samples were loaded onto the gels at 1.8 µL per lane.

GeneScan analysis software was used to analyse the AFLP markers. All marker loci were initially scored as 1s and 0s, indicating presence and absence of the bands. These data were converted to mapping data as As and Bs representing the maternal and paternal genotypes, respectively. The KeyGene standard primer list was used to name the AFLP markers (Table 1), as published at the web address: <http://wheat.pw.usda.gov/ggpages/keygeneAFLPs.html>. Relative molecular weight and the initial of the contributing parent were also used in the naming of the markers. Marker names with initials of both parents represented co-dominant markers. Molecular weight information given with the co-dominant markers represented the parental band with lower molecular weight.

SSR genotyping and analysis

Simple sequence repeat (SSR) analysis was conducted by PCR amplification of the DNA with primers known to bracket SSR regions. Candidate SSR markers were selected from current published barley maps (Becker *et al.* 1995; Liu *et al.* 1996; Ramsay *et al.* 2000) based on their distribution along the barley chromosomes.

PCR was conducted in 10-µL reaction samples, with MgCl₂ optimised for each primer to between 1.5 and 3.0 mM; 200 µM of each dNTP, 1 × PCR buffer (Boehringer Mannheim), and 0.4 U *Taq* DNA polymerase. Genomic DNA was optimised for each batch of samples but was generally 20 ng per sample. Thermal cycling conditions were as recommended with the publication of the primers (94°C for 3 min followed by 30–45 cycles of: 94°C for 30 s or 1 min; 45–58°C for 30 s or 1 min; and 72°C for 30 s or 1 min) (Ramsay *et al.* 2000) or by the touch-down method described above.

Either forwards or reverse primers were labelled with FAM, TET, or HEX ABI dyes. This allowed multiplexing of markers by colour and size on the same gel. Gel separation and detection of PCR products was performed on an ABI model 377 DNA sequencer or an ABI 310 Genetic Analyzer (capillary electrophoresis) using the internal standard GeneScan 500 (TAMRA). Genotyper 2.0 software (PE Applied Biosystems) was used for scoring of alleles.

Statistical analysis of marker data

SSR and AFLP marker data were collated for linkage analysis. All marker loci were subjected to a chi-square goodness-of-fit test for segregation analysis using Qgene software (Nelson 1997) to determine whether alleles were distributed in the 1 : 1 segregation ratio expected for a DH population. Linkage analysis of the markers was conducted using the software packages MapMaker/exp (Lander *et al.* 1987) and MapManager (Manly *et al.* 2001) using the Kosambi mapping function (Kosambi 1944). Linkages were established with a minimum LOD

(logarithm of odds ratio) score of 3.0. LOD scores <3.0 were used to predict loose linkages in some instances. Map positions of the AFLP markers were established through linkage with the SSR markers, using the previously published map locations of the SSRs as a reference.

The genetic map was used to identify QTLs associated with the phenotypic data. QTL analyses were performed using MapManager QTX (Manly *et al.* 2001) and Qgene (Nelson 1997) software packages. A threshold LOD score of 2.5 ($P = 0.001$) was chosen for declaring the existence of a QTL. Regression and interval mapping analyses were used to identify marker associations with the traits. Single and multiple site analyses were performed for each trait.

Results

Linkage map construction

Two hundred and nineteen AFLP loci and 39 SSR loci were revealed as polymorphic between the parental lines. Sixteen out of the total of 279 polymorphic loci were found to have considerable distortion from the expected ratios and were excluded from the linkage analysis. Linkage groups were assigned to the 7 barley chromosomes, by reference to the location of the SSR markers (Fig. 1). These groups accounted for 177 loci. Small linkage groups that included 59 AFLP markers could not be assigned to any of the barley chromosomes in the absence of SSR markers. Twenty-seven loci could not be assigned to any of the linkage groups.

QTL discovery

About a third of the across-site variation in grain yield was found to be associated with regions on chromosomes 2H and 3H. QTLs on 2H and 5H were associated with yield in 3 out of 5 environments. One QTL on each of chromosomes 2H and 3H was linked to lodging and broken straw traits (Table 2). Multiple regression analysis revealed that the same markers (p11m55K122 and p12m61TK118) from these regions had significant associations with lodging and broken straw, with total R^2 values of 42% and 40%, respectively. QTLs on 2H, 3H, 6H, 7H, and one marker not allocated to a linkage group explained 53% of the variation observed for basic vegetative period. Zadok score, a measurement of plant growth stage, appeared to be associated with QTLs on 2H and 3H in 2 environments. However, a location-specific QTL was also identified for Zadok score on chromosome 6H for the trial conducted at Blighty, NSW. The SSR marker EBmac0874 on 6H was linked to the maturity rating data collected from both Hermitage (Qld) and Toowoomba (Qld) (Table 2).

Several quality traits showed significant associations with marker loci. Based on the mean of data from all locations, both diastatic power and protein content were associated with regions on chromosomes 2H and 5H. Location-specific QTLs for diastatic power were identified on chromosomes 6H and 7H for the data obtained from Charlick, SA, and Blighty, NSW, respectively. Hot water extract was associated with a region on chromosome 6H ($R^2 = 30\%$) and a marker not yet assigned to a chromosome ($R^2 = 26\%$). Regions on

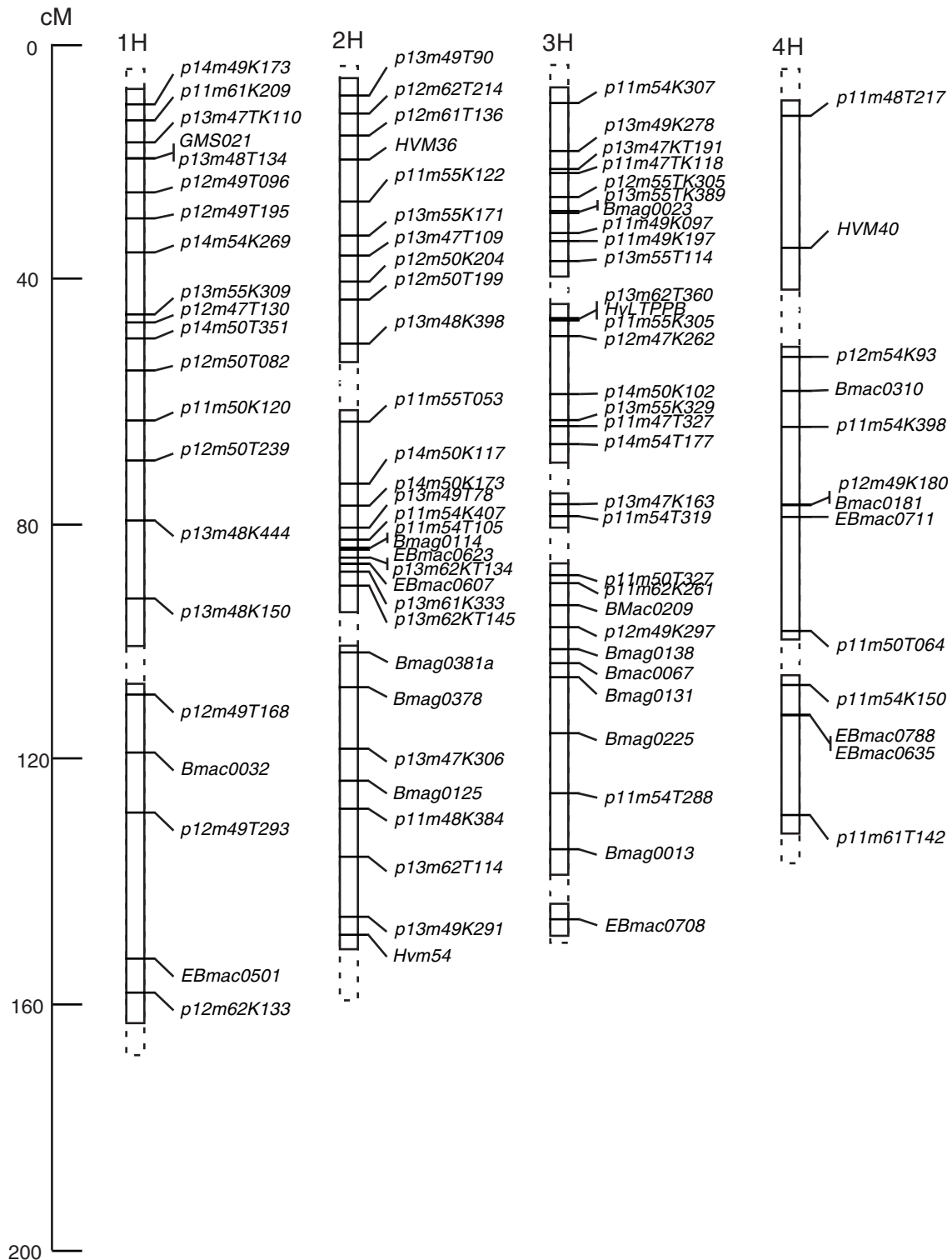


Fig. 1. Linkage map of barley chromosomes based on the Tallon × Kaputar mapping population. The scale in cM is shown on the left to indicate relative distances among the markers. Map distances were calculated with Kosambi function using software packages MapMaker and MapManager. AFLP markers were generated using *Pst*I and *Mse*I primer combinations (Table 1). Dashed lines were used to connect linkage blocks on chromosomes where linkages were less than a LOD score of 3.0. (Continued on next page.)

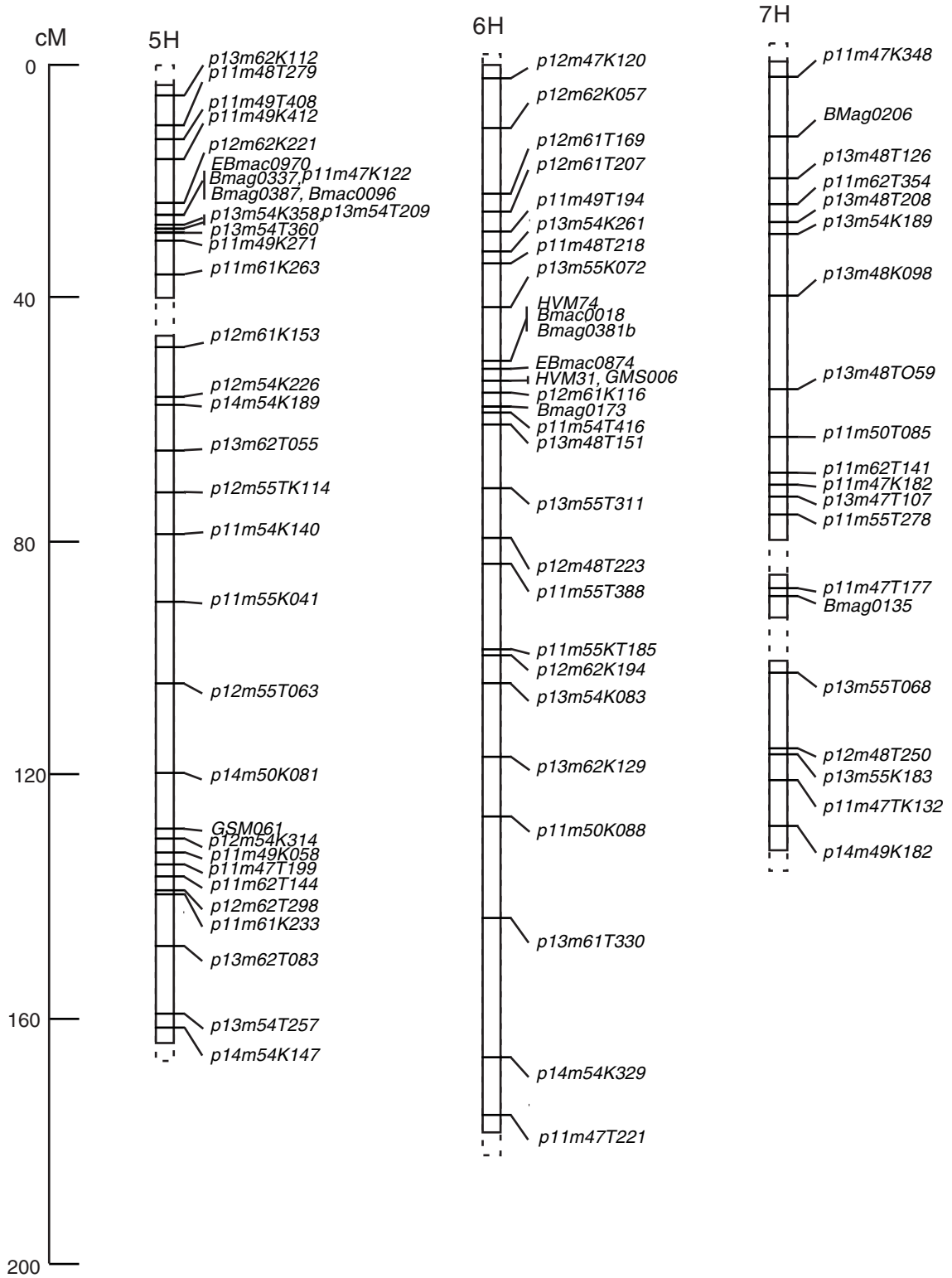


Fig. 1. (Continued).

Table 2. Chromosomal locations and percentage variation accounted for by major QTLs for agronomic, disease, and quality traits in the barley doubled haploid population Tallon × Kaputar

Trait	Location	Chrom.	LOD ^A score	% R ^{2,B} variation	Marker with greatest effect
Grain yield	Mean of locations	2H	2.95	20	p13m61K333
		3H	3.0	20	p11m54K307
		5H	2.60	18	p13m54KT257
Grain yield	Blighty, NSW	5H	1.95	12	p11m49K271
		5H	3.15	21	p13m54K058
Grain yield	Charlick, SA	2H	2.27	16	p13m61K333
Grain yield	Katanning, WA	5H	3.38	23	p14m50K081
		5H	2.11	15	p13m54T257
		NA	2.85	19	p11m54T262
Grain yield	Wongan Hills, WA	2H	3.03	25	p13m61K333
		3H	4.06	29	p11m54K307
		5H	3.53	24	p13m54T257
Lodging	Wagga Wagga, NSW	2H	4.68	30	p11m55K122
Broken straw	Wagga Wagga, NSW	3H	4.06	26	p11m47TK118
		2H	4.86	31	p11m55K122
Basic vegetative period	Shenton Park, WA	3H	3.20	21	p11m47TK118
		2H	3.98	28	p13m47T109
		6H	3.35	24	p12m61T207
Maturity	Hermitage, Qld	NA	3.89	28	p14m49K158
		6H	4.71	30	EBmac0874
		6H	6.6	40	EBmac0874
Maturity	Toowoomba, Qld	6H	6.6	40	EBmac0874
Zadok	Mean of locations	2H	8.39	46	p11m54T105
		3H	6.50	38	p11m47TK118
		2H	8.64	48	p11m54T105
Zadok	Hermitage, Qld	3H	4.86	30	p11m47TK118
		2H	5.49	39	p11m54T105
		3H	6.69	39	p11m47TK118
Zadok	Blighty, NSW	6H	3.41	23	Bmag0381
		2H	4.58	29	Bmag0114
		3H	5.04	31	p11m47TK118
Net type net blotch (seedling)	Glasshouse	6H	22.78	83	Bmag0381
		6H	13.71	65	EBmac0874
		2H	6.02	36	Bmag0114
Net type net blotch ^C (adult plant)	Hermitage, Qld	6H	13.71	65	EBmac0874
Stripe rust ^D (adult plant)	Toluca, Mexico	2H	6.02	36	Bmag0114
Leaf rust ^E (seedling)	Glasshouse	5H	15.20	68	p12m54K314
		2H	2.4	16	p14M50K117
		5HS	1.8	10	p12M54K226
Diastatic power	Mean of locations	5HL	3.1	21	p12M54K314
		1H	3.09	31	p13m48K444
		2H	2.84	28	Bmag0114
Diastatic power	Charlick, SA	5H	2.85	31	p12m54T257
		1H	3.87	32	p12m49T195
		2H	2.00	18	p14m50T209
Diastatic power	Blighty, NSW	6H	1.87	19	p14m54K329
		1H	5.43	37	p12m49T195
		1H	4.40	30	p12m49T195
Diastatic power	Wagga Wagga, NSW	5H	2.62	19	p12m54K226
		2H	4.46	40	Bmag0114
		5H	4.45	43	p12m54T257
α-Amylase activity	Mean of locations	2H	2.90	30	p11m55K122
Hot water extract	Mean of locations	7H	2.08	22	p11m50T085
		6H	3.00	30	p12m48T223
Protein content	Mean of locations	NA	2.50	26	p12m55T363
		2H	4.31	40	Bmag0114
		5H	3.82	37	p12m54K314

^A Logarithm of odds ratio.^B Phenotypic variation explained by each marker.^C For detailed discussion see Cakir *et al.* (2003a).^D For detailed discussion see Cakir *et al.* (2003b).^E For detailed discussion see Park *et al.* (2003).

chromosomes 2H and 7H were associated with α -amylase activity, with an R^2 of 30% and 20%, respectively.

Discussion

This study has generated a set of precisely sized AFLP markers that could be used in comparative mapping of other populations—a process that allows quick and cost-effective mapping of traits in different genetic material. In conjunction with bulk segregant analysis we have applied this approach for detecting and validating QTL regions in other populations (Cakir *et al.* 2003a, this issue).

All of the SSR markers were mapped to their published locations (Ramsay *et al.* 2000), with the exception of Bmag0381. This SSR marker was mapped on both chromosome 2H and 6H, in contrast to 2H only as reported by Ramsay *et al.* (2000). Possible reasons for this discrepancy are discussed elsewhere (Ablett *et al.* 2003, this issue).

Some of the AFLP markers were assigned to a specific chromosome based on their map locations in other genetic maps. This occurred in cases where the AFLP marker was not linked to any of the SSR markers. Previous research has shown that homologous AFLP fragments map on the same chromosomes in different populations (Waugh *et al.* 1997). The same conclusion was reached in our mapping of AFLP markers in different barley populations (data not shown).

Several significant QTL regions were identified from the Tallon × Kaputar population for different traits based on means of locations or years (Table 2). Loci were also identified that were associated with genotype × environment interaction for some of these traits. For example, principal component analysis of diastatic power identified a second component in which Katanning contrasted with the other sites studied (Charlick, Blighty, Wagga Wagga, and Wongan Hills). The expression of this component was associated with a region on chromosome 5H ($R^2 = 43\%$). In other words, the data indicated that an allele could be selected in this region of the genome that would confer specific adaptation to environments similar to Katanning. A similar pattern was found for hot water extract (data not shown).

DNA markers are being used as tools in marker assisted selection of barley in breeding programs throughout Australia (Barr *et al.* 2000; Cakir *et al.* 2003c). Several markers that were associated with important traits have been identified in the mapping of the Tallon × Kaputar population and reported here and elsewhere in this issue (Cakir *et al.* 2003a, 2003b; Park *et al.* 2003). In addition, the use of SSR markers to assign AFLP linkage groups to the individual chromosomes of barley was demonstrated. The regions of the chromosomes in which significant markers were located in this study will be focal points of further research for validation and implementation of the markers for routine selection in breeding programs.

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