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Mapping and QTL analysis of the barley population Alexis × Sloop

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Abstract. Two populations between the German malting variety Alexis and the Australian malting variety Sloop were constructed, mapped, phenotyped, and subjected to quantitative trait loci analysis. One population consisted of 153 F_4-derived recombinant inbred lines and the other of 111 doubled haploid lines. This paper describes 18 field and laboratory experiments conducted with the populations and summarises the traits mapped and analysed. The genetic basis of 5 traits (malt extract, resistance to leaf rust, resistance to powdery mildew, early flowering, plant stature) important to Australian efforts to improve malting barley varieties was elucidated. Detailed maps for these populations are shown in this paper, while a consensus map incorporating these maps and further experiments on the populations are described elsewhere in this issue.

Additional keywords: molecular marker assisted selection, quantitative trait loci.

Introduction

The single most important issue facing breeders of malting barley in Australia is the improvement of malt extract. Australian breeders have targetted Canadian, Japanese, and European varieties as the most likely donors of genes to improve malt extract. Hence, Alexis was chosen as one potential donor of such genes. Alexis was bred by Saatzucht Joseph Breun GDBR in Germany from the cross Br1622d54 × Trumpf (Friedt *et al*. 2000). Alexis has been a leading variety in Germany and neighbouring countries since its release in 1986. Its key features include spring growth habit, semi-dwarf stature (*sdw* gene), mildew resistance (*mlo* allele), wide adaptation, long basic vegetative period (bvp), high malt extract, low wort viscosity, and good diastatic power (it carries the *SD1* allele for β-amylase, Eglinton *et al*. 1998).

The other parent is the Australian variety Sloop (Barr 1998). Sloop was bred by R. C. M. Lance, D. H. B. Sparrow, and A. R. Barr in South Australia and released in 1995. Sloop has many desirable features for malting including good diastatic power (*SD1* allele for β-amylase, Eglinton *et al*. 1998), low wort viscosity, very low wort β-glucan, moderately high fermentability, and good free amino nitrogen. However, its malt extract, although equal to the Australian industry standard Schooner, is lower than Alexis. Sloop has moderately tall straw, plump grain, short basic vegetative period, early maturity, and adult plant resistance to net form net blotch. Sloop was reselected in F_6 from the F_2-derived breeders' line WI2875. It was previously known as WI2875-22. Another reselection, WI2875-1, was also considered, but ultimately rejected, for release. WI 2875-22 was used as one parent of the doubled haploid population, and WI 2875-1 was used as a parent of the recombinant inbred population. Table 1 shows the comparison of Alexis and Sloop for key traits when grown under South Australian conditions.

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Table 1. Comparison of Alexis and Sloop (WI2875-22, WI2875-1) for key traits when grown under South Australian conditions

Phenotype	Alexis	Sloop (WI2875-22, WI2875-1)
	Malt	
Extract	High	Moderate
Diastatic power	Moderate	Moderate high
Viscosity	Low	Very low
β-amylase isoform	SD1	SD1
Post-harvest dormancy	Moderate–high	Low
	Disease resistance	
Leaf rust	Moderately resistant	Susceptible
Mildew	Resistant	Susceptible
Spot form net blotch	Moderately susceptible	Susceptible
Net form net blotch	Moderate–resistance	Moderate adult-plant resistance, seedling susceptible
	Plant type	
Stature	Semi-dwarf	Moderately tall
Spikelet	2 row	2 row
Basic vegetative period	Long	Short
Early growth	Prostrate	Erect
	Grain size	
Size	Moderately small (av. 42 mg)	Moderately large (av. 48 mg)

Population construction

Initially, the cross WI2875-1 × Alexis was made at the Waite Campus in 1994. The population was progressed through F_2 and F_3 by single-seed descent. Two hundred recombinant inbred lines (RIL) were derived from F_4 single seeds. These were multiplied in 2-row by 4-m plots in 1998, to provide seed for the NBMMP field experiments in 1999 and 2000.

In Western Australia, the cross Alexis × Sloop (WI2875-22) was made in 1996 and the doubled haploid (DH) lines were produced from F_1 donor plants using the anther culture method in 1997 (S. Broughton and P. Priest, Western Australian Department of Agriculture, pers. comm.).

Hence, of the 264 individuals chosen for map construction and phenotyping, 153 were RIL from WI2875-1 × Alexis and 111 were DH lines from Alexis × Sloop. Table 2 shows markers used in construction of maps.

Construction of map

RFLP analysis

Plant DNA extraction was by a DNA mini-prep method adapted from Rogowsky *et al*. (1991). Restriction endonuclease digestion and Southern hybridisation followed standard methods. Total genomic DNA was digested with *Bam*HI, DraI, *Eco*RI, *Eco*RV, *Hind*III, and *Xba*I.

Table 2. Markers used in construction of maps

Marker type	Doubled haploids	Recombinant inbred lines
AFLP	187	177
RFLP	53	51
SSR (Uni. of Adelaide)	5	0
SSR (SCRI)	45	45
Other	1	1
Total	291	274

Deoxyribonucleic acid clones used as probes to detect restriction fragment length polymorphisms (RFLPs) were obtained through the Australian Triticeae Mapping Initiative (P. Sharp, University of Sydney) and from the repository of the International Triticeae Mapping Initiative. RFLP markers are typically designated Xabc, Xabg, Xawbma, Xbcd, Xbg, Xcdo, Xmwg, Xksu, Xpsr, XpTAG, Xwg.

Simple sequence repeat (SSR) analysis

Microsatellite markers were obtained from the published sequences of Maroof *et al*. (1994), Becker *et al*. (1995), Liu *et al*. (1996), Struss and Plieske (1998), Pillen *et al*. (2000), and Ramsay *et al*. (2000). Twelve new SSR markers prefixed Xawbms (Adelaide Waite Barley Microsatellite) were developed by Karakousis (2002). Most markers were chosen on the basis that they mapped to a single locus and gave high quality polymerase chain reaction (PCR) results. SSR markers are typically designated Xawbms, Xbmac, Xbmag, XEBmag, XEBmatc, Xgms, and XHV. PCR amplification and electrophoresis of microsatellites are described in Ramsey *et al*. (2000) or using the methods described by Karakousis *et al*. (2003, this issue) and Ablett *et al*. (2003, this issue). In brief, amplification was performed by a standard thermal cycling profile or by touchdown-PCR (Don *et al*. 1991). Separation of PCR products was performed on agarose gels, polyacrylamide gels, or by capillary electrophoresis.

Adapters and primers used in amplified fragment length polymorphism (AFLP) analysis

The AFLP method developed by Vos *et al*. (1995) was followed with some modifications. Genomic DNA (1 mg) was digested with the restriction endonucleases *Pst*I and *Mse*I and double-stranded *Pst*I and *Mse*I adaptors were ligated to the ends of the restriction fragments. Pre-amplification was performed using primers specific for the *Pst*I and *MseI* adaptors, including one selective nucleotide, followed by selective amplification using similar primers with 3 selective bases. The pre-amplification mix was diluted 1:5 in water before being used in the selective amplification step. Pre-amplification PCR conditions consisted of 20 cycles of 94°C for 30 s, 56°C for 1 min, and 72°C for 1 min. PCR reaction conditions for selective

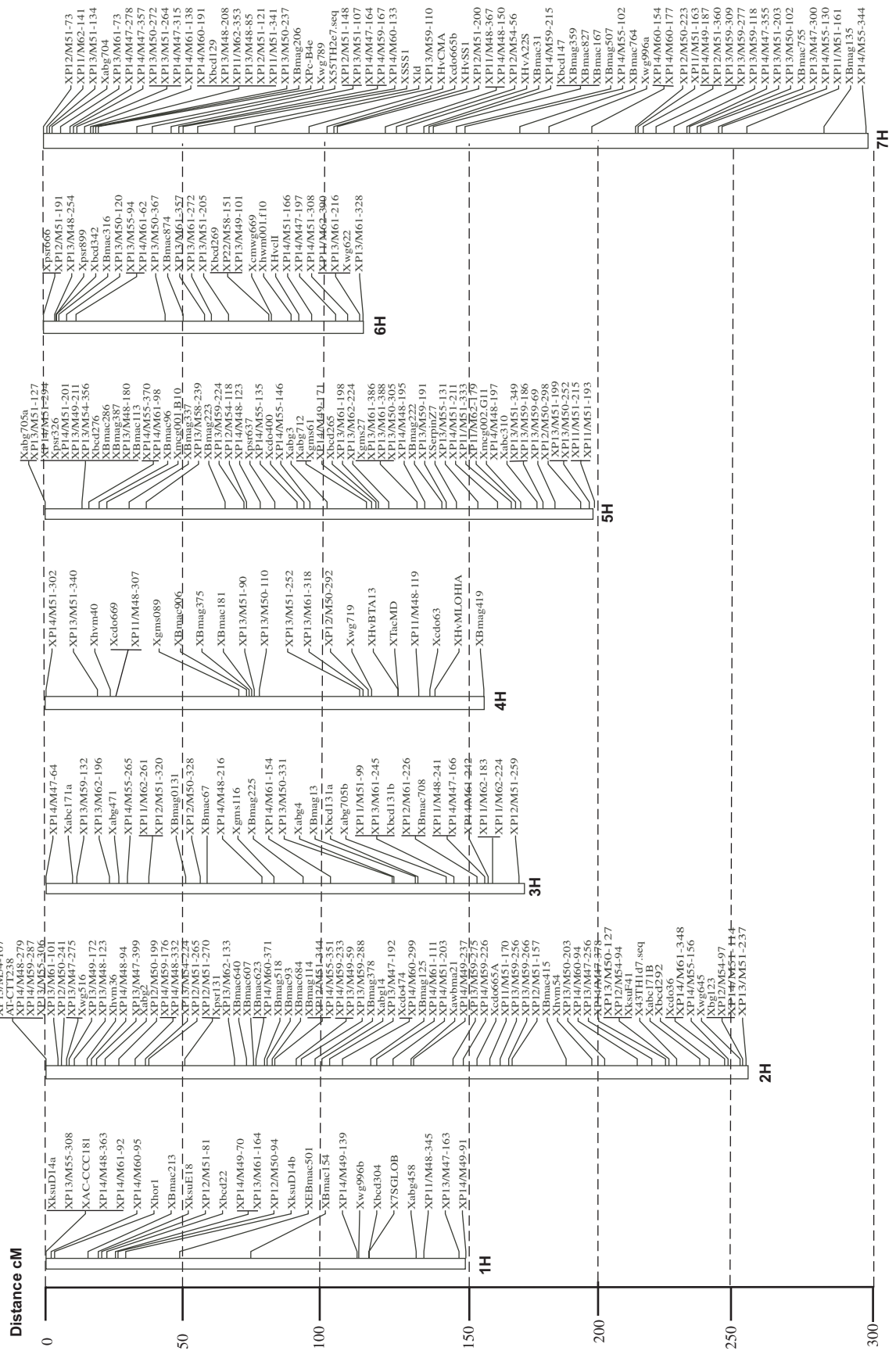

Fig. 1. Linkage map derived from the doubled haploid Alexis × Sloop lines.

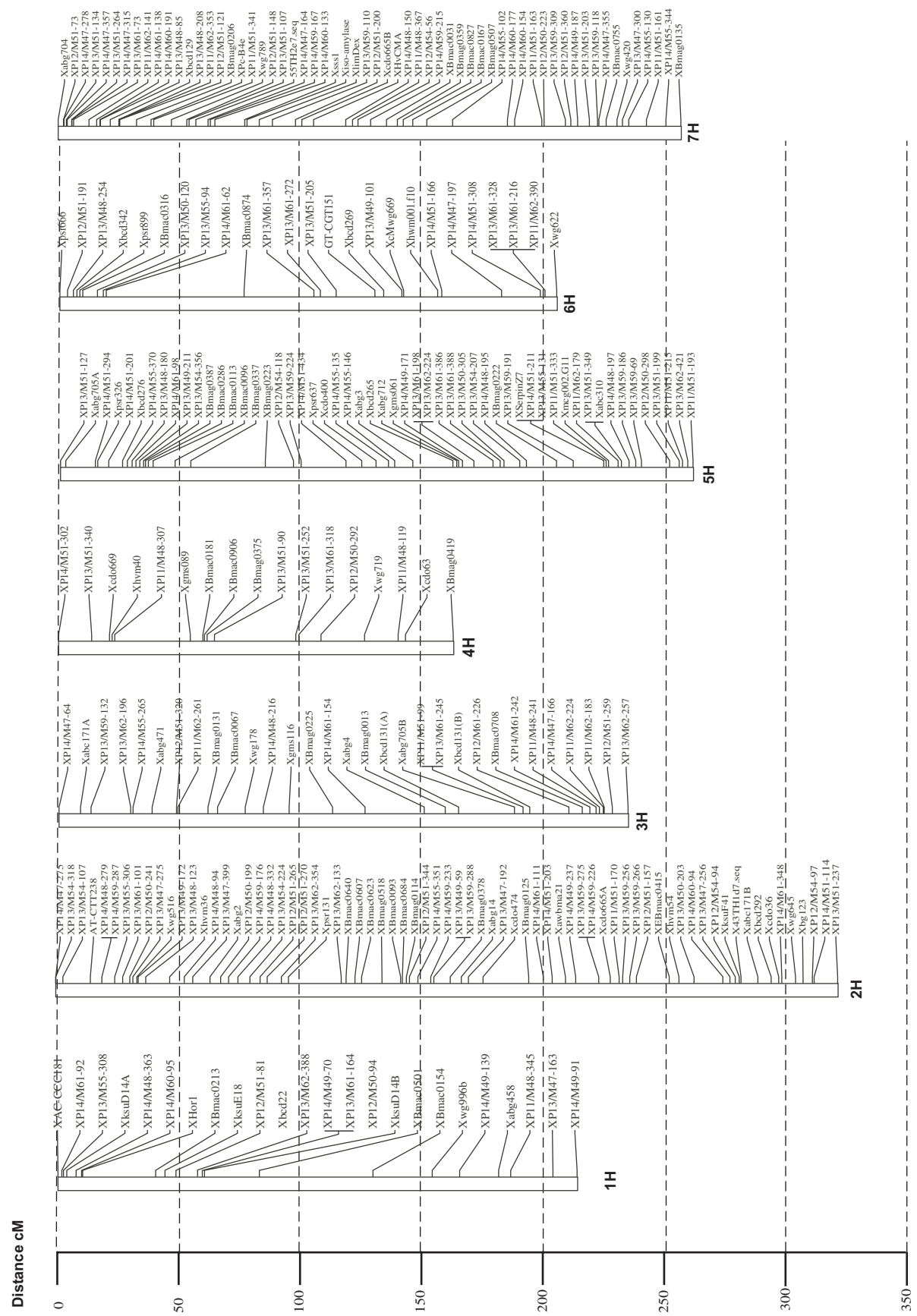

Fig. 2. Linkage map derived from the recombinant inbred Alexis × Sloop-sib (WI2875-1) lines.

Table 3. Experiments conducted with the Alexis × Sloop population

Type	Locations	State	Lat.	Long.	Aim of experiment
			1998		
Field	Charlick	SA	35	139	Seed multiplication, mildew resistance, maturity, plant structure
			1999		
Field	Charlick	SA	35	139	Grain yield and quality
Field	Katanning	WA	34	117	Malt quality, grain yield, development, field score, physiological spot, height, mildew
Field	Wongan Hills	WA	32	117	Malt quality, grain yield, development, field score, physiological spot, height, mildew
Field	Horsham	Vic.	37	142	Yield, development score
Field	Wagga	NSW	35	147	Yield, straw strength
Field	Hermitage	Qld	28	152	Yield, height, flowering, powdery mildew, physiological leaf spot
Field	STR				Yield, height, development, growth habit
			2000		
Field	Charlick, early sown	SA	35	139	Malt quality, yield, development, grain size
Field	Wongan Hills	WA	32	117	Yield, development, field score
Field	Katanning	WA	34	118	Yield development, field score
Field	Horsham	Vic.	37	142	Malt quality, yield, development score
Field	Bith	NSW			Yield, sprouting, height
Field	Charlick, late sown	SA	35	119	Yield, development, grain size
Glass-house	Hermitage	Qld	28	152	Net form net blotch, spot form net blotch
			2001		
Field	Minnipa	SA		135	Yield, grain size, development
Field	Paruna	SA	34	141	Yield, grain size, development
Field	Pt Wakefield	SA	34	138	Yield

amplification consisted of one cycle at 94°C for 30 s, 65°C for 30 s, and 72°C for 1 min, followed by 9 cycles over which the annealing temperature was decreased by 1°C per cycle with a final step of 25 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 1 min.

Three alternative protocols were used for visualisation of AFLP products. These were silver staining, radio-labelling with [γ^{32}P] ATP, or fluorescent tagging of the oligonucleotide primers.

Silver staining was carried out as described by Bassam and Caetano-Annollés (1993). For radio-labelling, the *Pst*I primer used in selective amplification was end-labelled with [γ^{32}P] ATP and T4 polynucleotide kinase. Amplified fragments were separated on 6% denaturing polyacrylamide gels. The gels were transferred to 3MM paper for drying and autoradiography was carried out with Fuji RX medical X-ray film at room temperature for 24–48 h.

For multifluorophore fragment analysis, *Pst*I primers were either labelled with 6-carboxy-fluorescein (6-FAM), tetrachloro-fluorescein (TET), or hexachloro-fluorescein (HEX), whereas the *Mse*I primer was unlabelled. One µL of each of the labelled PCR products was pooled. The combined sample was dried then mixed with 0.25 µL of a N,N,N′,N′-tetramethyl-6-carboxyrho-damin (TAMRA)-labelled internal length standard GeneScan-500 TAMRA (PE/Applied Biosystems) and 1.75 µL of formamide, denatured for 3 min at 90°C, and quickly chilled on ice. Electrophoresis of samples was carried out on 6% denaturing polyacrylamide gels in an ABI Prism 373XL DNA sequencer (PE/Applied Biosystems). For raw data collection, the ABI Prism Collection Software version I.I was employed. AFLP fragment analysis was performed with GeneScan analysis software version 3.1 (PE/Applied Biosystems), as described in the user's manuals. The nomenclature for an AFLP marker is derived from the enzyme combination, the primer combinations, and the relative molecular weight of the product. KeyGene provides the standard list of AFLP nomenclature at the following web address: http://wheat.pw.usda.gov/ggpages/keygeneAFLPs.html.

Linkage map construction

To maintain data quality, all molecular marker data were scored independently by at least 2 individuals. Data obtained from scoring segregation patterns of DNA markers among the DH and RI populations were analysed using MapManager QT (versions QTb27ppc and QTb28ppc, Manly and Cudmore 1997) using the Kosambi mapping

Table 4. Key trait–marker associations implemented in Australian Breeding programs

Trait	Locus	Chrom.	Positive allele	Reference
Malt extract	XEBmac 0501, AWBMS80	1H	Alexis	Collins *et al*. (2003, this issue)
	Xabg14	2H	Alexis	
	XAG-CTA191	5H	Alexis	
Leaf rust resistance	BMAC0113, Bmag387	5H	Alexis	Park *et al*. (2003, this issue)
	AG/CCA203	7H	Alexis	
Powdery mildew resistance *mlo*	HVmlophia	3H	Alexis	
Early flowering	Xabg2	2HS	Sloop	Coventry *et al*. (2003, this issue)
	Xabg14	2HL	Sloop	
Plant stature	Denso, Xbcd 131a, Xabg4	3H	Sloop, tall; Alexis, semi-dwarf	Coventry *et al*. (2003)

function (Kosambi 1944; Lander *et al*. 1987). Initial linkage groups were constructed using the 'rearrange report' function to determine the best order of the loci at a threshold value of $P = 0.001$. Subsequent markers were then integrated using the 'find best location' function. The number of double crossover events was then calculated for each linkage group, i.e. parental scores, which are flanked by alternative scores of the alternative parent (Säll and Nilsson 1994). Where an excess of 5 double crossover events were identified, the segregation data were re-inspected and, if necessary, corrected. Cross-referencing markers to published maps allowed the assignment of linkage groups to chromosomes. The maps for both the DH and recombinant inbred lines are shown in Figs 1 and 2, respectively.

Phenotypic data collected

Following seed multiplication in 1998, the full population was available for field experiments in 1999 and 2000. Experiments were conducted in up to 6 locations spread over 5 states (Table 3). Two experiments were chosen from each year on the basis of grain protein (preferably 9.5–12.0%) and grain plumpness for malting.

QTL analysis

MapManager QTX (Manly *et al*. 2001) was used to develop associations between markers and quantitative trait loci (QTLs). Statistical associations were based on regression analysis. The likelihood ratio statistic (LRS) was calculated using the interval mapping functions in MapManager QTX. Permutation analyses (1000 iterations) were carried out to determine whether a particular value of the LRS was highly significant (99.9%). The Q-gene analytical package (Nelson 1997) was used to confirm associations using interval mapping and maximum likelihood statistics [logarithm of odds ratio (LOD) values] and to generate graphical representations of maps and marker–trait associations.

Validation

Two major studies have been conducted to validate the effect of alleles identified from Alexis × Sloop. They involve the Alexis alleles associated with malt extract (Collins *et al*. 2003, this issue) and the effects of 3 loci controlling days to heading/photoperiod response on grain yield and stability (Coventry *et al*. 2003, this issue). These studies confirm that loci of crucial importance to breeding malting barley for Australian conditions were identified in this population.

Implementation

The most important traits identified in the Alexis × Sloop population are the QTLs controlling malt extract, although several other traits are also significant (Table 4).

Conclusions

The Alexis × Sloop population has been a valuable source of 9 marker loci, which have been implemented in Australian breeding programs (Table 4). Further, it has been a useful tool for comparing the suitability of DH and recombinant inbred lines for map construction and QTL analysis.

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