DEVELOPING MARKER-TRAIT ASSOCIATIONS IN THE POPULATION CHIEFTAN/BARQUE//MANLEY/VB9104, USING NOVEL TECHNIQUES

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INTRODUCTION

Genetic mapping in cereals has been exclusively applied to populations derived from simple crosses. Genetic studies are now beginning to target other population structures to take advantage of association mapping and whole genome analysis techniques. This paper describes an experiment in which 841 double haploid (DH) lines derived from the complex cross Chieftan/Barque//Manley/VB9104 are being genotyped with microsatellite (SSR) markers. The germplasm used in this experiment is a significant departure from typical mapping population structures. Phenotypic information is available for all 841 DH lines. More extensive phenotypic data is available for sub groups of these lines that advanced to successive stages in the breeding program. This population has been extensively phenotyped for malt quality and adaptation characteristics, through evaluation as a mainstream breeding population within the South Australian Barley Improvement Program (SABIP).

MATERIALS AND METHODS

Germplasm

Initial crosses between Chieftan and Barque, Manley and VB9104 were made, resulting in 'single cross' F_1 plants. These F_1 plants were then intercrossed, and 841 DH lines were derived from 9 different intercross F_1 plants. The number of these DH lines generated per plant ranged from 4 to 332 with 206 lines and 332 lines being the two largest sets.

Phenotyping

837 of the 841 DH lines were evaluated in double row trials in 1999, all lines were harvested, data collected on screenings and quality traits were assessed by near infra-red spectroscopy (NIR). 350 selected individuals were promoted to stage 1 yield trials in 1999, grown as 1 replicate at 3 sites, with 7 cultivars as grid checks. Agronomic observations were recorded, yield measured and IOB wet-chemistry quality data obtained. Under the same growing conditions as for stage 1 trials, 70 individuals were advanced to stage 2 yield trials in 2000. Data was collected as in stage 1 but with more detailed quality analyses. Ultimately 10 individuals were advanced to stage 3 yield trials in 2001.

Genotyping

The DNA was extracted based on a standard lab protocol using ball bearing method to grind the frozen leaf material. An SSR based marker system was chosen to genotype this population using tagged and untagged primers. These primers were designed for a touchdown PCR reaction with an annealing temperature of either 50^{o} C or 55^{o} C. Three tags FAM, HEX and NED were used to facilitate multiplexing, or simultaneous analysis of these markers. Labelled samples are electrophoresed on the ABI 3100 and analysed with Genotyper software. Unlabelled SSRs were analysed on 8% acrylamide gels, then visualised by staining

with ethidium bromide and photographed using a gel documentation system. Due to the size of the population a 384 well plate format was used in conjunction with the Corbett CAS-3800 pipetting robot.

RESULTS

The minimum density of SSR markers for linkage map construction have been selected based on their map positions around known QTL and specific traits of interest (Table 1).

Table 1. Quality scores and chromosome locations for barley varieties, Chieftan, Barque, Manley and VB9104.

	Chieftan	Barque	Manley	VB9104	Chromosome
Scald	R	S	S	MR	3H
SFNB	S	R	S	MR	7H
Mildew	R	MR	S	S	2H,4H
Height	SD	T	T	T	3H
Maturity	L	E	L	E	2H
Leaf rust	R	S	S	S	5H,7H
Lodging	R	MS		MS	2H
Extract	H	L	H	M	1H,2H,5H
DP	M	L	VH	M	1H,4H,5H
Viscosity	L	H	L	MH	1H
Fermentability	M	L	VH	M	4H,6H
CCN(Ha4)	S	R	S	S	5H
Yield	M	H	L	H	2H,3H,4H,7H
Grain size	SM	ML	M	VL	2H
ppd		sens		sens	2H
eps		E		E	2H

SFNB – spot form net bloch; DP - diastatic power; CCN – cereal cyst nematode; *ppd* – photo period response; *eps* – earliness *per se*; R – resistant; S – susceptible; MR – moderately resistant; MS – moderately susceptible SD – semi-dwarf; T – tall; L – late; E – early; H – high; L – low; M – moderate; VH – very high; MH – moderately high; SM – small to medium; ML – medium to large; M – medium; VL – very large; sens - sensitivivity

226 markers were screened across the parental germplasm of which 134 were polymorphic (88 fluorescent labelled and 46 unlabelled). 20 of these 134 polymorphic markers allowed us to distinguish between each parental barley genotype in this study i.e. produced 4 alleles (Figure 1). The polymorphism distribution between the four parents is detailed in Table 2.

We have been successful in optimising multiple PCR reactions, thus allowing up to 8 markers per reaction to be analysed simultaneously on the ABI 3100 (Figure 2). This group of 8 markers is referred to as a "kit" and each kit is formed based on the primers' tag and size of product generated. For this study we have organized the 88 labelled markers into 11 kits.

68 SSR markers were chosen, to form the basis of an initial skeletal map (48 fluorescent labelled, 20 unlabelled). To date, we have completed 4 kits, which have resulted in 24 labelled primers producing reliable results. Of the 20 unlabelled SSR markers, 10 have been completed and analysed. From these initial results it appears that there up to six alleles present for some markers, rather than the maximum of four that would be expected.

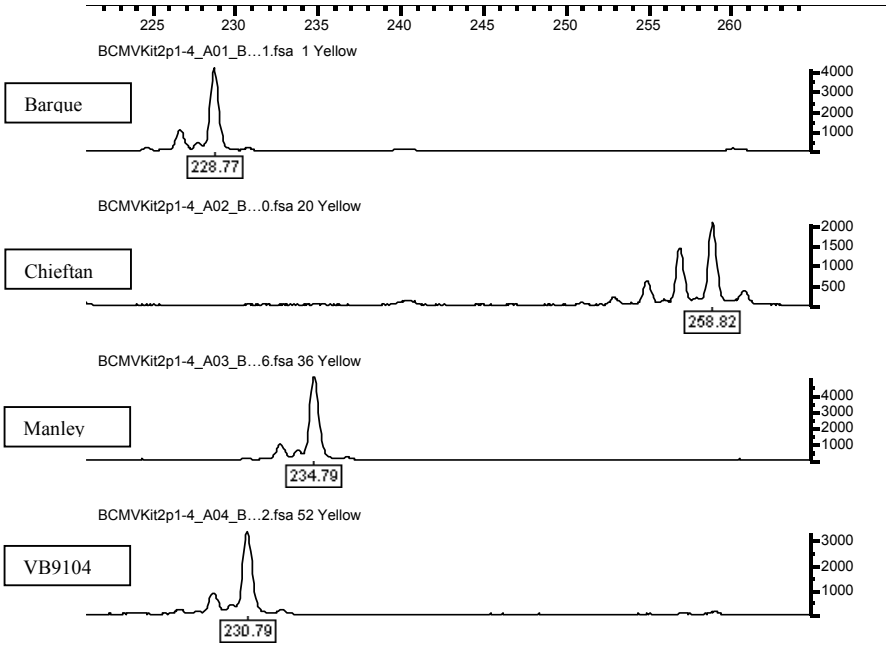

Figure 1. Results of parental screen using a fluorescent labelled SSR marker which distinguishes between the four barley genotypes.

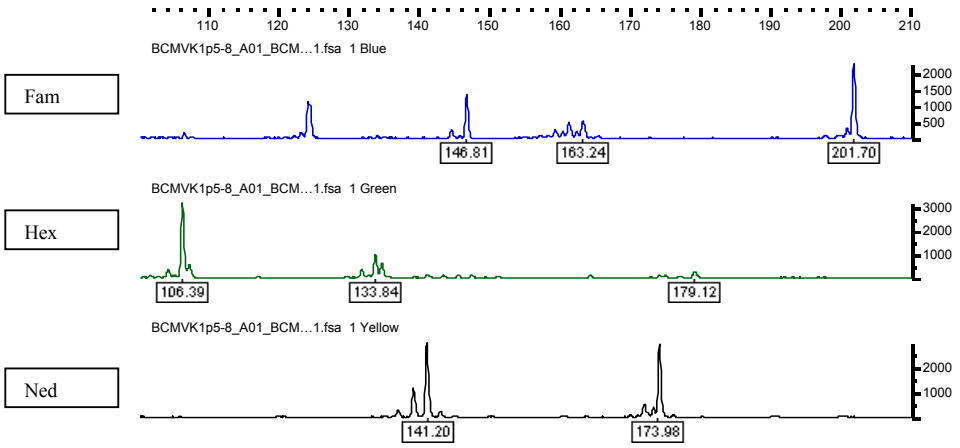

Figure 2. Multiplexing results with 8 SSR markers on parental genotype Barque.

Table 2. The polymorphism distribution between the four parents.

No. of markers	No. of alleles	Chieftan	Barque	Manley	VB9104
12	2	A	b	b	b
13	2	b	a	b	b
13	2	b	b	a	b
14	2	b	b	b	a
3	2	a	a	b	b
8	2	a	b	a	b
2	2	a	b	b	a
3	3	a	b	c	b
6	3	a	b	c	b
9	3	a	b	a	c
3	3	a	b	c	a
7	3	a	a	b	c
6	3	a	b	b	c
20	4	a	b	c	d

The same letter indicates no polymorphism. 15 polymorphic SSRs are yet to be fully analysed an are not included.

DISCUSSION

The full set of DH lines is similar to a very large conventional mapping population except that it is derived from one or more double-cross F_1 plants and from four parents that are not all necessarily homogeneous and homozygous. From the results to date, some markers appear to have more than four alleles present, this may suggest that one, or more than one of these parents could be heterogeneous and/or heterozygous. Preliminary analysis suggests the additional marker alleles are derived from Barque.

Using additional SSR markers, a high density map will be generated on a sub group of the initial 837 DH lines, comprising the 350 stage 1 lines. This will facilitate measurement of frequency changes to alleles in response to pragmatic selection, and to identify conserved linkage blocks associated with superior agronomic or quality phenotypes.

This analysis will determine the genetic basis of the elite line WI3408, which was a member of the largest family of 332 lines. WI3408 is currently being considered for commercial release, targeting medium-low rainfall production environments and premium export markets.

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