

## Mapping and QTL analysis of the barley population Chebec × Harrington

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**Abstract.** A doubled haploid population of 120 individuals was produced from the parents Chebec, an Australian 2-row barley of feed quality with resistance to the cereal cyst nematode, and Harrington, a 2-rowed, Canadian variety of premium malting quality. This paper describes 18 field and laboratory experiments conducted with the population and summarises the traits mapped and analysed. The genomic location of 25 traits and genes is described and marker–trait associations for 5 traits (malt extract, diastatic power, resistance to cereal cyst nematode, early flowering, resistance to pre-harvest sprouting) important to Australian efforts to improve malting barley varieties have been used in practical breeding programs. 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.

### 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, Harrington was chosen as one potential donor of such genes. Harrington was bred by the University of Saskatchewan and released in 1981 (Harvey and Rosnagel 1984). Harrington has been a leading variety in Canada and neighbouring states of the USA since its release in 1981 until 2001. Its key features include spring growth habit, tall stature, wide adaptation, long basic vegetative period (bvp), high malt extract, low wort viscosity, and very high diastatic power (SD1 allele for  $\beta$ -amylase, Eglinton *et al.* 1998). The genetic control of key traits in Harrington has also been studied in the population Harrington × TR 306 by the North American Barley Genome Mapping Project (Tinker *et al.* 1996; Hayes *et al.* 1997; Mather *et al.* 1997).

The other parent is the Australian variety Chebec. Chebec was bred by D. H. B. Sparrow and R. C. M. Lance in South Australia and released in 1992. Chebec has some desirable features for malting, including moderate levels of malt extract, high free amino nitrogen, low wort viscosity, and low wort  $\beta$ -glucan, but it was not approved by the Australian malting industry because of its low diastatic power (*SD2L* allele for  $\beta$ -amylase, Eglinton *et al.* 1998) and low fermentability. Chebec has moderately tall straw, plump grain, short basic vegetative period, early maturity, adult plant resistance to net form net blotch, and resistance to the cereal cyst nematode (*Heterodera avenae* Woll.). Chebec was previously known as WI2737.

### Population construction

Initially, the cross Chebec × Harrington was made at the Waite Campus in 1990. Doubled haploid plants were produced from F<sub>1</sub> donors using the anther culture system. These were multiplied in 2-row by 4-m plots in 1993, to

**Table 1. Comparison of Chebec and Harrington for key traits when grown under South Australian conditions**

Trait type	Phenotype	Chebec	Harrington
Malt	Extract	Moderate	High
	Diastatic power	Low	Very high
	Viscosity	Low	Low
	$\beta$ -amylase isoform	SD2L	SD1
	Post harvest dormancy	Low	Very low
Disease	Cereal cyst nematode	Resistant	Susceptible
	Spot form net blotch	Moderate seedling resistance	Susceptible
Plant type	Stature	Moderately tall	Tall
	Spikelet	2 row	2 row
	Bvp	Short	Long
	Early growth	Erect	Erect
Grain size	Size	Moderately large (av. 48 mg)	Moderately small (av. 42mg)

provide seed for the South Australian and National Barley Molecular Marker Program (a nationally coordinated and funded program in Australia) field experiments in the period 1994–96.

One hundred and twenty individuals were chosen for map construction and phenotyping. Table 1 shows the comparison of Chebec and Harrington for key traits when grown under South Australian conditions.

**Table 2. Markers used in construction of maps**

Marker type	Numbers
AFLP	47
RFLP	258
SSR	41
Other	2
Total	348

**Table 3. Experiments conducted with the Chebec  $\times$  Harrington population**

Type	Locations	State	Lat.	Long.	Aim of experiment
			<i>1996</i>		
Field	Strathalbyn	SA	35	139	Yield, grain, malt quality <sup>A</sup>
Field	Pinery	SA	34.5	138.5	Yield, grain, malt quality <sup>A</sup>
Field	Tarranyurk	Vic.	36	142	Yield, grain, malt quality <sup>A</sup>
Field	Hermitage	Qld	28	152	Yield
Field	Kaimkillenbun	Qld	27	151	Yield, decimal growth stage
Field	Gairdner River	WA	34	119	Yield
Field	Wongan Hills	WA	32	117	Yield, decimal growth stage
Field	Wagga	NSW	35	147	Yield, decimal growth stage
Field	Blighty	NSW	35.5	145	Yield, decimal growth stage
			<i>1995</i>		
Field	Balliang	Vic.	38	144	Yield
Field	Horsham	Vic.	37	142	Yield
Field	Kaimkillenbun	Qld	27	151	Yield
Field	Tipton	Qld			Yield
Field	Strathalbyn	SA	34	139	Yield
Field	Roseworthy	SA	34.5	138.5	Yield
Field	Wagga	NSW	35	117	Yield, decimal growth stage
Field	Wongan Hills	WA	32	117	Yield, decimal growth stage
			<i>2001</i>		
Field	Esperance	WA	34	122	Black point, pre-harvest sprouting, grain colour

<sup>A</sup>Grain and malt quality includes hardness, grain diameter, and grain weight (measured with SKCS system); grain protein; malt traits including soluble protein, wort  $\beta$ -glucan, Kolbach Index, free  $\alpha$ -amino nitrogen, diastatic power, IOB and EBC hot water extract and viscosity (J. F. Panozzo *et al.*, unpublished data).



**Table 4. Physiological traits and known gene markers mapped in Chebec × Harrington**

Trait	Locus	Chrom.	Reference
<i>Physiological</i>			
Cereal cyst nematode resistance	Ha2	2H	Kretschmer <i>et al.</i> (1997)
Powdery mildew resistance	PM	1H	B. Stephenson, pers. comm. (1998)
Leaf rust resistance gene	Rph19	7H	Park and Karakousis (2002)
<i>Mapped genes</i>			
$\alpha$ -L-arabinofuranosidase I (isoform I)	XAraI	2H	Lee <i>et al.</i> (2001)
Pollen specific gene family phalaris	XAWPPCB7B	5H	Baumann (1995)
Midgit clone from rye	Xawrm1	1H	Franki (1995)
WM1 gene family	XAwwm1	3H	Whitford (2001)
$\beta$ -amylase	Xbmy (1–2)	2H, 4H	Li <i>et al.</i> (1996); Li <i>et al.</i> (2002)
Cellulose synthase 5–3' fragment	XCesA(1,2,5a,6)	2H, 3H, 5H, 6H	R. Burton and G. B. Fincher, pers. comm. (2002)
Cellulose synthase-like gene	XCslE1(a,b)	3H, 5H	R. Burton and G. B. Fincher, pers. comm. (2002)
$\beta$ -glucan exo hydrolase isoenzyme exo 1	XEXO1a	5H	A. Harvey and G. B. Fincher, pers. comm. (1999)
$\beta$ -glucan exo hydrolase isoenzyme exo 2	XEXO2(c,d,e,f)	4H, 5H	A. Harvey and G. B. Fincher, pers. comm. (1999)
Sucrose transporter	Xfh11	4H	P. Whitfield, CSIRO, pers. comm. (2000)
$\beta$ -Gluconase	XGIV	3H	Li <i>et al.</i> (1996)
Galactosyl transferase gene	XhvtGalT(a,b,c)	4H, 3H, 4H	N. Farrokhi and G.B. Fincher, pers. comm. (2001)
Xylanase gene	X-I	5H	Banik <i>et al.</i> (1997)
Iso-amylase 5'	Xiso-amyl5p	7H	Burton <i>et al.</i> (2002)
Limit dextrinase	XLD	7H	Li (1997)
Managanese efficiency	XMnea,b,c	4H,7H	Pallotta <i>et al.</i> (2000)
Ds flanking region fragment from transposon-tagged population	XpA(2,3,6)	4H, 2H, 3H	T. Koprek, pers. comm. (2001)
Protein phosphotase	XPP	7H	Li (1997)
Disease resistance gene analogues	XRlch4N(a,c,d,e)	5H, 7H, 7H, 2H	Seah <i>et al.</i> (1998)
Resistance gene analogue, cDNA probe	XS120	2H	L. Madsen, pers. comm. (2000)
cDNA for soluble starch synthase 1	XSSS1	7H	S. J. Coventry, pers. comm. (2000)

**Table 5. Key trait–marker associations implemented in Australian Breeding programs**

Trait	Locus	Chrom.	Positive allele	Reference
Malt extract	Xabg57, XGMS01	5H	Harrington	Collins <i>et al.</i> (2003, this issue)
Diastatic power	Xabg57, XGMS01	5H	Harrington	Coventry <i>et al.</i> (2003a, this issue)
Cereal cyst nematode resistance	Aawbma21, Bmag125	2H	Chebec	Kretschmer <i>et al.</i> (1997)
Early flowering	Xabg2	2HS	Chebec	Coventry <i>et al.</i> (2003b, this issue)
	Xabg14, Xbmag140	2HL	Chebec	Coventry <i>et al.</i> (2003b)
Resistance to pre-harvest sprouting	Xabg57, XGMS01	5H	Chebec	Li <i>et al.</i> (2003, this issue)

### Construction of map

Methods used for DNA extraction, type of markers assayed, linkage map construction, and quantitative trait loci (QTL) analysis are as described by Barr *et al.* (2003, this issue). Table 2 summarises the markers used to construct the map. The total genetic length of the map is ~1330 cM. Distorted segregation ratios to the expected 1:1 were detected for specific chromosome segments located on 2H, 5H, 6H, and 7H. This observation has been previously documented by

Logue *et al.* (1995). Detailed maps of the 7 chromosomes are shown as Fig. 1.

### Phenotypic data collected

Following seed multiplication in 1993, the full population was available for field experiments in 1994, 1995, and 1996 and 2001. Experiments were conducted at 16 locations spread over 5 states (Table 3). Two experiments were chosen from the 1995 and 1996 experiments on the basis of grain

protein (preferably 9.5–12.0%) and grain plumpness for malting.

In addition to the field trial program, Chebec × Harrington has been extensively used in mapping, genetic, and physiological studies (Table 4) which, when combined with the North American Barley Genome studies of Harrington × TR306 and Harrington × Morex (Tinker *et al.* 1996; Mather *et al.* 1997), make the Harrington genome a very well characterised model.

### QTL analysis

MapManager QTX (Manly *et al.* 2001) was used to develop associations between markers and 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.

The yield data from 15 experiments conducted in 5 states of Australia were analysed using spatial models. Yield and yield stability were associated with the basic vegetative period locus on 2H (B. R. Cullis and A. B. Smith, unpublished data). Additional detailed analyses of the malt quality data by Patrick Lim, Joe Panozzo, Monica Radcliffe, Belinda Evans, Brian Cullis, and Alison Smith have also been conducted.

### Validation

Two major studies have been conducted to validate the effect of alleles identified from Chebec × Harrington. They involve the Harrington alleles associated with diastatic power (Coventry *et al.* 2003a, this issue) and malt extract (Collins *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.

A QTL for pre-harvest sprouting has been identified on chromosome 5H at the marker locus ABG57-GMS01, which explains over 70% variation for pre-harvest sprouting (Li *et al.* 2003, this issue). This marker was validated in a Stirling × Harrington doubled haploid population.

### Implementation

The most important traits identified in the Chebec × Harrington population are resistance to cereal cyst nematode (Kretschmer *et al.* 1997) and the QTL controlling malt extract and diastatic power, although other traits are also significant (Table 5).

### Conclusions

The Chebec × Harrington population has been a valuable source of many genes, QTLs, and marker loci, 5 of which have been implemented in Australian breeding programs (Tables 4 and 5). These marker loci have been very valuable in Australian breeding programs as breeders attempt to transfer resistance to cereal cyst nematode from Chebec and the malt quality traits from Harrington into new malting barley lines. Through the work of Karakousis *et al.* (2003, this issue), several simple sequence repeat markers are now available for monitoring these traits in Australian breeding programs.

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