

Agronomic and Breeding Value of Major Genes in an Introgression Program

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Introduction

To ensure the reliability of production and consistency of quality required by international markets, new malting quality varieties must offer growers agronomic advantages over both established malting and feed quality varieties. The newly released variety Sloop will assist in ensuring that Australian barley is more competitive in international markets but will not offer barley growers any significant agronomic advantage over Schooner. Consequently, a strategy has been initiated to improve the agronomic characteristics of Sloop. This strategy involves the progressive introgression of genes of agronomic importance into Sloop background. It is important, therefore, to understand and determine the agronomic value, extent of linkage drag and/or pleiotropic effects associated with each of the major genes to be introgressed. Preliminary results of this study are presented and discussed here.

Methodology

Genetic Material

Backcross populations involving the recurrent parent Sloop and the donor parents listed in Table 1 were developed. Molecular marker assisted selection was used to develop BC₂ populations for cereal cyst nematode (CCN) and Barley Yellow Dwarf Virus (BYDV) resistance, boron (B) tolerance and manganese (Mn) efficiency. Phenotypic selection methods were required for semi-dwarf plant type (BC₁) and scald resistance (BC₁).

Table 1. Traits and donor parents used in the backcrossing program

Trait	Donor Parent
Cereal Cyst Nematode resistance	Chebec
Leaf Scald resistance	Guardian, Halcyon, Sultan and Waveney
Barley Yellow Dwarf Virus resistance	Franklin
Boron tolerance	Sahara
Manganese efficiency	Amaji nijo
Semi-dwarfism	Skiff

From each of the backcross populations, except B-tolerance, 10-20 individuals carrying the gene for the desired trait, or closely linked marker, and an equivalent number of individuals not carrying the desired trait, were selected. For the B-tolerance stream, 72 backcross lines homozygous for all possible combinations of three marker alleles associated with QTLs found to confer B-tolerance in barley (Jefferies *et al.*, 1999), were selected.

Field experiments

The B-tolerance population was sown at a high B site at Minnipa in 1998 as a randomised complete block with two replicates and with Sloop check plots every fifth plot. Ten whole shoots were cut from every plot and B concentration determined by ICP. Plots were scored for leaf symptoms at anthesis. The CCN resistance and semi-dwarf populations were sown at two sites (Strathalbyn and Pinery) in 1998 as randomised complete blocks with two replicates. The Mn efficiency population was sown at a Mn deficient site at Marion Bay in 1998 as a split-plot (+/- Mn fertiliser), randomised complete block with two replicates and with Sloop every fifth pair of plots. The BYDV population was late sown (August) at Strathalbyn in 1998 as a split-plot (+/- disease), randomised complete block with two replicates and with Sloop check plots every fifth pair of plots. Straw from oat plants carrying heavy infection of viruliferous aphids were cut and spread on the '+ disease plots' while the '-disease' plots were sprayed with contact and systemic insecticides. Wheat plots were sown as a disease free buffer between every barley plot. Scald resistance populations were sown near Gawler in 1998, as a split-plot (+/- disease), single replicate nursery with Sloop every fifth pair of plots. Wheat plots were used as a disease free buffer. The '+ disease' plots were inoculated with infected straw at early tillering. Seed of the '-disease' split was treated with 1.5g/kg Baytan ® (tridimenol) and plots were treated at mid-tillering and early anthesis with 500 ml/ha Tilt ® (propiconazole) fungicide. Untreated plots were scored for disease severity on a 1-9 scale. All field experiments were harvested with a small plot harvester and grain yield and screenings percentages (<2.5 mm) were determined from the harvested samples.

Micro-malting and analysis

Samples of grain harvested from trials at Pinery were screened on a 2.5 mm screen and a sixty gram sub-sample micro-malted using a Phoenix System automated micro-malting facility. Malted samples were analysed for a full malting quality profile (Waite Barley Quality Evaluation Laboratory, Annual Report, 1997).

DNA extraction and marker screening

DNA extraction for marker screening was achieved using a DNA mini-prep method adapted from Rogowsky et al. (1991). Restriction endonuclease digestion, Southern hybridisation and polymerase chain reactions followed standard methods. RFLP markers associated with CCN resistance (*Xawbma21*), B-tolerance (*Xcdo370*, *Xwg405*, *Xwg114*, *Amy-1*) and manganese efficiency (*Xabg714*) and a PCR marker (*YLM*) for Barley Yellow Dwarf Virus resistance, were used to genotype the respective populations.

Statistical Analysis

Means for grain yield and screenings percentage were calculated, allowing for extraneous variation using spatial techniques developed by Cullis and Gleeson (1991). For each population, lines were grouped into classes based on either their marker genotype or their phenotype (scald resistance, semi-dwarf). Least-squares means were calculated on the spatially adjusted individual line means using a single factor ANOVA. The means for each class were compared using contrasts.

Results and Discussion

Boron Tolerance

While there were substantial differences between backcross lines for B-toxicity leaf symptoms, no significant difference in grain yield was observed. 21% ($P < 0.001$) and 14% ($P < 0.01$) of variation in screenings percentage (grain size), however, was accounted for by

leaf symptom score and whole shoot B concentration respectively. Jefferies et al. (1999) identified loci on chromosome 2H, 3H, 4H and 6H important in the control of B-tolerance in barley. Backcross lines carrying donor parent (Sahara) RFLP marker alleles at the 3H and 6H loci were not significantly different for grain size from lines carrying a Sloop allele at these loci (Table 2). In contrast, individuals carrying the 2H and 4H donor parent alleles produced significantly larger grain than those carrying the Sloop allele at these loci. In combination, the 2H and 4H loci accounted for a 17.2% difference ($P < 0.001$) in screenings losses (Table 3).

Table 2. Effect of donor and recurrent parent marker alleles on screenings losses (<2.5 mm) in BC_2 , F_2 derived lines at Minnipa, 1998.

	2H locus	3H locus	4 H locus	6H locus
Sahara allele	33.7	38.7	32.9	35.9
Sloop allele	41.1	36.4	39.9	40.4
Sign diff (P)	<0.005	N/S	<0.005	N/S

Table 3. Effect of donor and recurrent parent marker alleles at the 2H and 4H loci on screenings losses (<2.5 mm) in BC_2 , F_2 derived lines at Minnipa, 1998.

2H-Sloop, 4H-Sloop	2H-Sloop, 4H-Sahara	2H-Sahara, 4H-Sloop	2H-Sahara, 4H-Sahara
45.9 a ¹	36.3 b	36.0 b	28.7 c
¹ Different letters represent significant differences, 'a' and 'b' ($P < 0.005$), 'b' and 'c' ($P < 0.05$), and 'a' and 'c' ($P < 0.0005$)			

Semi-Dwarfness and CCN Resistance

There was no significant difference for grain yield between the donor and recurrent parent allele classes for either CCN resistance or semi-dwarfness. Grain size differences have, to this date, not been assessed. Significant differences ($P < 0.05$) between Chebec and Sloop were identified for DP, β -amylase and malt extract. Despite the difference between parents there was no significant difference between CCN resistant and CCN susceptible backcross lines for any of these traits. Significant differences between Skiff and Sloop were identified for DP, α -amylase, β -amylase, viscosity and FAAN. There was no significant difference between standard height and semi-dwarf backcross lines for viscosity, FAAN and β -amylase. Standard height backcross lines averaged 16% higher DP than semi-dwarf backcross lines. It is possible that a chromosome region associated with high DP, from Sloop, is in the proximity of the *denso* gene for semi-dwarf plant stature on chromosome 3H. It appears that neither the CCN resistance gene from Chebec nor the semi-dwarf gene from Skiff are associated with factors important in the control of malt extract, α -amylase, viscosity and FAAN.

Mn efficiency

Significant differences ($P < 0.001$) in grain yield were observed between the Mn treated and untreated plots for all control varieties including the Mn efficient Amaji nijo (untreated - 63% of treated) and WA73S276 (untreated - 77% of treated). There was, however, no relationship between marker genotype and Mn efficiency. Sloop is more efficient than the very inefficient cultivars Galleon, Barque and WI2585 and, while it is polymorphic for the RFLP marker at the Mn efficiency 4H locus, it may, in fact carry a different allele for the same gene at this locus or an additional gene of similar effect at an alternative locus. Seven of the backcross lines were found to be as efficient as Amaji nijo. Amaji nijo must differ from Sloop by at least one additional unmapped/unidentified Mn efficiency locus. It appears that the chromosome 4H, Mn efficiency locus from Amaji nijo, is not associated with either deleterious or advantageous factors related to grain yield or grain size.

Table 4. Effect of CCN marker allele and semi-dwarfness gene on the malt quality of BC₁ F₂ derived lines, Pinery 1998. (Significance of difference based on pairwise comparison between parents, and between classes within experiment only. Different letters represent significant difference at P<0.05).

CCN	No.	Extract	DP	α -amylase	β -amylase	Viscosity	FAAN
Sloop	2	77.3 a	436 a	93.5 a	340 a	1.91 a	122 a
Chebec	2	76.7 b	386 b	93.0 a	293 b	1.97 a	128 a
-marker	9	77.2 a	419 a	90.6 a	335 a	1.99 a	126 a
+marker	11	77.1 a	402 a	91.7 a	317 a	1.97 a	119 a
S-dwarf							
Sloop	2	76.6 a	463 a	90.0 a	372 a	1.72 a	123 a
Skiff	2	75.1 b	407 b	67.5 b	340 a	2.31 b	79 b
Tall	8	75.8 a	423 a	87.2 a	336 a	1.80 a	116 a
S-Dwarf	5	76.6 a	364 b	80.5 a	283 a	1.83 a	112 a

BYDV Resistance/Tolerance

A 71% difference in grain yield and 39% difference in screenings percentage was related to the presence of a PCR marker allele (*YLM*) for the Yd2 BYDV resistance gene derived from Franklin (Table 5). The +*YLM* class was significantly higher yielding than the –*YLM* class in plots where disease was controlled. Some disease symptoms were observed in the –disease plots and the yield difference could be attributed to low level of disease infection. It appears that the Yd2 gene from Franklin is not associated with deleterious factors related to grain yield or grain size.

Table 5. Effect of *YLM* marker allele on grain yield and screenings losses (<2.5mm) in BC₂, F₂ derived lines infected with BYDV at Strathalbyn, 1998. Significance of difference (P) between ‘+disease’ and ‘-disease’ provided in table. Different letters represent significant difference of pairwise comparison between marker allele classes at P<0.001.

	Marker allele	- Disease	+ Disease	% of - Disease	Sign of diff (+/-) (P)
Grain yield (g/plot)	- <i>YLM</i>	1023 a	316 a	29	<0.0001
	+ <i>YLM</i>	1257 b	1096 b	87	<0.05
Screenings	- <i>YLM</i>	37 a	71 a	34	<0.0001
Losses %	+ <i>YLM</i>	28 a	31 b	3	N/S

Scald Resistance

While severe leaf scald infection was achieved, very dry spring conditions resulted in relatively large effects of disease control on grain size but only moderate grain yield responses. The spatially adjusted mean yield difference between diseased and disease controlled Sloop plots was 17% while the difference in screenings losses was 34% (Table 6). Leaf symptom score accounted for 27% of the variation in grain yield and 48% of variation in grain size in the disease infected plots. No significant difference in grain yield was observed between backcross families in the disease free plots. The Sultan BC₁ family, however, averaged only 90% (P<0.0001) of the grain yield of the recurrent parent Sloop. The Sultan family also produce significantly less screenings losses than the other families but not Sloop. The Guardian family produced significantly higher screenings losses than Sloop. There was no significant difference in grain yield or screenings losses between resistant and susceptible lines within each family in the disease free plots (Table 7). It appears therefore, that the scald resistance genes are not associated with deleterious factors related to lower grain yield in the Sultan family and smaller grain size in the Guardian family.

Table 6. Mean grain yield and screenings losses of BC₁ families in disease free plots, Kinsford, 1998

Family	Number of lines	Grain Yield (kg/plot)	Screenings losses (2.5mm)
Guardian BC₁	18	3.27 a,b	22.0 a
Halcyon BC₁	15	3.27 a,b	18.5 a,c
Sultan BC₁	18	3.07 b	13.1 b,c
Waveney BC₁	13	3.25 a,b	19.6 a,c
Sloop	10 reps	3.39 a	16.3 a,b,c

Table 7. Mean grain yield and screenings losses (<2.5mm) of BC₁ families in leaf scald infected and disease free plots, Kinsford, 1998. Significant differences for pairwise comparisons between resistance classes (S-MS and MR-R) for individual families only.

Trait	Family	Res/Sus	+ Fungicide	Sign (P)	- Fungicide	Sign (P)
Grain yield (kg/plot)	Guardian BC ₁	S-MS	3.35	N/S	2.50	<0.05
		MR-R	3.18		2.77	
	Halcyon BC ₁	S-MS	3.20	N/S	2.65	N/S
		MR-R	3.27		2.86	
	Sultan BC ₁	S-MS	3.29	N/S	2.53	N/S
		MR-R	3.00		2.65	
	Waveney BC ₁	S-MS	3.23	N/S	2.73	<0.01
		MR-R	3.15		3.13	
Screenings losses (2.5mm)	Guardian BC ₁	S-MS	23.3	N/S	58.9	<0.0001
		MR-R	20.9		37.2	
	Halcyon BC ₁	S-MS	22.8	N/S	51.9	<0.0001
		MR-R	18.6		23.3	
	Sultan BC ₁	S-MS	13.3	N/S	36.1	<0.0001
		MR-R	12.9		20.0	
	Waveney BC ₁	S-MS	19.4	N/S	43.9	<0.001
		MR-R	19.6		29.7	

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