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S.P Jefferies · M.A. Pallotta · J.G. Paull
A. Karakousis · J.M. Kretschmer · S. Manning
A.K.M.R Islam · P. Langridge · K.J. Chalmers

Mapping and validation of chromosome regions conferring boron toxicity tolerance in wheat (*Triticum aestivum*)

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Abstract Boron is an essential plant micro-nutrient which can be phytotoxic to plants if present in soils in high concentration. Boron toxicity has been recognised as an important problem limiting production in the low rainfall areas of southern Australia, West Asia and North Africa. Genetic variation for boron toxicity tolerance in wheat has been well-characterised. The efficiency of breeding for boron toxicity tolerance could be greatly enhanced by the development of molecular markers associated with QTLs for tolerance in wheat. A population of 161 doubled haploids from a cross between the tolerant cultivar Halberd and the moderately sensitive cultivar Cranbrook was used to identify chromosomal regions involved in boron tolerance. A combined RFLP and AFLP linkage map of the Cranbrook x Halberd population was used to identify chromosomal regions involved in the boron tolerance traits measured. Regions on chromosome 7B and 7D were associated with leaf symptom expression. The region on chromosome 7B was also associated with the control of boron uptake and with a reduction in the effect of boron toxicity on root-growth suppression. RFLP markers at the chromosome 7B and 7D loci were shown to be effective in selecting for improved boron tolerance in an alternative genetic background. Halberd alleles at the chromosome 7B locus were associated with the concentration of boron in whole shoots and grain. The concentration of boron in whole shoots and in grain were both related to grain yield in a field trial conducted on soil containing toxic levels of boron. Implications relating to marker-assisted selection for boron toxicity tolerance in wheat are discussed.

Key words Boron toxicity · Boron tolerance · Mapping · Wheat · Marker-assisted selection

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S.P. Jefferies (✉) · M.A. Pallotta · J.G. Paull · A. Karakousis
J.M. Kretschmer · S. Manning · A.K.M.R. Islam · P. Langridge
K.J. Chalmers
Department of Plant Science, Waite Campus,
University of Adelaide, South Australia 5064 and CRC
for Molecular Plant Breeding, Waite Campus,
South Australia 5064, Australia

A. Karakousis · J.M. Kretschmer · S. Manning · P. Langridge
K.J. Chalmers
ARC Special Research Centre for Basic
and Applied Plant Molecular Biology, Waite Campus,
University of Adelaide, South Australia 5064, Australia

Introduction

Boron (B) is an essential plant micro-nutrient which can be phytotoxic to plants if present in soils in high concentration. Boron toxicity to crop plants has been recognised since the early 1930s (Christensen 1934), yet it was not until 1984 that it was first recognised in southern Australia in barley growing under dryland conditions (Cartwright et al. 1984). High concentrations of B have been recorded from soils and plant samples collected from widespread regions of the cereal growing districts of southern Australia (Ralph 1992). Boron toxicity has also been recognised as a problem in the dry regions of West Asia and North Africa and a problem associated with irrigation water in many other parts of the world (Gupta et al. 1995).

A 17% difference in the grain yield of adjacent areas of barley was related to differences in the concentration of B in shoots just prior to anthesis (Cartwright et al. 1984). Moody et al. (1993) estimated that wheat yield losses of up to 11% could be attributed to B toxicity in southern Australia. Paull (1990) found that wheat plants exposed to high concentrations of B, under glasshouse conditions, responded with reduced vigour, delayed development, leaf symptoms which include yellowing of leaf tips of older leaves followed by non-specific necrosis continuing down the leaves and reduced total dry matter and grain yield.

The concentration of B in soils in southern Australia has been shown to increase with depth (Cartwright et al. 1984, 1987). The occurrence of B at depth in the soil profile precludes amelioration through soil modification.

The breeding of tolerant wheat cultivars, therefore, offers the most feasible option for reducing losses in yield and quality associated with B toxicity.

Genetic variation in tolerance to B toxicity in wheat has been reported by Paull (1985) and Moody et al. (1993). The bread wheat cultivar Halberd is among the more tolerant Australian cultivars identified. Halberd and other related cultivars, tracing back to cvs. Federation and Currawa, dominated wheat production in southern Australia for most of the twentieth century (Paull 1990). Paull (1990) related genetic variation in the concentration of B in grain with the extent and persistence of bread wheat cultivars commercially grown in Australia.

The inheritance of boron tolerance of wheat has been studied (Paull 1990, Chantachume 1995). Tolerance is expressed as a partially dominant character and controlled by at least three major genes acting in an additive manner and named *Bo1*, *Bo2* and *Bo3* (Paull et al. 1991).

Methods for screening and selection for B tolerance in breeding populations include the growing of plants in pots with soil containing toxic concentrations of B (Paull et al. 1988), by solution culture in filter paper (Chantachume et al. 1995) and in field trials conducted on toxic soils (Moody et al. 1993, Jenkin 1993). Most selection methods are highly labour intensive and susceptible to experimental error. Jefferies et al. (1999) identified four chromosome regions associated with the expression of B tolerance in barley and found that not all four could be readily identified in a single assay system. It is likely that a combination of assay systems, or plant response characteristics, would also be required to select, with relative accuracy, for important chromosome regions associated with B tolerance in wheat. The location of genes conferring B tolerance to chromosomes and then to specific chromosome regions would facilitate the selection of B-tolerant germplasm using linked molecular markers (marker-assisted selection), thereby overcoming many of the limitations associated with alternative assay systems.

Recently, a linkage map was constructed in a doubled haploid (DH) population of 161 individuals created from a cross between the moderately tolerant parent Halberd and the moderately intolerant parent Cranbrook (Chalmers et al. in preparation). The objectives of the study reported here were to use this population and marker dataset to identify chromosomal regions associated with response to toxic concentrations of boron and test the relative expression of those regions when introgressed into an alternative genetic background.

Materials and Methods

Genetic material

The genetic material used in the mapping component of this study was a population of 161 DH lines derived from a cross between the moderately B-tolerant cultivar Halberd and the moderately B-sensitive cultivar Cranbrook. The validation studies consisted of two components. The first component was a repeat of the soil-based assay using a population of 98 DH lines derived from a cross between Halberd and the B-sensitive breeders line Warigal/MMC. The mapping population parents, 15 common Australian wheat cultivars and a North African cultivar known to be very sensitive to B toxicity were also included in the assay. The second component involved field trials and marker screening of 25 cultivars important either as commercial cultivars or as parents to southern Australian wheat breeding programmes during the mid-1980s.

Mapping – plant response to boron toxicity

Two assay systems were used for assessing plant response to B toxicity in the Cranbrook x Halberd mapping population:

1) a filter paper, solution culture assay (Chantachume et al. 1995) in which the relative root length (RRL) of seedlings grown on filter papers soaked in solutions containing toxic and non-toxic concentrations of B was determined.
2) a soil-based assay in which plants were grown in soil to which toxic concentrations of B were added. Leaf symptom expression, total dry matter and whole-shoot B concentration were measured.

Detailed assay methods followed those described by Jefferies et al. (1999). The relative root-length assay was conducted as a randomised complete block with three replicates. Similarly, the soil-based assay was also conducted as a randomised complete block with 3 replicates (2 plants per line, per replicate); each replicate containing within a single large soil box.

Validation – response to boron toxicity

The response of the Halberd x Warigal/MMC population and selected cultivars to B toxicity was assessed using two replicates of the soil-based assay (Jefferies et al. 1999) alone. Field experiments were conducted at Two Wells (approximately 40 km N of Adelaide) in 1985 and 1986 on a red clay soil with naturally occurring high concentrations of B in the subsoil. The concentration of B in the soil at this site, in 1983, ranged from 3.6 mg kg^{-1} in the first 10 cm, increasing to 101–104 mg kg^{-1} in the 20–40 cm range of the profile (Cartwright et al. 1987). Plots were 4.2 m long and 60 cm wide (4 rows) and sown with approximately 60 kg/ha of seed. The experiments were arranged as a randomised complete block design with six replicates in 1985 and seven replicates in 1986. The B-sensitive genotype Warigal/MMC was grown as a control grid and included every fifth plot. In 1985, five whole shoots per plot were harvested 78 days after sowing when most plants were at or near the boot stage (Zadoks 41–45). Samples were rinsed with deionized water, oven-dried, ground in a stainless steel mill and analysed for concentration of B by nitric acid digestion and ICP-spectrometry (Zarcinas et al. 1987). Field experiments were harvested at maturity and grain yield per plot and the concentration of B in grain were determined for both experiments.

Statistical analysis

All statistical analyses except for interval and multiple regression marker analyses of B response were performed with JMP (v3.0, SAS Institute Inc, 1995) software. The least squares means for relative root length (root length at 0 ppm B as a percentage of root length at 100 ppm B) were calculated using an ANOVA model. Factors for the ANOVA model were doubled haploid line, replicate and plant number. Least squares means for B concentration in whole shoots, leaf symptom score and dry matter production were also calculated using an ANOVA model with doubled haploid line and replicate forming the factors for the model. No raw data transformation was required as residuals were normally and independently distributed. Comparisons between B tolerance parameters were conducted by calculating simple pairwise correlation coefficients.

Heritability for each trait was estimated from a linear model incorporating data from the 161 DH lines over three replicates. Factors were doubled haploid line and replicate. Heritabilities were calculated from an estimate of the genetic variance component as a proportion of the total variance for each trait.

A total of 545 marker loci, 112 restriction fragment length polymorphisms (RFLPs) and 433 amplified fragment length polymorphisms (AFLPs), covering the majority of the wheat genome (Chalmers et al. in preparation), were used for simple and interval regression analysis, the latter by the method of Haley and Knott (1992). A minimum LOD threshold of 3.0 was used. A marker locus thought to be associated with a gene or chromosomal region conferring B tolerance was tested for two-way interaction with all other markers in the dataset using the method described by Nelson et al. (1998). Initial marker analyses was conducted using an additive regression model with MAP MANAGER QT software (Manly and Cudmore 1997). Interval analysis and marker interaction tests were performed with the computer programme QGENE (Nelson 1997).

Validation – DNA extraction, restriction endonuclease digestion and Southern hybridisation

DNA extraction was achieved using a DNA mini-prep method adapted from Rogowsky et al. (1991). Variations to the method were as described below. For the initial extraction, 750 µl of extraction buffer and phenol-chloroform-isoamyl alcohol (25:24:1) were used. The extraction buffer was 0.1 *M* TRIS-HCl (pH 8.5),10 m*M* EDTA, 0.1 *M* NaCl, 1% sarkosyl and 2% polyvinylpolypyrrolidone (insoluble). After the second phenol-chloroform-isoamyl alcohol extraction the aqueous phase was extracted once with an equal volume of chloroform. DNA was precipitated by the addition of 0.1 vol. of 3 *M* sodium acetate (pH 4.8) and 1 vol. of propan-2-ol.

Restriction endonuclease digestion and Southern hybridisation followed standard methods. RFLP markers identified as having a significant association with B tolerance in the Cranbrook x Halberd population were subsequently used to genotype the validation population and commercial cultivars. Paull (1990) proposed that boron tolerance genes present in Australian cultivars were of common origin tracing to parents of Halberd. It was assumed, therefore, that Halberd was the only source of B tolerance in the set of Australian cultivars assessed.

Locus effects

Q-GENE (Nelson 1997) was used to produce "graphical genotypes" (Young and Tanksley 1989) for the entire mapping population. In this function of Q-GENE it is assumed that a marker locus represents a chromosomal segment of the same parental genotype, extending halfway to the next marker locus on either side. Each of the 161 DH mapping population lines were scored for the likely presence of either a Halberd or Cranbrook chromosome segment at the locus most significantly associated with each trait. From this, the predicted genotype of each line was determined. Lines of identical genotype were grouped into chromosome segment classes. Least squares means for each class were calculated using a single factor (segment class) ANOVA. Means of chromosome segment class were compared using contrasts. This method was also used for the validation population except that the genotype of lines were assigned on the basis of the most closely associated RFLP marker allele.

Results

Comparison between boron tolerance parameters

Table 1 details the pairwise correlation coefficients between the four B tolerance traits measured (RRL, leaf symptom score, concentration of B in whole-shoots and whole shoot dry matter production). Pairwise correlations between three of the traits, RRL, leaf symptom score, and B concentration in whole shoots were highly significant ($P<0.001$). The concentration of B in whole shoots was highly negatively correlated with RRL (–0.80). Correlation coefficients between leaf score and RRL and B concentration in whole shoots were relatively low at –0.51 and 0.54, respectively. This is consistent with the relatively poor association previously found between leaf symptom expression and grain yield (Jenkin 1993; Riley and Robson, 1994) and leaf symptom expression and concentration of B in plant tissue (Mahalakshmi et al. 1995; Jefferies et al. 1999). In contrast, whole-shoot dry matter production was significantly correlated (-0.17) with whole-shoot B concentration, but only at the $P<0.05$ significance level.

Mapping

Root-length assay

A significant ($P<0.001$) reduction in root growth at 100 ppm B was observed in all DH lines and both parents. Significant ($P<0.001$) genetic variation for seedling root length at both 0 ppm B and 100 ppm B was observed within the mapping population (Fig. 1). The heritability of root length at 0 ppm was estimated as $h^2=0.55$, while the heritability of root length at 100 ppm B was significantly greater at $h^2=0.83$. Relative root length was calculated as root length at 100 ppm B expressed as a percentage of root length at 0 ppm B and was used to provide a measure of the root-length response independent of genetic variation for absolute root length. Frequency distributions for root length at 0 ppm B, 100 ppm B, and for relative root length are provided in Fig. 1. The 0-ppm trait approximated a continuous distribution around a mean of 158 mm. The frequency distributions for the 100-ppm trait and RRL were consistent with that of traits controlled by single genes (not tested for fit). With the exception of 1 line which recorded a significantly lower ($P<0.001$) RRL than Cranbrook, the RRL of all DH lines fell within the parental range.

Table 1 Pairwise correlation coefficients between four boron tolerance traits measured on the Cranbrook x Halberd mapping population

Boron tolerance traits	Whole-shoot boron concentration	Relative root length (RRL)	Leaf symptom score
Relative root length	–0.80***		
Leaf symptom score	0.54***	–0.51***	
Whole-shoot dry weight	–0.17*	0.08	–0.02

* Significant at $P<0.05$,
*** significant at $P<0.001$

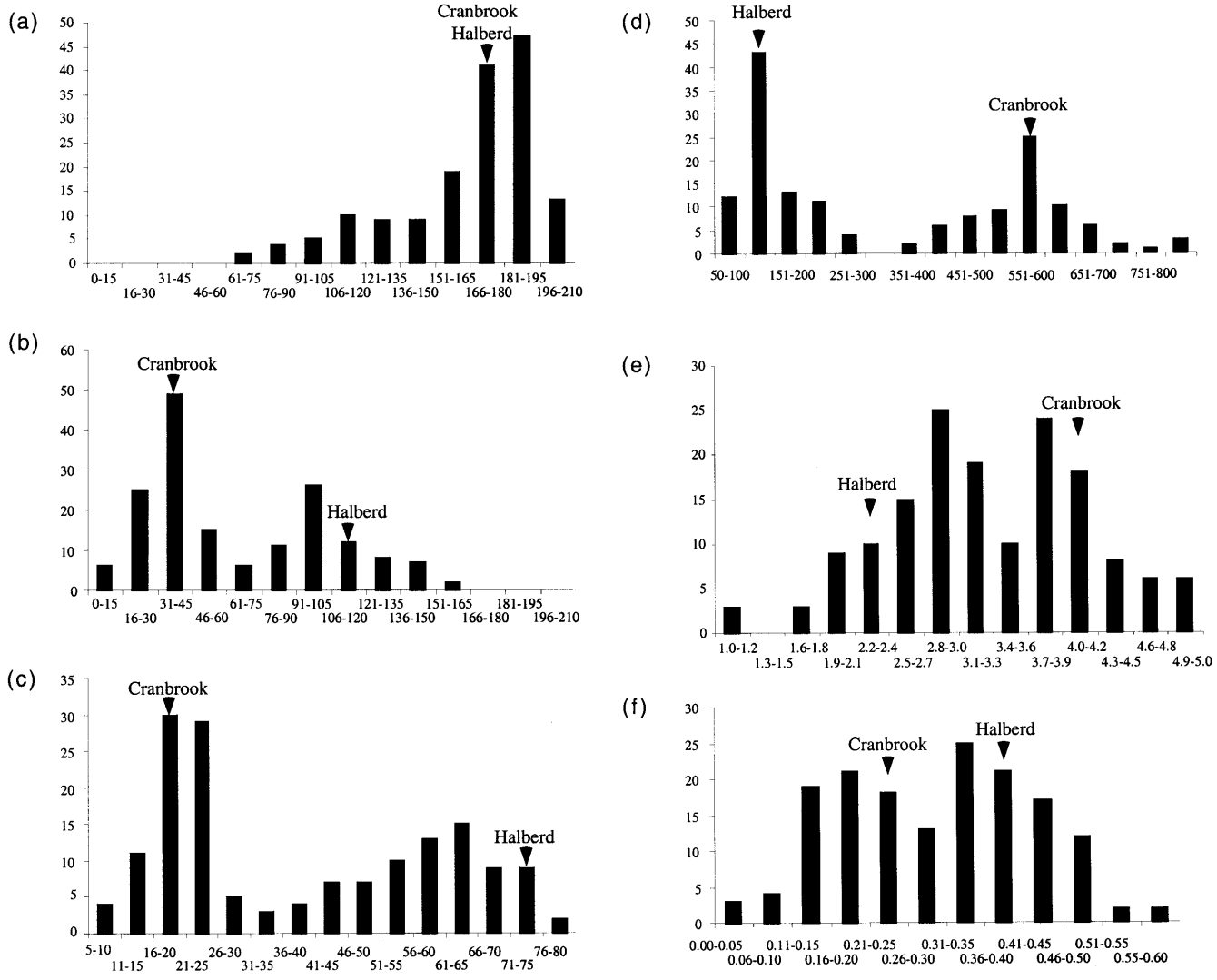

Fig. 1a–f Frequency distributions for all traits measured on the Cranbrook x Halberd mapping population. The *y-axis* on all parts represents the number of DH lines. **a** Length (mm) at 0 ppm B, **b** root length (mm) at 100 ppm B, **c** relative root length (%), **d** whole-shoot B concentration (ppm), **e** leaf symptom score (1–6), **f** whole-shoot dry matter (gm)

Marker analysis of seedling root length at 0 ppm B revealed no significant marker-trait association. Marker analysis for seedling root length at 100 ppm B revealed a significant association (LOD=23.6) with markers in a linkage group on chromosome 7B (Fig. 2). A region within this same linkage group was found to be strongly associated (LOD=35.1) with RRL (Fig. 2). Multiple regression analysis showed that this locus accounted for approximately 49% of the variation in root length at 100 ppm B and 64% of the variation in RRL. Halberd marker alleles were associated with high root length at 100-ppm B and high RRL. Marker analysis of the low (0 ppm) B concentration failed to show significant relationships with the region associated with root length at 100 ppm B and RRL (LOD=0.8). It can be concluded, therefore, that the region identified on chromosome 7B linkage group is significantly associated with root-length response to B concentration. The RFLP marker most strongly associated with root length at 100 ppm B and high RRL was *Xpsr680–7B*.

Soil assay

In the soil assay, significant ($P<0.001$) differences between mapping population parents and DH lines were observed (by ANOVA) for leaf symptom score, whole-shoot dry matter and concentration of B in shoots. The frequency distributions for the three traits were mostly bi-modal with the DH lines falling within the range of the parents (Fig. 1). The heritabilities of leaf symptom score, whole-shoot dry matter and whole-shoot B concentration were 0.53, 0.23 and 0.81, respectively.

Marker analysis of leaf symptom data revealed significant associations (LOD=8.8) between leaf symptoms and the region on the chromosome 7B linkage group found to be associated with RRL (Fig. 3). A significant association (LOD=4.7) was also revealed with a chromosome 7D linkage group (Fig. 3). Based on multiple re-

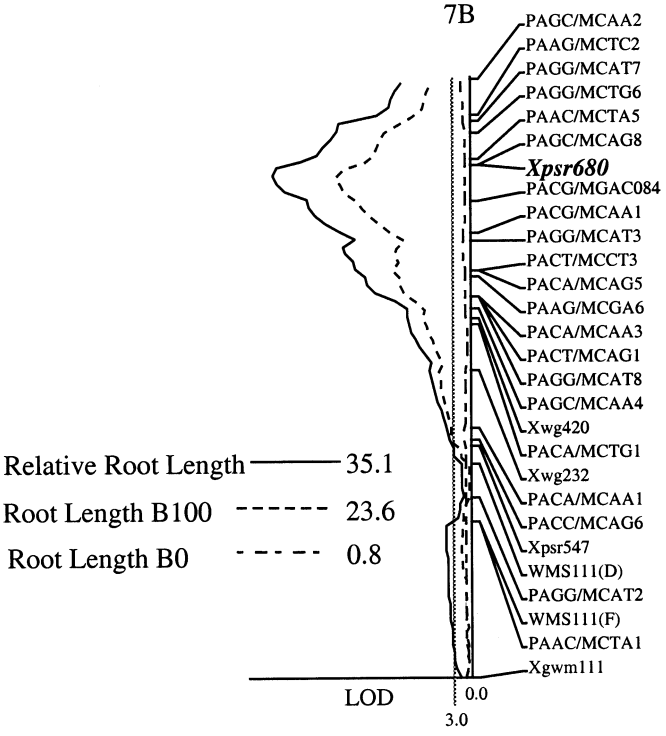

Fig. 2 Chromosome location of regions associated with root length at 0 ppm B and 100 ppm B and relative root length expressed in DH lines of the cross Cranbrook x Halberd based on a partial interval map of chromosome 7B. The short arm is towards the *top* is the chromosome. Maximum LOD score is provided *adjacent* to the legends. The RFLP marker most closely associated with the traits is presented in *bold italics.* The *finest dashed line* shows the LOD 3.0 threshold

gression analysis, loci on chromosomes 7D and 7B, in combination, accounted for approximately 35% of the variation in leaf symptom data. The RFLP markers most strongly associated with leaf symptom score were *Xpsr680–7B* and *Xpsr160–7D.* Halberd marker alleles at both loci were associated with low leaf symptom scores. Despite a significant difference between parents and DH lines, shown by ANOVA, both regression analysis and interval mapping techniques failed to identify a significant association between markers and whole-shoot dry weight response to B toxicity.

Markers in a linkage group on chromosome 7B were found to be associated (LOD=38.4) with whole-shoot B concentration of plants grown in B toxic soil. Based on multiple regression analysis, the chromosome 7B locus accounted for approximately 69% of variation in whole-shoot B concentration data. Halberd marker alleles at this locus conferred low whole-shoot boron concentration. The RFLP marker on chromosome 7B most strongly associated with whole shoot B concentration was also *Xpsr680–7B.*

Marker interactions

The 2 marker loci (*Xpsr160–7D* and *Xpsr 680–7B*) that were significantly associated with chromosomal regions involved in the control of B tolerance traits were tested for two-way interaction with each other and all other markers in the dataset. In addition, each marker in the complete dataset was tested for interaction with all other markers. No significant (P<0.001) interactions were identified.

Fig. 3a, b Chromosome locations of regions associated with leaf symptom expression and whole-shoot B concentration expressed in DH lines of the cross Cranbrook x Halberd based on a partial interval maps of chromosome 7B (**a**) and 7D (**b**). The short arm is towards the *top* of the chromosomes. Maximum LOD score is provided in *parenthesis.* The RFLP marker most closely associated with the trait is presented in *bold italics.* The *finest dashed line* shows the LOD 3.0 threshold

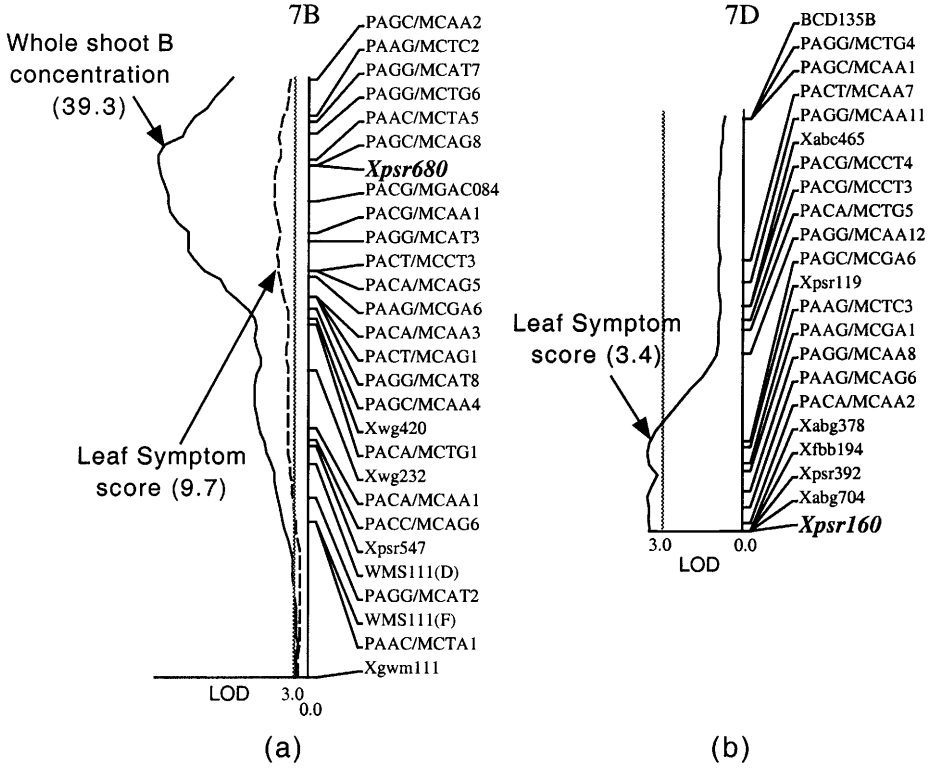

Table 2 Mean whole-shoot boron concentration and leaf symptom score in 17 commercial wheat cultivars grown in soil with toxic levels of boron (B toxic soil)

Cultivar	Halberd (H) or Alternative (A) allele at 2 loci		Leaf symptom score (1–9)		Whole-shoot boron concentration (ppm)	
	Xpsr160–7D	*Xpsr680–7B*	Least squares mean	Standard error	Least squares mean	Standard error
Aroona	H	H	2.13	0.60	402.3	26.2
BT-Schomburgk	H	H	3.63	0.60	229.8	26.2
Barunga	H	–	3.13	0.60	234.0	26.2
Cranbrook	A	A	4.38	0.60	517.3	26.2
Dagger	H	H	2.75	0.60	299.8	26.2
Excalibur	A	H	5.25	0.60	408.6	30.3
Frame	H	H	4.63	0.60	244.8	26.2
Halberd	H	H	2.95	0.27	193.3	12.3
Janz	A	A	3.25	0.60	447.3	26.2
Kenya Farmer	–	–	8.50	0.60	487.3	26.2
Krichauff	–	–	2.75	0.60	212.0	26.2
Molineux	H	H	3.25	0.60	414.8	26.2
Schomburgk	H	H	3.38	0.60	419.8	26.2
Silverstar	–	A	5.13	0.60	494.8	26.2
Spear	H	H	3.25	0.60	239.8	26.2
Stiletto	H	H	3.88	0.60	272.3	26.2
Trident	H	H	3.13	0.60	254.8	26.2
Wl/MMC	A	A	7.60	0.27	528.4	12.9
Warigal	H	H	4.00	0.60	469.8	26.2
LSD (P<0.05)			1.65		79.6	

Table 3 Mean concentration of boron in whole shoots and grain of 25 cultivars important to southern Australian breeding programmes, grown in field trials at Two Wells, South Australia, 1985 and 1986

Cultivar	Halberd (H) or Alternative (A) allele at two loci		Boron concentration in whole shoots (mg/kg)	Boron concentration (mg/kg) in grain		Grain yield (g/plot)	
	Xpsr160–7D	*Xpsr680-7B*		1985	1986	1985	1986
Halberd	H	H	35.6	5.84	3.32	291	320
Olympic	A	–	44.2	8.48	5.27	291	229
Spear	H	H	44.2	7.84	4.57	235	288
Aroona	H	H	46.0	7.39	6.07	214	275
Raven	H	H	51.8	9.08	–	176	–
Dagger	H	H	52.4	9.03	4.95	196	277
Millewa	H	H	58.4	9.76	5.08	145	316
Sunstar	H	–	60.0	11.60	9.15	165	239
Banks	A	A	62.0	10.51	–	154	–
Vulcan	A	–	64.8	13.99	9.38	206	206
Cook	A	A	64.9	11.30	10.01	190	213
Condor	A	A	68.8	12.01	8.82	181	234
Meering	A	A	69.1	11.27	6.77	186	243
Kite	A	A	73.7	15.96	10.02	152	245
Madden	A	A	73.7	14.35	–	138	–
Cranbrook	A	A	74.9	12.68	6.36	171	242
Warigal	H	H	75.5	9.72	6.56	222	322
Schomburgk	H	H	75.7	9.89	5.80	227	263
Festiguay	A	–	78.4	12.42	7.91	177	217
Gabo	A	–	81.9	15.84	–	126	–
Oxley	H	A	85.5	12.51	7.71	134	231
Bindawarra	H	A	88.3	17.15	8.64	185	266
Egret	H	A	89.1	13.41	6.14	155	268
Machete	A	A	95.5	13.61	7.14	152	276
W1*MMC	A	A	99.4	14.38	7.35	125	208
LSD (P<0.05)			30.0	4.20	1.95	62	99

Table 4 Pairwise correlation coefficients between concentration of boron in shoots and grain and grain yield of 25 Australian cultivars grown in a field experiment on B toxis soils at Two Wells, South Australia, 1985 and 1986

	Whole-shoot B concentration 1985	B concentration in grain, 1985	B concentration in grain, 1986	Grain yield, 1985
B concentration in grain, 1985	0.76**			
B concentration in grain, 1986	0.54**	0.77**		
Grain yield, 1985	–0.43**	–0.50**	–0.38**	
Grain yield, 1986	–0.42**	–0.45**	–0.57**	0.36**

** Significant at $P<0.01$

Table 5 Mean effect of the presence of either the Halberd or the Cranbrook chromosome segment on relative root length (%), leaf symptom score and whole-shoot B concentration of the Cranbrook x Halberd mapping population

RFLP marker most closely associated with trait	Parent segment- Halberd (H) Cranbrook (C)	Number of DH lines within class	Least squares mean (%)	Standard error of mean
RRL (%)				
Xpsr680–7B	H	83	54.4 a[b]	1.5
	C	75	22.3 b	1.6
Leaf symptom score (1–6)[a]				
Xpsr160–7D	H	89	2.99 a[b]	0.08
	C	68	3.49 b	0.10
Xpsr680-7B	H	80	2.83 a	0.08
	C	77	3.59 b	0.08
Xpsr160–7D/	HH	43	2.69 a	0.11
Xpsr680–7B	CC	34	3.88 b	0.12
	HC	45	3.31 c	0.11
	CH	35	3.06 a*, c	0.12
Whole-shoot B concentration (mg/kg)				
Xpsr680–7B	H	80	171.4 a[b]	15.4
	C	75	527.9 b	15.9

[a] Mean of leaf symptom score (1–6 scale: 1=no symptoms, 6>90% leaf necrosis)
[b] Means with different letters are significantly different at $P<0.001$; a* is significantly different from a at $P<0.05$

Validation

Through regression analysis, highly significant ($P<0.0001$) relationships were established between marker allele and both leaf symptom expression and whole-shoot B concentration in the validation population, Halberd x Warigal/MMC. The 2 loci, *Xpsr160–7D* and *Ypsr680-7B*, were estimated to account for 22% and 14% of the variation in leaf symptom data, respectively. Similarly, *Xpsr680–7B* accounted for 84% ($P<0.0001$) of the variation in whole-shoot boron concentration data. As expected, there was no significant relationship between *Xpsr160–7D* and whole-shoot B concentration.

Seventeen wheat cultivars were assessed for differences in leaf symptom score and whole-shoot boron concentration of plants grown on soil containing toxic concentrations of B (Table 2). Significant ($P<0.0001$) relationships were established between the Halberd allele at the *Xpsr680–7B* locus and whole-shoot B concentration and leaf symptom score. Similarly, a significant ($P<0.0001$) relationship was established between the Halberd allele at the *Xpsr160–7D* locus and leaf symptom score.

A sub-set of 25 of the cultivars used in the field study were screened for the Halberd allele at the *Xpsr680–7B* and *Xpsr160–7D* loci. Table 3 presents whole-shoot B concentration for these 25 cultivars grown in the 1985 field experiment and the concentration of B in grain from the 1985 and 1986 experiments. Significant relationships ($P<0.001$) between marker allele and B concentration in both whole shoots and grain were established. There was no significant relationship between *Xpsr160–7D* and B concentration in whole shoots or in grain in 1985, but there was a significant relationship in 1986 ($P<0.005$). Table 4 provides the correlation coefficients between the B concentration in shoots (1985) in grain (1985 and 1986) and grain yield (1985 and 1986).

Locus effects

The mean effect of the presence of either a Halberd or Cranbrook chromosome 7B segment in the region of *Xpsr680–7B* on RRL, whole-shoot B concentration and the leaf symptom score in the Cranbrook x Halberd map-

Table 6 Mean effect of the presence of either Halberd or Warigal/MMC marker allele on whole-shoot boron concentration and leaf symptom score of the Halberd//Warigal/MMC population grown in soil containing toxic concentrations of B

RFLP marker	Marker allele Halberd (H) Warigal*MMC(W)	Number of DH lines within class	Least squares mean	Standard error of mean
Leaf symptom score (1–9)[a]				
Xpsr160–7D	H	48	4.87 a[b]	0.22
	W	50	5.52 b	0.20
Xpsr680–7B	H	44	4.77 a	0.22
	W	54	5.57 b	0.20
Xpsr160-7D/ Xpsr680–7B	HH	25	4.30 a	0.30
	WW	31	5.69 b	0.25
	HW	19	5.41 b	0.30
	WH	23	5.26 b	0.31
Whole-shoot B concentration (mg/kg)				
Xpsr680–7B	H	44	283.8 a[c]	16.4
	W	54	432.1 b	14.5

[a] Mean of leaf symptom score (1–9 scale; 1=no symptoms, 9>90% leaf necrosis)
[b] Means with different letters are significantly different at $P<0.05$
[c] Means with different letters are significantly different at $P<0.001$

ping population is provided in Table 5. The presence of the Halberd segment at the *Xpsr680–7B* locus conferred an average 32.1% increase in RRL, a 68% reduction in whole-shoot B accumulation and a 0.76 point reduction in leaf symptom score over those individuals carrying the Cranbrook segment. The presence of the Halberd segment at the *Xpsr160–7D* locus conferred an average 0.5 point reduction in leaf symptom score. Individuals carrying the Halberd segment at both the *Xpsr160–7D* and *Xpsr680–7B* loci, on average, received a 1.19 point lower leaf symptom score than individuals carrying Cranbrook alleles at both loci. It appears that leaf symptom expression is predominantly controlled by two loci behaving in a largely additive manner, with the chromosome 7B locus having a slightly larger effect than the chromosome 7D locus.

The mean effect of the presence of either a Halberd on Warigal/MMC allele for *Xpsr680–7B* on whole-shoot B concentration and leaf symptoms and either allele for *Xpsr160–7D* on leaf symptom score is presented in Table 6. The presence of the Halberd allele for *Xpsr680-7B* conferred a 74% reduction in whole-shoot B concentration. The presence of the Halberd allele at either or both loci conferred significant reductions in leaf symptom score, but only at $P<0.05$.

Discussion

Using aneuploid analysis, Paull (1990) identified the chromosomal location of B tolerance genes derived from cvs. Federation and Halberd (moderately tolerant) and cv. G16450 (very tolerant). Chromosomes of homoeologus groups four and seven were found to be involved in the expression of B tolerance. Chromosome 7B was thought to be the most probable location of genes for tolerance to B derived from Federation and, therefore, Halberd, and chromosomes 7D and 4A were the most probable locations of genes derived from G16450. Genetic analysis using a Condor monosomic series [B tolerance in Condor is believed to differ from that of Halberd at 1 locus (Paull 1990)] also located a tolerance gene of Halberd on chromosome 7B (Chantachume, 1995). Similarly, monosomic analysis of G16450, also using the Condor series, supported the location of a major gene for tolerance on chromosome 4A but did not support the hypothesis of a further gene from G16450 on chromosome 7D (Chantachume 1995). In these genetic studies, Paull (1990) used variation in leaf symptoms, while Chantachume (1995) used variation in seedling root length.

While an RFLP marker associated with a gene for B tolerance in the line G16450 has been identified (Paull et al. 1995), any marker-trait association for a gene derived from Halberd, located on chromosome 7B (Paull 1990, Chantachume 1995), had no previously been found. Halberd and a number of related cultivars have been used widely in southern Australian wheat breeding programmes. Identification of a marker closely associated with this gene, or chromosomal region, may have a significant impact on the breeding and selection of B-tolerant cultivars in southern Australia. Additional minor genes conferring B tolerance may be present in Halberd, but the quantitative nature of the traits' expression and the choice of assay system may have inhibited detection using aneuploid analysis.

Chromosome locations conferring boron tolerance derived from Halberd, and relationships to proposed mechanisms

Nable (1991) found that the pattern of B distribution in plant parts was very similar between tolerant and intolerant barley genotypes despite great differences in the total B accumulated. The chromosome 7B locus identified in this study appears to be involved in the control of boron accumulation in whole plants or, more precisely, the control of a B exclusion mechanism determining the relative accumulation of B in whole plants. The exclusion mechanism is likely to reduce the effect of toxic concentra-

tions of B in solution on root growth, reflected in improved RRL. This is supported by the high correlation (r=0.80, P<0.001) found between RRL and whole-shoot boron concentration (Table 1).

The chromosome 7D locus was associated with leaf symptom expression only. Nable (1991) found that the concentration of B in leaves of barley increases from young to old leaves and from base to tip. This is the general pattern followed by the progression of leaf symptoms in both barley and wheat. Therefore, it is likely that the chromosome 7D locus is involved in the translocation of boron in leaf tissue, which may contribute to differences in leaf symptom expression.

Nable (1988) attributed genetic variation for leaf symptom expression in wheat and barley to differences in B concentration in plant tissue. Similar relationships between these traits were established in this study, and a genetic basis to this relationship was demonstrated. However this study, and a similar study in barley (Jefferies et al. 1999), identified chromosome regions on chromosome 7D in wheat and chromosome 2H in barley involved in the control of leaf symptom expression independent of boron accumulation. Chantachume (1995) failed to locate genes controlling boron tolerance, derived from Halberd, to chromosome 7D. This investigator used an assay system which only measured variation in root length and hence overlooked the 7D locus. Paull (1990), using leaf symptom scores, proposed that a region on chromosome 7D, derived from G61450, is associated with boron tolerance. It is possible that the Halberd 7D locus identified in the present study and the 7D locus derived from G61450 are common loci.

Effect of boron tolerance loci on grain yield response

The chromosome 7B and 7D loci identified in this study were shown to have a significant effect on leaf symptom expression and whole-shoot boron concentration in the mapping population, validation population and a number of other, mostly Australian, cultivars. The effect of these loci on grain yield response to boron toxicity was not tested. Significant correlation, however, was observed between the grain yield of a set of Australian cultivars and both whole-shoot B concentration and grain B concentration (Table 4). Paull (1990) proposed that boron tolerance genes present in Australian cultivars are of common origin tracing to parents of Halberd. Australian cultivars carrying the Halberd allele at the *Xpsr680-7B* locus are likely to be carrying the identical chromosome region conferring reduced B accumulation. Concentration of B in whole shoots and grain in 25 Australian cultivars was shown to be strongly related to the Halberd allele for *Xpsr680-7B* (Table 3). There is correlative evidence, therefore, that the *Xpsr680–7B* locus is associated with improved grain yield on boron toxic soils.

The locus conferring improved RRL (chromosome 7B) was transferred from Halberd into cv. Schomburgk to develop BT-Schomburgk through three cycles of backcrossing with selection for vigour in B toxic soil. BT-Schomburgk had a grain yield advantage over Schomburgk of up to 11% in several field trials conducted on soils subject to boron toxicity (Moody et al. 1993). No significant difference was observed between the leaf symptom scores of Schomburgk and BT-Schomburgk, but a significant difference in whole-shoot B accumulation was observed (Table 2). Poor relationships between leaf symptoms and grain yield response have been reported in both wheat and barley (Paull et al. 1988; Jenkin 1993; Riley and Robson 1994). The relative contribution of each major region and/or gene, particularly in relation to grain yield response or ‘field tolerance’, warrants further investigation.

Number of loci involved in the control of boron tolerance in wheat

Two regions involved in the control of B tolerance in wheat were identified in the Cranbrook x Halberd mapping population. Jefferies et al. (1999) identified four chromosomal regions involved in the control of B tolerance in barley. Regions on chromosome 4H and 6H were found to be involved in the control of B accumulation in plants, a region on chromosome 2H was found to be involved in the control of leaf symptoms and a region on chromosome 3H was found to be associated with variation in RRL independent of B accumulation. In light of this, it is possible that there are more than two regions in the wheat genome involved in B tolerance.

Exotic wheat germplasm, more tolerant than Halberd, has been identified (Moody et al. 1993). Paull (1990) proposed that genes for B tolerance, derived from an exotic Greek line, GK1450, were located on chromosomes 4A and 7D. Chantachume (1995) supported the location of tolerance genes found in G61450 to chromosome 4A but not 7D.

Paull et al. (1995) tested 110 F_7 derived lines of a cross between G61450 (very tolerant) and Kenya Farmer (very sensitive) for segregation in response to B and for 43 RFLP markers. A highly significant association between response to B and an RFLP marker (*XksuG10*) was found. The *XksuG10* locus is located on chromosomes of homoeologous group 4 (Gill et al. 1991). Jefferies et al. (1999) identified a region on chromosome 4H of barley involved in the control of B accumulation also closely associated with *XksuG10*. It is possible that wheat and barley may posses a common B tolerance gene on A4 and 4H, respectively (Jefferies et al. 1999). In addition, transgressive segregation was observed in progeny of a cross between Halberd and G61450 (Paull et al. 1991). This evidence supports the conclusion of at least two different genes controlling B tolerance between the two genotypes.

The cultivar Kenya Farmer and the breeders’ line Warigal/MMC produced significantly greater leaf symptom scores than Cranbrook when grouwn on B toxic soil (Table 2). At least 1 additional locus controlling leaf

symptom expression is, therefore, likely to be present in both Halberd and Cranbrook and many other Australian cultivars, but these could not be identified through quantitative trait locus (QTL) mapping of the Cranbrook x Halberd population.

Marker-assisted selection for boron tolerance

Molecular markers closely linked to genes of agronomic importance have been demonstrated to be useful tools for indirect selection in a barley breeding programme (Jeffereries et al. 1997). Marker-assisted selection is time-efficient, non-destructive and, depending on linkage relationships, characterised by low selection error.

The role and agronomic value of the chromosome 7D locus, derived from Halberd and involved in the control of leaf symptom expression, is uncertain. Selection for B tolerance on the basis of leaf symptom score is also not desirable as the trait is controlled by at least two genes and is subject to high experimental error. Selection on the basis of whole-shoot B concentration is destructive, time-consuming and expensive. The estimated heritability of RRL in the mapping population was very high, exceeding 0.8. The relative time and resource costs of the RRL assay and marker-assisted selection would be comparable. Benefits of marker-assisted selection for the chromosome 7B locus alone would include increased flexibility. The RRL assay is either destructive or requires the transplantation of seedlings. DNA, for marker-assisted selection, can be harvested and extracted from plants grown in the field, glasshouse, or other trait screening assays without significant damage to growing plants.

The major benefit of marker-assisted selection for B tolerance in wheat, particularly in Australia where the frequency of B tolerance genes derived from the same source as Halberd is high, would be in combining, or pyramiding, genes for tolerance from different sources. Transgressive segregation for B tolerance in crosses between Halberd and G61450 has been observed (Paull et al. 1991). Markers associated with important chromosome regions conferring B tolerance in Halberd and G61450 have been identified. The introgression of selected chromosome regions conferring improved B tolerance from both parents into elite quality and agronomic backgrounds could significantly improve the grain yield and quality of wheat grown on soil prone to B toxicity.

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