BORON TOXICITY IN BARLEY: PROSPECTS FOR DELIVERING A YIELD ADVANTAGE

J.K. Eglinton[1], R.J. Reid[2], J.E. Hayes[2], P. Davies[3], D. Jha[3] and G. McDonald[1].

[1]School of Agriculture and Wine, Waite Campus, and [2]School of Earth and Environmental Sciences, The University of Adelaide, Glen Osmond, South Australia, 5064.
[3]South Australian Research and Development Institute, Glen Osmond, South Australia, 5064.

INTRODUCTION

The physiological role of boron is not clearly understood, however it has long been established that it is essential for the growth of higher plants (Aghulon 1910). Boron can be phytotoxic if present in soils at high concentrations and has been recognised as a production constraint in regions of southern Australia, West Asia and North Africa. The genetic basis of tolerance to boron toxicity has been examined in a mapping population derived from Sahara 3771 and Clipper (Jefferies et al 1999). This study identified four significant QTL involved in aspects of boron tolerance. A region on chromosome 2H was associated with leaf symptom expression, a 3H QTL influenced root growth suppression by boron toxicity, a region on 6H was associated with the level of boron uptake, and a region on 4H influenced boron uptake, root growth response, dry matter production, and leaf symptom expression. A backcross population was developed to validate the effect of the Sahara QTL (Jefferies et al 2000). A boron tolerant individual from the Clipper x Sahara population was backcrossed through two cycles of combined phenotypic and marker assisted selection to the recurrent parent Sloop. 450 BC_2F_2 plants were screened with RFLP markers linked to the chromosome 2H and 4H QTL, and 68 individuals homozygous for either Sloop or Sahara alleles at these loci were selected for field experiments. These lines were grown in boron toxic soils at Minnipa, South Australia, in 1998 and 1999. Significant boron toxicity symptoms were observed in the trials, and Sahara marker alleles at the 2H and 4H loci were associated with reduced leaf symptoms. However, the chromosome 4H locus was associated with a modest yield and grainsize increase in 1999 only, and the 2H locus was only associated with improved grainsize in 1998 (Jefferies et al 2000).

This paper presents results of studies aimed at further characterising yield responses associated with Sahara derived boron tolerance alleles, and examining the basis for potential yield penalties associated with these loci. The backcross lines were evaluated in further field trials that included analysis of rooting depth and water use in the soil profile. The sizes of introgression segments derived from Sahara were determined in this germplasm, highlighting linkage problems that potentially confound any yield advantage. Recent advances in our understanding of the mechanisms of boron toxicity tolerance are considered in relation to the genetic control of the trait. A novel method of selecting for boron tolerance is presented and discussed in the context of developing breeding strategies to maximise recombination.

FIELD PERFORMANCE OF BORON TOLERANT BACKCROSS LINES

Backcross lines of the B-intolerant parental lines Sloop and VB9104 were grown at Minnipa and Birchip in 2001 and at Minnipa in 2002. Soil B concentrations below 40 cm were 16-20 mg kg^{-1} at Minnipa in 2001, 24-33 mg kg^{-1} at Minnipa in 2002. The genotypes of the

backcross lines are designated according to their 2H/4H combination as: -/-, -/+, +/- or +/+. Only results from Minnipa are presented as the Birchip data showed similar trends in yield. Foliar symptoms of boron toxicity appeared early in 2001, but above average spring rainfall resulted in few symptoms developing later in the year. The season was much drier in 2002 with foliar symptoms being more severe and progressively worsening during spring. Selection for B tolerance reduced foliar symptoms in both years, but more consistently in the Sloop lines (Table 1). Tissue analysis also showed that shoot B concentrations of the backcross lines were similar to or less than the parental lines in both years (data not shown). Dry matter production at head emergence was not significantly affected by genotype in either year. Despite reduced expression of B toxicity symptoms, yields of the backcross lines were no higher and in some cases significantly less that the parents (Table 2). There was no consistent effect of the 2H and the 4H QTL on yield.

Table 1. Scores of visual symptoms of B toxicity of Sloop and VB9104 backcross lines. Observations were made on 23 August 2001 and 3 October 2002. Scoring is based on a 0-10 scale where 0 = no symptoms and 10 = severe leaf necrosis.

	2001		2002	
Genotype	Sloop	VB9104	Sloop	VB9104
Parent	7.7	5.3	4.8	1.7
-/-	5.6	4.6	5.1	5.0
-/+	4.2	4.4	4.3	2.2
+/-	4.7		2.1	4.6
+/+	3.8	4.0	1.1	2.1
Sahara	3.3		0	
Gairdner	6.7		3.4	
Mundah	5.3		6.4	
SED	0.73		1.06	

Table 2. Grain yield of Sloop and VB9104 backcross lines (t Ha^{-1}).

	2001		2002	
Genotype	Sloop	VB9104	Sloop	VB9104
Parent	2.63	3.10	1.12	1.13
-/-	2.31	2.53	1.02	1.11
-/+	2.11	2.88	1.08	1.18
+/-	2.37		1.14	0.85
+/+	2.05	2.18	1.08	1.22
Sahara	1.32		1.02	
Gairdner	3.68		1.30	
Mundah	2.27		1.13	
SED	0.363		0.134	

In 2001, there was a significant positive correlation between the severity of B toxicity symptoms in August and yield within backcross families. The correlation in 2001 for Sloop derivatives was $r=0.93$ ($P<0.05$) and for VB9104 lines was $r=0.84$ ($P<0.10$). When B toxicity was severe in 2002, there was no correlation.

Analysis of root growth gave similar results in 2001 and 2002. The maximum depth of rooting in 2002 was about 70 cm and Sahara tended to have more roots below 50 cm. However, there was no evidence that selection based on the Sahara QTL improved root growth in the subsoil, where high concentrations of B were measured. There was no difference in either the depth of water extraction or total amount of water used within the backcross lines (data not shown).

In 2001 the Sloop backcross lines were significantly taller than Sloop and height increased progressively with the number of B-tolerant genes from Sahara that were introgressed into Sloop (Fig. 1). The mean height of the +/+ lines was not significantly different to that of Sahara. There was no significant difference in height among the lines derived from VB9104. The data from 2001 suggest that the level of introgression from Sahara influences other agronomic characteristics of the backcross lines independently of B tolerance. The positive correlation between symptoms and yield in 2001 may reflect the deleterious effect on yield of the Sahara introgression.

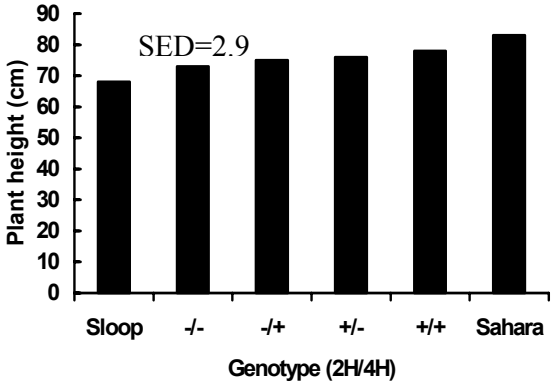

Figure 1. Plant height at anthesis of backcross lines of Sloop selected on the basis of the presence (+) or absence (-) of B tolerance QTL on chromosomes 2H and 4H.

INTROGRESSION SEGMENT LENGTH ANALYSIS

The introgression segment length for 21 Sloop backcross lines and 25 VB9104 backcross lines was determined using molecular markers. The lines were screened with 16 SSR and RFLP markers on chromosome 2H, and a further 16 markers on chromosome 4H. 12 of these markers were not polymorphic in these crosses, resulting in incomplete chromosome coverage. The backcross lines were characterised by large linkage blocks, with chromosome 2H introgression segments ranging from 14 – 71 cM (Figure 2). Similar results were obtained from the VB9104 backcross lines (data not shown). This is likely to be an underestimate of introgression segment size due to the lack of informative markers on the short arm of chromosome 2H. Significant Sahara derived segments distinct from the B tolerance QTL were also identified on the long arm of chromosome 2H (Figure 2). Chromosome 4H was characterised by smaller introgression segments, ranging from 7 – 49 cM in both the Sloop and VB9104 backcross lines (data not shown).

The large introgression segments detected on chromosome 2H are likely to influence the agronomic performance of the backcross lines due to the presence of key developmental loci

in this region (Figure 2). PpdH1 and eps2 exert significant influence on grain yield and grain size, particularly in low rainfall environments in southern Australia (Coventry et al 2003). The positive relationship between plant height and the presence of Sahara alleles (Figure 1) also suggests linkage drag is reducing agronomic fitness and confounding any yield advantage conferred by B toxicity tolerance.

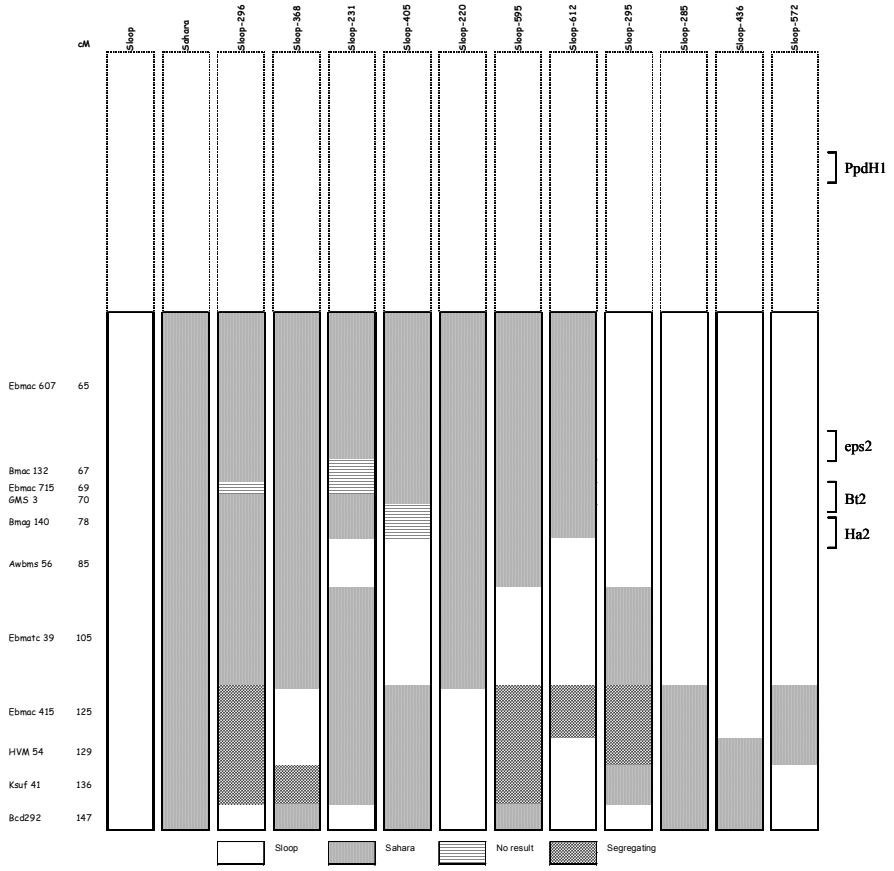

Figure 2. Schematic representation of Sahara derived introgression segments on chromosome 2H observed in a range of Sloop backcross lines. The approximate locations of PpdH1, eps2, Bt2 and Ha2 are shown on the right axis.

MECHANISMS OF BORON TOXICITY AND TOLERANCE

Despite considerable research effort, the precise mechanisms for B toxicity and tolerance in plants are still unclear. Boron toxicity results in a broad range of physiological effects, including decreased shoot and root growth (Lovatt and Bates 1984: Nable et al 1990), root cell division (Liu et al 2000) and RNA content (Fawzia et al 1994), reduced leaf chlorophyll, photosynthesis and stomatal conductance (Lovatt and Bates 1984), reduced levels of lignin and suberin (Ghanati et al 2002). Effects appear to be correlated with the accumulation of high concentrations of B in the shoot, which is a function both of the concentration of external B and time of exposure. Leaf symptom expression of toxicity in barley, characterised by necrotic spots developing from the leaf tip, occurs only when the affected parts of the leaf reach concentrations of at least 50 mM B. Tissue sensitivity does not differ between Sahara (B-tolerant) and Schooner (B-sensitive) varieties, although leaf symptom expression of B toxicity was not strongly correlated with shoot B concentrations in the Clipper x Sahara DH population (Jefferies et al 1999).

Where there is early toxicity, tolerance to high B is associated most strongly with reduced B accumulation into the shoot (Nable et al 1990; Jefferies et al 1999; Figure 3). Evidence to date suggests that this is due to reduced uptake at the root level. In solution culture, B-tolerant barley is able to exclude B so that root concentrations are up to 40% lower than in the external solution. This is somewhat unexpected because at soil pH levels of anything less than 9, uncharged boric acid is the predominant form of B and, in this form, rapidly equilibrates across cell membranes (Stangoulis et al 2001). For example, in the roots of Schooner (B-sensitive) barley, B equilibrates with the external solution within three hours. Hypotheses concerning why some plants are able to exclude B are wide-ranging and include reduced membrane permeability (Dordas and Brown 2000) or the presence of physical barriers such as callose or suberin layers in the root, complexation of B in the rhizosphere, and active efflux of borate or boric acid (Takano et al 2002). Exclusion of B does not appear to be inducible, but is evident both at high and low external concentrations of B.

Restricted B entry to roots is unlikely to be the only mechanism influencing B tolerance under field conditions. For example, WI3408 is exceptionally sensitive under field conditions, but short-term solution culture experiments indicate that it has higher tolerance than Schooner (Figure 3). In addition to the four QTL known to influence B tolerance, a number of recent studies have suggested differences in root growth or morphology may form the basis for escape from B toxicity in some situations. Further studies on the Sahara derived Sloop backcross lines are underway to clarify the relationships between field tolerance and the mechanisms underlying the B tolerance QTL.

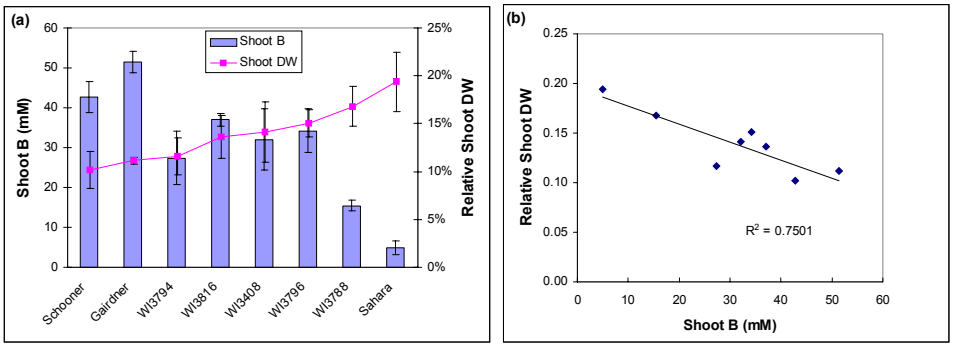

Figure 3. (a) Relative shoot dry weights (compared to low B-grown plants) and shoot B concentrations of eight barley cultivars following growth for 16 days in nutrient solution (pH 5.5) containing 5 mM B, (b) correlation between relative shoot dry weight and shoot B concentrations for the 8 varieties.

NOVEL SELECTION METHODS FOR BORON TOLERANCE

The feasibility of selecting for boron tolerance *in vitro* during the isolated microspore culture procedure used for generating doubled haploid breeding lines was evaluated. Lines were selected from the Clipper/Sahara doubled haploid mapping population to contain Sahara alleles at one of the four possible B tolerance loci located on chromosomes 2H, 3H, 4H & 6H (Jefferies et al 1999). Microspore derived regenerants have been obtained from the lines possessing either the 3H or 4H alleles using selection medium where the concentration of

boron was 2-3 times higher than those used by Paull et al (1988) in soil and 20 times higher than those used by Nable (1988) in solution culture. A study of the relative rates of regeneration in the presence of boron indicated a higher percentage of regeneration for the lines having 3H & 4H alleles over those having none (Table 3). No regenerants were obtained from either Clipper or lines possessing the 2H or 6H alleles at higher concentrations of boron in the selection medium. The results obtained to date suggest that this screening protocol could be used for breeding purposes to select for the boron tolerance alleles on chromosomes 3H and 4H. An important feature of this method is that large numbers of genotypes can be tested at relatively low cost. The demonstrated effect of linkage drag in the Sloop and VB9104 backcross lines indicates the importance of screening large populations to identify specific recombination events. This may be achieved by using *in vitro* screening to increase the frequency of desirable alleles in the subsequent DH population, in conjunction with MAS approached for introgression segment size.

Table 3. Relative regeneration per spike (%)

	Selection Pressure (Boron mM)				
B tolerance allele	**0**	**5**	**10**	**15**	**20**
-	100	55	5	0	0
2H,3H,4H,6H	100	76	28	14	3
2H	100	84	3	0	0
3H	100	54	29	11	0
4H	100	100	23	15	0
6H	100	15	5	0	0

ACKNOWLEDGEMENTS

The authors acknowledge the contribution of Leigh Davis, Patricia Warner and Rebecca Fox to this work. This work has been supported by the South Australian Grains Industry Trust Fund (SAGITF), the ARC, and Australian farmers and the Commonwealth Government through the GRDC.

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