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Mapping of chromosome regions conferring boron toxicity tolerance in barley (*Hordeum vulgare* L.)

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Abstract Boron toxicity has been recognised as an important problem limiting production in the low-rainfall regions of southern Australia, West Asia and North Africa. Genetic variation for boron toxicity tolerance in barley has been characterised but the mode of inheritance and the location of genes controlling tolerance were not previously known. A population of 150 doubled-haploid lines from a cross between a boron toxicity tolerant Algerian landrace, Sahara 3771, and the intolerant Australian cultivar Clipper was screened in four tolerance assays. An RFLP linkage map of the Clipper × Sahara population was used to identify chromosomal regions associated with boron tolerance in barley. Interval regression-mapping allowed the detection of four chromosomal regions involved in the boron tolerance traits measured. A region on chromosome 2H was associated with leaf-symptom expression, a region on chromosome 3H was associated with a reduction of the affect of boron toxicity on root growth suppression, a region on chromosome 6H was associated with reduced boron uptake, and a region on chromosome 4H was also associated with the control of boron uptake as well as being associated with root-length response, dry matter production and symptom expression. The benefits and potential of marker-assisted selection for boron toxicity tolerance are discussed.

Key words Boron toxicity · Boron tolerance · RFLP mapping · Barley

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Introduction

Boron (B) is an essential plant micronutrient which can be phytotoxic to plants if present in soils at high concentration. Boron toxicity to crop plants has been recognised since the early 1930s, but it was not until 1983 that it was first recognised in southern Australia in barley growing under dryland conditions (Cartwright et al. 1984). A 17% difference in the grain yield of adjacent areas of barley (cv Clipper) was related to differences in the concentration of boron in shoots just prior to anthesis (Cartwright et al. 1984). Boron toxicity has also been recognised as a problem in the dry regions of West Asia and North Africa (ICARDA Annual Report 1993) and is a problem associated with irrigation water in many other parts of the world (Gupta et al. 1995). Soil amelioration of boron toxicity appears to be impractical.

Genetic variation for tolerance of boron toxicity exists within a number of crops, including wheat and barley (Cartwright et al. 1987; Moody et al. 1988; Paull et al. 1988 a) field peas and pasture medics (Paull et al. 1992). Exploitation of this genetic variation through plant breeding in barley may significantly improve both the productivity and quality of barley growing in areas prone to boron toxicity problems.

Using leaf-symptom data, Jenkin (1993) studied the inheritance and location of genes conferring boron tolerance in barley in three cross combinations and their reciprocals. Jenkin proposed that boron tolerance (reduced leaf symptoms) was controlled by three major gene loci behaving in a largely additive manner. These

"genes" were located on barley chromosomes using an RFLP linkage map of 43 doubled-haploid lines from a cross between the intolerant cultivar Clipper and the tolerant Algerian landrace Sahara 3771 (Sahara). Regions on chromosomes 2H and 7H were reported to be significantly associated with leaf-symptom expression. The associations reported were weak (LOD <3.0) since the number of doubled-haploid lines screened (43) were, most likely, insufficient to establish significant relationships with confidence.

The most obvious symptoms of boron toxicity in barley are chlorosis and necrosis extending from leaf tips, as well as the formation of brown lesions initially at the leaf margins but extending over the distal half, or more, of the leaf. While chlorosis and necrosis of leaf tips can occur on new leaves, brown lesions appear on the oldest leaves first and successive leaves become affected in sequence. In severe cases, brown lesions can appear on leaf sheaths, stems and awns.

It appears that boron tolerance in cereals involves more than one mechanism. Nable (1988) reported that the sensitivity of wheat cultivars to toxic concentrations of boron was governed solely by their ability to exclude boron from plant tissue. Cartwright et al. (1987) considered that, in addition to a genetic exclusion mechanism, other mechanisms related to root morphology may assist tolerant cultivars to extend roots into boron-toxic sub soils. Huang and Graham (1990) demonstrated variation for boron tolerance by culturing excised root tips on high-boron media. They found that tolerant genotypes either produced a callus or else developed longer root axes than intolerant genotypes. In addition, seedlings of tolerant wheat genotypes grown on filter paper soaked in toxic concentrations of boron have been shown to produce significantly longer roots than more-sensitive genotypes (Chantachume et al. 1995).

It appears that leaf-symptom expression may only be a minor component of the overall tolerance mechanism. Jenkin (1993) assessed differences in both grain yield and leaf symptoms in three populations derived from crosses between tolerant and intolerant parents grown on soil high in boron concentration and yet found no consistent relationship between leaf-symptom expression and grain-yield response. Riley and Robson (1994) also showed that severe levels of leaf symptoms could be produced in later stages of plant growth with minimal effect on grain yield. Mahalakshmi et al. (1995) found that some barley genotypes produced very few leaf symptoms yet accumulated high concentrations of boron in plant tissue. It is therefore likely, that some or all of the mechanisms involved in boron tolerance are under separate genetic control. Consequently, attempting to map major genes or quantitative trait loci (QTLs) associated with boron tolerance based on data from leaf symptoms alone is unlikely to identify all the important loci involved in the tolerance mechanism.

Recently, an RFLP map of barley chromosomes was constructed in a doubled-haploid population of 150 individuals created from a cross between the boron-tolerant parent Sahara 3771, a North African landrace, and the intolerant Australian cultivar Clipper (Langridge et al. 1995). The objective of our study was to identify chromosomal regions associated with differential plant response to toxic concentrations of boron using this population and the RFLP marker data set.

Materials and methods

Genetic material

The genetic material used in this study was a population of 150 doubled-haploid lines derived from a cross between the boron-tolerant Algerian landrace Sahara 3771 and a boron-sensitive Australian cultivar Clipper. The Clipper × Sahara population was produced by the *Hordeum bulbosum* method (Islam and Shepherd 1981) using embryo culture followed by chromosome doubling through colchicine treatment.

Screening for boron tolerance in a solution culture–root length assay

Treatment levels chosen for the solution culture–root length assay were 100 mg $B\,l^{-1}$ (B100) and 0 mg $B\,l^{-1}$ (B0). Chantachume et al. (1995) found that seedling root lengths of wheat varieties responded consistently at three concentrations: 50, 100 and 150 mg $B\,l^{-1}$. A control treatment (B0) was included to account for genetic variation of root length in the absence of boron toxicity.

Seeds of each doubled-haploid line were surface-sterilized with 5.0% sodium hypochlorite and pre-germinated for 8 days at 4°C. Three evenly germinated seeds, per doubled-haploid line, were placed embryo-downwards at a spacing of 2 cm across the middle of a filter paper (Ekwip 32 × 46 cm grade R6) soaked in either the B0 or B100 solutions. The base solution used in both the control (B0) and high-concentration treatment (B100) also included 0.5 mM $Ca(NO_3)_2 \cdot 4H_2O$, 0.0025 mM $ZnSO_4 \cdot 7H_2O$ and 0.015 mM H_3BO_3, following the method of Chantachume et al. (1995). The filter papers were rolled and covered with aluminium foil, then stored upright at 15°C for 12 days. The longest root of each seedling was measured.

The experiment was conducted as a randomised complete block with two replicates in 1997 and a further two replicates in 1998.

Screening for boron tolerance in soil–leaf symptoms, tissue boron concentration and dry matter production

The methods used for seed preparation followed those described for the solution culture–root length assay.

The soil-based assay was conducted in two boxes (2 m × 1 m × 0.25 m) containing soil to which boron was applied at 100 mg $B\,kg^{-1}$. The soil was a bulk sample of silt clay loam from the surface (0–10 cm) of a red-brown earth (Paull et al. 1988 b). The concentration of boron extractable in hot $CaCl_2$ (Spouncer et al. 1992) was 65 mg $B\,kg^{-1}$.

Two evenly germinated seeds of each doubled-haploid line were planted approximately 2 cm apart, each pair of plants forming a distinct plot. The 150 doubled-haploid lines and parents were all sown in a single box, representing a complete replicate with two boxes (replicates) planted in 1997 and a further two in 1998. The

parent varieties, Clipper and Sahara, were sown in every alternate seventh plot.

Four weeks after planting each plant was scored for severity of leaf symptoms on the basis of leaf damage on a scale of 1–6 where 1 gave no visual symptoms and 6 showed greater than 90% necrosis.

Each plot was harvested 1 cm above ground level 5 weeks after planting. The plants were dried at 80°C for 48 h and weighed. Dried shoots were ground, digested in nitric acid and analyzed for boron concentration by inductively coupled plasma spectrometry (ICP) (Zarcinas et al. 1987).

Statistical analysis of raw data

All statistical analyses, except for interval and multiple-regression marker analyses of boron response, were performed with JMP (v3.0, SAS Institute Inc.) software. The least-square means for relative root length (root length at B0 as a percentage of the root length at B100), were calculated using an ANOVA model. Factors for the ANOVA model were the doubled-haploid line, replicate and the plant number. Least-square means for boron concentration in whole shoots, leaf-symptom score and dry matter production were also calculated using the ANOVA model with the doubled-haploid line and replicate forming the relevant factors. Least-square means were calculated for each of the four traits from both the 1997 and 1998 data sets independently and for the 2-years data combined into a single four-replicate analysis for each trait. No raw data transformation was required.

The heritability for each trait was estimated from a linear model incorporating data from the 150 doubled-haploid lines over four replicates (two in 97 and two in 98). The factors were the doubled-haploid line and the 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 168 RFLP markers, covering all barley chromosomes, 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 employed. Significant relationships between trait expression and RFLP markers for the 1997, 1998 and combined data sets were compared. A marker locus thought to be associated with a gene or chromosomal region conferring boron tolerance was tested for two-way interaction with all other markers in the data set using the method described by Nelson et al. (1998). All marker analyses were performed with the computer program 'Qgene' (Nelson 1997).

Gene effects

Each of the 150 doubled-haploid lines was scored for the presence of either a Clipper or Sahara marker allele for the RFLP markers most significantly associated with each trait, one marker representing each unique chromosomal region identified. From this, the marker-allele genotype of each doubled-haploid line was determined. Lines of identical genotype were grouped into marker-allele classes. Least-square means for each class were calculated using a single-factor (marker class) ANOVA. Means of marker classes were compared using contrasts.

Results

Screening for boron tolerance in the solution culture–root length assay

A significant ($P < 0.001$) reduction in root growth at B100 was observed in all doubled-haploid lines and both parents. Significant ($P < 0.001$) genetic variation for seedling root length at both B0 and B100 was observed within the mapping population (Fig. 1A and B). The heritability of root length at B0 was estimated as $h^2 = 0.51$. The heritability of root length at B100 was significantly greater at $h^2 = 0.89$. Relative root length (RRL) was chosen as the appropriate variable for mapping as it was expected to provide a measure of tolerance independent of genetic variation for absolute root length. RRL was calculated as the root length at B100 expressed as a percentage of the root length at B0.

The frequency distribution for relative root length is provided in Fig. 1C. With the exception of six lines which exceeded the RRL of Sahara, all other lines fell within the parental range.

Screening for boron tolerance in soil–leaf symptoms, tissue boron concentration and dry matter production

Significant ($P < 0.001$) differences between doubled-haploid lines were observed for all three traits measured in the soil-based assay (Fig. 1D–F). The estimated heritability for whole-shoot boron concentration was 0.89, 0.72 for leaf-symptom score and 0.32 for whole-shoot dry weight. Frequency distributions for leaf-symptom score, whole-shoot dry weight and whole-shoot boron concentration are provided in Fig. 1D–E, and F respectively. Distributions for leaf- symptom score (Fig. 1D) and whole-shoot dry weight (Fig. 1E) were slightly skewed towards the lower values. Whole-shoot dry weight followed a bi-modal distribution (Fig. 1F) (data not tested for fit) consistent with either a single major gene or more than one gene but with a single gene having a major effect. Only two lines significantly ($P < 0.05$) exceeded Clipper for leaf-symptom score, three lines exceeded Clipper for whole- shoot boron concentration and two lines were significantly below Clipper for whole-shoot dry weight. There was no evidence of positive transgressive segregation for any of the traits measured.

Comparison between boron tolerance parameters

Table 1 details the pairwise correlation coefficients between the four boron tolerance traits measured. While all pairwise correlations were highly significant ($P < 0.001$), none exceeded 0.55. Leaf-symptom score generally had the lowest overall pairwise correlation coefficients ranging from -0.27 with whole-shoot dry weight to 0.43 with whole-shoot boron concentration. This is consistent with the lack of association previously found between leaf-symptom expression and grain yield (Jenkin 1993; Riley and Robson 1994), and leaf-symptom expression and the concentration of boron in plant tissue (Mahalakshmi et al. 1995).

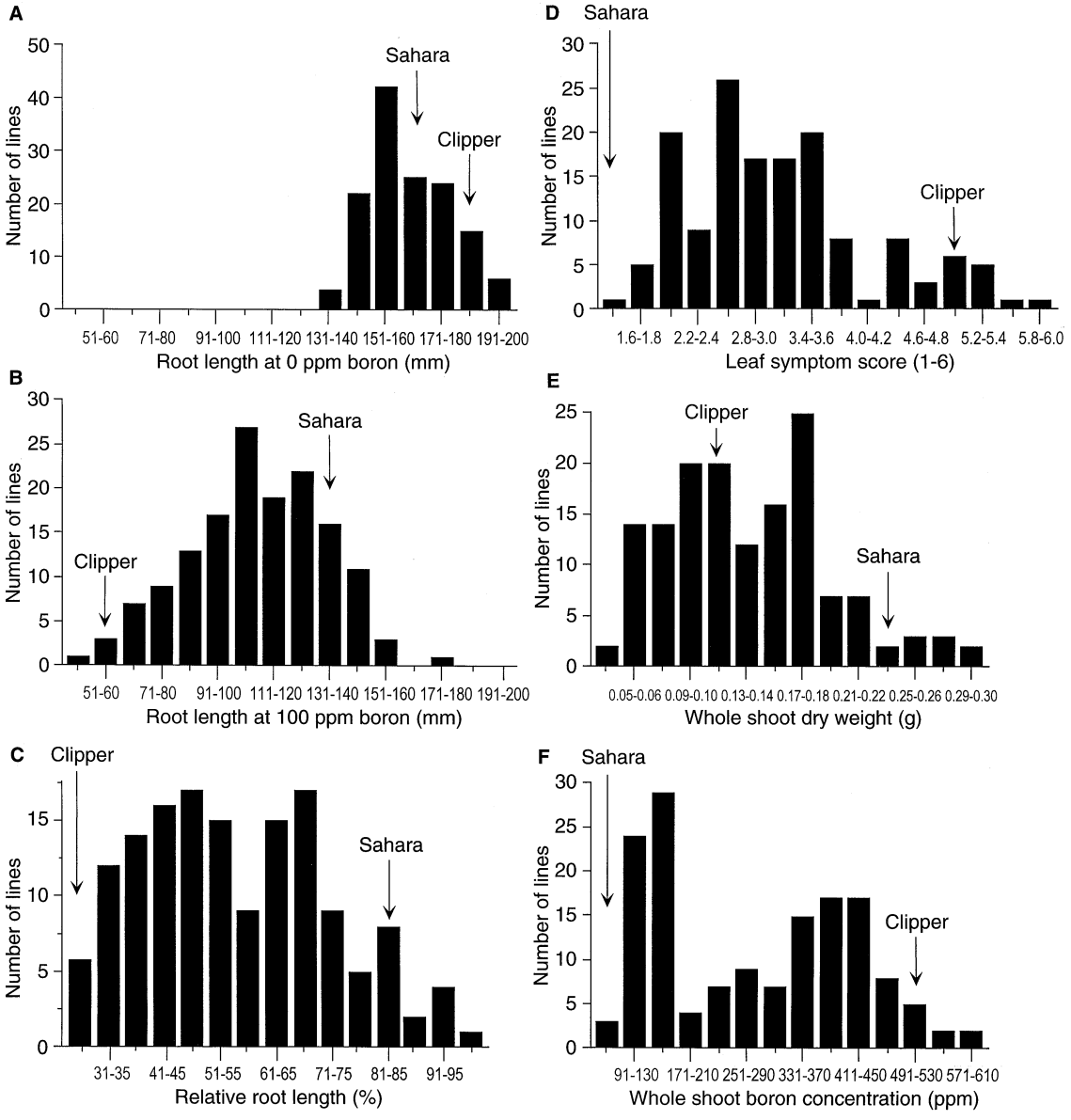

Fig. 1A–F Frequency distributions for all traits measured on the Clipper × Sahara 3771 mapping population. Data is derived from a combined analysis of the 1997 and 1998 data sets. **A** Root length at 0 ppm B solution, **B** root length at 100 ppm B solution, **C** relative root length (RRL) (%), **D** leaf-symptom score (1–6), **E** whole-shoot dry weight (g), **F** whole-shoot boron concentration (%)

Whole-shoot boron concentration had the overall highest pairwise correlation coefficients ranging from 0.43 with leaf-symptom score to -0.55 with whole-shoot dry weight.

Mapping

Relative root length

Marker analysis of the combined (1997 and 1998) data sets for seedling root length at the low (B0) and high (B100) boron concentration identified a region on the long arm of chromosome 5H (Fig. 2A) associated with root length per se (RL). A region on the long arm of chromosome 4H and the short arm of chromosome 3H were strongly associated with RRL (Fig. 2B and C respectively). Multiple regression analysis showed that these two loci, in combination, accounted for approximately 39% of the variation in RRL. Sahara marker alleles were associated with a high RRL. Marker analysis of the low (B0) boron concentration failed to show significant relationships with these same regions on chromosomes 4H and 3H. It can be concluded, therefore, that the regions identified on these chromosomes are associated with root-length response to high boron concentration.

Significant associations between RRL and regions on chromosomes 4H and 3H were identified in both the 1997 and 1998 data sets. There were only minor

Table 1 Pairwise correlation coefficients between four boron tolerance traits measured on the Clipper × Sahara 3771 mapping population

Boron tolerance traits	Whole-shoot boron concentration	Relative root length	Leaf-symptom score
Relative root length	− 0.53*		
Leaf-symptom score	0.43*	− 0.30*	
Whole-shoot dry weight	− 0.55*	0.37*	− 0.27*

* Significant at $P < 0.001$

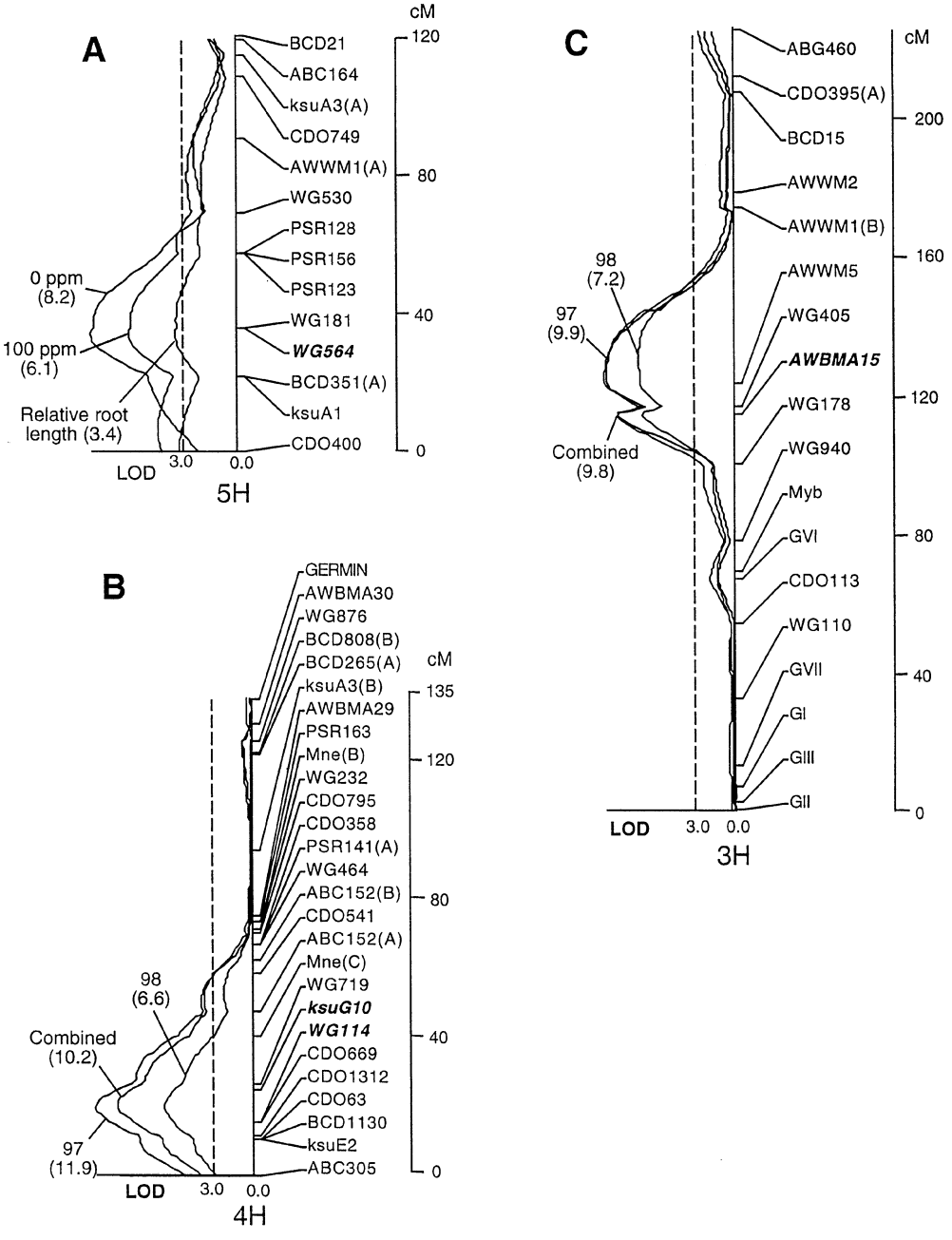

Fig. 2A–C Chromosome location of regions associated with relative root length (RRL) expressed in doubled-haploid lines from the cross Clipper × Sahara 3771 based on interval mapping of the 1997, 1998 and combined data. Short arms are towards the tops of chromosomes. Maximum LOD scores for each peak are provided in *parentheses*. The RFLP marker most significantly associated with the trait is presented in *bold italics*. *Dashed lines* show the LOD 3.0 threshold. Interval map of chromosome 5H based on root length at 0 ppm B, 100 ppm B and relative root length (1997 and 98 combined data) (**A**). Interval map of chromosomes 4H (**B**) and 3H (**C**) based on RRL from the 1997, 1998 and combined data sets

LOD-score variations between the analyses of the 1997 and 1998 data sets. Analysis of the 1998 data set identified an association between the region conferring RL, previously identified on chromosome 5H, and RRL. The conversion of root length to RRL appears to have failed to account for all the variation attributed to RL in the 1998 and combined data sets (Fig. 2A).

The RFLP markers on chromosomes 3H and 4H most strongly associated with relative root length were *xAWBMA15* and *xWG114* respectively.

Leaf-symptom score

Markers on regions of chromosomes 2H (Fig. 3A) and 4H (Fig. 3B) were associated with the expression of leaf symptoms on plants grown in boron toxic soil based on the 1997, 1998 and combined data sets. Based on multiple-regression analysis, loci on chromosomes 2H and 4H, in combination, accounted for approximately 38% of the variation in leaf-symptom data. The marker on chromosome 4H most strongly associated with leaf-symptom score was *xWG114*. *xWG114* was also the chromosome-4H marker most strongly associated with RRL (Fig. 2B). Sahara marker alleles at this locus were associated with a low leaf-symptom score.

Whole-shoot dry weight

Markers on a region of chromosomes 4H were strongly associated with whole-shoot dry weight. The strongest relationship was again with marker *xWG114* (Fig. 4). Based on simple marker-regression analysis, the chromosome-4H locus accounted for approximately 34% of the variation in dry matter data. Sahara marker alleles at this locus conferred high dry weight.

Whole-shoot boron concentration

Markers on regions of chromosomes 4H (Fig. 5A) and 6H (Fig. 5B) were associated with the whole-shoot boron concentration of plants grown in boron toxic

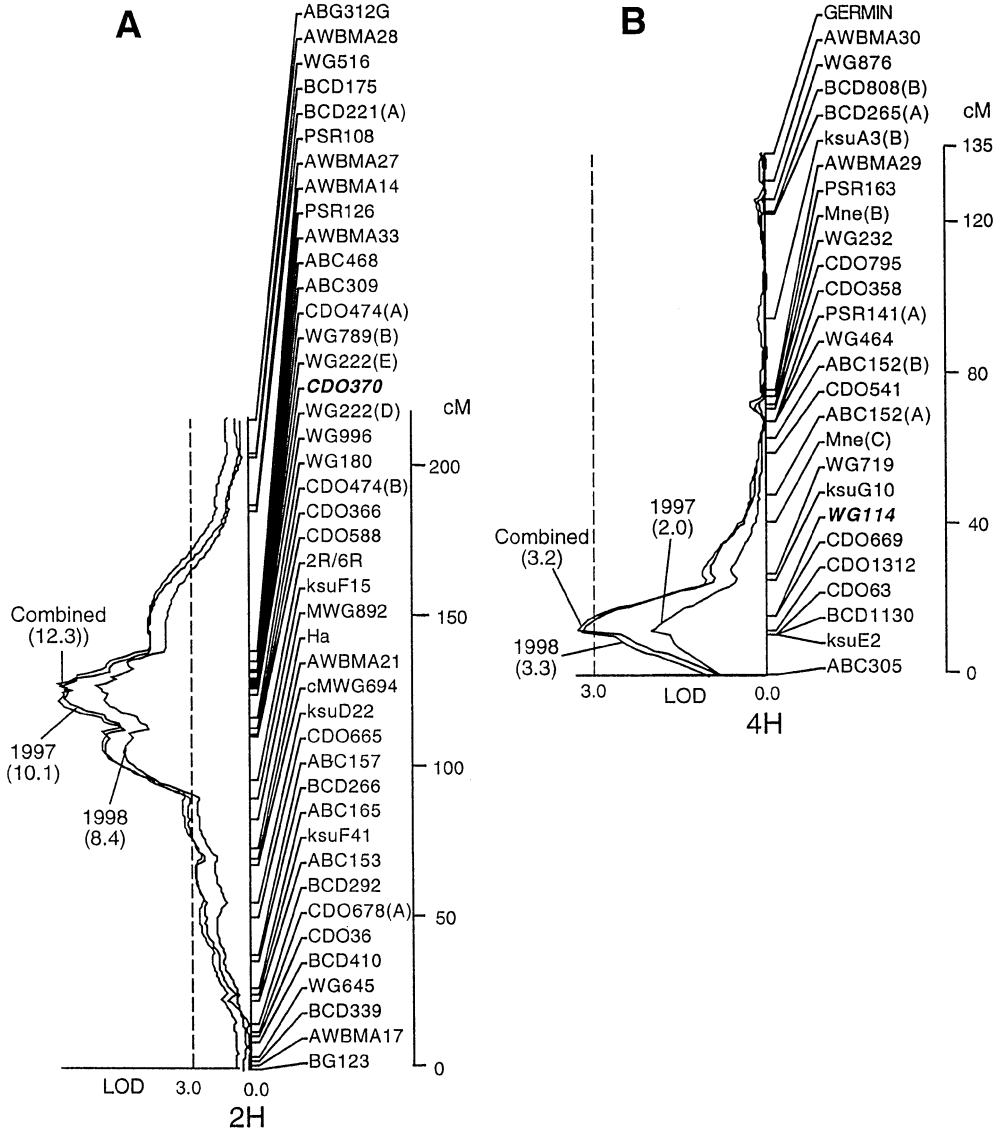

Fig. 3A, B Location on chromosomes 2H (**A**) and 4H (**B**) of regions associated with leaf-symptom score expressed in doubled-haploid lines from the cross Clipper × Sahara 3771 based on interval mapping of the 1997, 1998 and combined data. Short arms are towards the tops of chromosomes. Maximum LOD scores for each peak are provided in *parentheses*. The RFLP marker most significantly associated with the trait is presented in *bold italics*. *Dashed lines* show the LOD 3.0 threshold

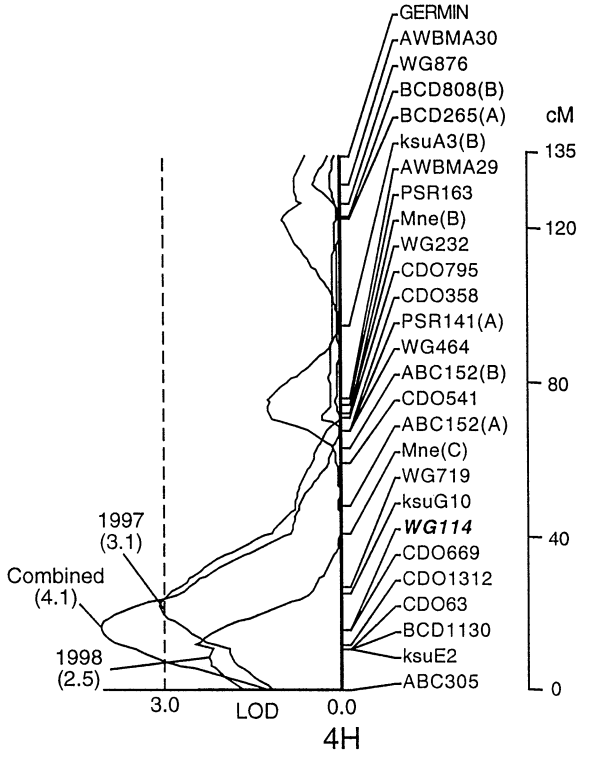

Fig. 4 Chromosome location of a region associated with dry matter response to a toxic concentration of boron expressed in doubled-haploid lines from the cross Clipper × Sahara 3771 based on interval mapping of the 1997, 1998 and combined data. The short arm is towards the top of the chromosome. Maximum LOD scores for each peak are provided in *parentheses*. The RFLP marker most significantly associated with the trait is presented in *bold italics*. *Dashed lines* show the LOD 3.0 threshold

soil. Based on multiple-regression analysis, loci on chromosomes 4H and 6H combined, accounted for approximately 53% of the variation in whole-shoot boron concentration data. Sahara marker alleles at these loci conferred low whole-shoot boron concentration. RFLP markers on chromosomes 4H and 6H most strongly associated with leaf-symptom score were *xWG114* and *xAmy-1(A)* respectively.

Marker interactions

Marker loci thought to be associated with a chromosomal region involved in the control of any of the four boron tolerance traits were tested for two-way interaction with all other markers in the data set. The markers tested for interaction with all other markers were *xCDO370* (2H), *xAWBMA15* (3H), *xWG114* (4H) and *xAmy-1(A)* (6H). No significant ($P < 0.001$) interactions were identified.

Gene effects

Relative root length

Table 2 details the mean effect of the presence of either a Clipper or Sahara marker allele on RRL. As expected, there was no significant ($P < 0.05$) difference between individuals carrying the Clipper or Sahara marker alleles at the chromosome-2H locus, previously identified

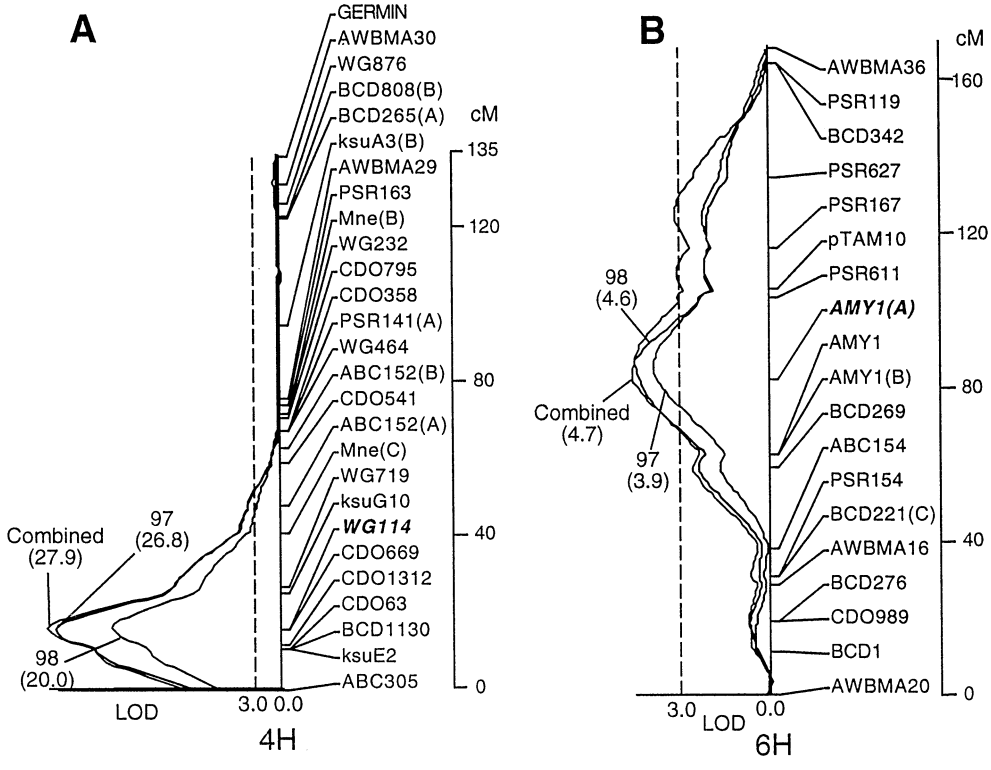

Fig. 5A, B Location, on chromosomes 4H (**A**) and 6H (**B**) of regions associated with whole-shoot boron concentration expressed in doubled-haploid lines from the cross Clipper × Sahara 3771 based on interval mapping of the 1997, 1998 and combined data. Short arms are towards the tops of chromosomes. Maximum LOD scores for each peak are provided in *parentheses*. The RFLP marker most significantly associated with the trait is presented in *bold italics*. *Dashed lines* show the LOD 3.0 threshold

Table 2 Mean effect of the presence of either the Clipper or the Sahara marker allele on the relative root length (%) of the Clipper × Sahara 3771 mapping population seedlings grown on filter paper soaked in low and toxic concentrations of boron

Marker	Allele(s) – Clipper (C) Sahara (S)	Number of doubled-haploid lines within class	Relative root length [least squares mean (%)]	Standard error of mean
xAWBMA15 (3H)	C	84	50.1 a[a]	1.7
	S	64	63.6 b	2.0
xWG114 (4H)	C	82	47.4 a	1.5
	S	64	66.4 b	1.8
xAWBMA15 (3H)/*xWG114* (4H)	CC	48	40.9 a, e	1.8
	CS	36	62.3 b, f	2.1
	SC	34	56.6 c, f	2.1
	SS	28	71.8 d, g	2.4

[a] Means with letters 'a', 'b', 'c' and 'd' are significantly different at $P < 0.05$, means with letters 'e', 'f' and 'g' are significantly different at $P < 0.005$; comparisons within major class groups only

to be associated with leaf-symptom score only. The presence of the Sahara allele at the chromosome-3H locus (*xAWBMA15*) conferred an average 13.5% increase in RRL. The presence of the Sahara allele at the chromosome-4H locus (*xWG114*) conferred an average 19.0% increase in RRL over those individuals carrying the Clipper marker allele only. Individuals carrying the Sahara marker allele at both the 3H and 4H loci produced, on average, a 30.7% greater relative root length than individuals carrying the Clipper allele at both loci.

The majority of variation in RRL is controlled by two major loci, on chromosomes 3H and 4H, behaving in a largely additive manner, with the chromosome-4H locus having a slightly larger effect.

Leaf-symptom score

Table 3 details the mean effect of the presence of either a Clipper or Sahara marker allele on leaf-symptom score. The presence of the Sahara marker allele at the chromosome-2H locus (*xCDO370*) conferred an average 1.2-point reduction in leaf-symptom score while a Sahara marker allele at the chromosome-4H locus (*xWG114*) conferred an average 0.6-point reduction. Individuals carrying the Sahara marker allele at both the 2H and 4H loci, on average, received, on average, a 1.8-point lower leaf-symptom score than individuals carrying Clipper alleles at both loci. It appears that leaf-symptom expression is predominantly controlled by two major loci behaving in a largely additive manner, with the chromosome-2H locus having a substantially larger effect than the chromosome-4H locus.

Whole-shoot dry weight

Table 4 details the mean effect of the presence of either a Clipper or Sahara marker allele on whole-shoot dry weight. The presence of the Sahara marker allele at the

Table 3 Mean effect of the presence of either the Clipper or the Sahara marker allele on the leaf symptoms of the Clipper × Sahara 3771 mapping population grown in soil containing toxic concentrations of boron

Marker	Allele(s) – Clipper (C) Sahara (S)	Number of doubled-haploid lines within class	Leaf-symptom score [least squares mean (score 1–6][a]	Standard error of mean
xCDO370 (2H)	C	75	3.7 a[b]	0.1
	S	72	2.5 b	0.1
xWG114 (4H)	C	82	3.4 a	0.1
	S	64	2.8 b	0.1
xCDO370(2H)/*xWG114*(4H)	CC	41	4.1 a	0.1
	CS	33	3.2 b	0.1
	SC	42	2.7 bc	0.1
	SS	30	2.3 c	0.1

[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 $P < 0.005$, comparisons within major-class groups only

Table 4 Mean effect of the presence of either the Clipper or the Sahara marker allele on the whole-shoot dry weight of the Clipper × Sahara 3771 mapping population grown in soil containing toxic concentrations of boron

Marker	Allele(s)	Number of doubled-haploid lines	Whole-shoot dry weight (least squares mean, g)	Standard error of mean
xWG114 (4H)	Clipper (C)	82	0.12 a[a]	0.01
	Sahara (S)	64	0.17 b	0.01

[a] Means a and b significantly different at $P < 0.001$

Table 5 Mean effect of the presence of either the Clipper or the Sahara marker allele on the whole- shoot boron concentration of the Clipper × Sahara 3771 mapping population grown in soil containing toxic concentrations of boron

Marker	Allele(s) – Clipper (C) Sahara (S)	Number of doubled-haploid lines within class	Whole-shoot B concentration [least squares mean (ppm)]	Standard error of mean
xWG114 (4H)	C	82	372.5 a[a]	7.4
	S	64	133.2 b	8.7
xAmy-1(A) (6H)	C	87	311.8 a	13.9
	S	61	215.7 b	17.1
xWG114/xAmy-1(A)	CC	51	412.1 a	7.0
	CS	31	302.4 b	9.4
	SC	34	158.3 c	8.8
	SS	30	101.3 d	9.9

[a] Means with different letters are significantly different at $P < 0.000$, comparisons within major class groups only

chromosome-4H locus (*xWG114*) conferred an average 42% increase in whole-shoot dry weight over individuals carrying the Clipper marker allele.

Whole-shoot boron concentration

Table 5 details the mean effect of the presence of either a Clipper or Sahara marker allele on whole-shoot boron concentration. The presence of the Sahara marker allele at the chromosome-4H locus (*xWG114*) conferred an average 64% reduction in whole-shoot boron concentration over those individuals carrying the Clipper marker allele only. The presence of the Sahara allele at the chromosome-6H locus (*xAmy-1(A)*) conferred an average 31% reduction in whole-shoot boron concentration. Individuals carrying the Sahara marker allele at both the 4H and 6H loci showed, on average, a 76% lower whole-shoot boron concentration than individuals carrying the Clipper allele at both loci. It appears that whole-shoot boron concentration is predominantly controlled by two major loci behaving in a largely additive manner, with the chromosome-4H locus having a substantially larger effect than the chromosome-6H locus.

Discussion

Chromosomal locations conferring boron tolerance, and relationships to proposed tolerance mechanisms

This study has identified and located four chromosomal locations involved in tolerance to boron toxicity as measured by the four traits discussed.

The relative rating of barley genotypes for boron tolerance has conventionally been based on the expression of leaf symptoms. Genetic variation for boron tolerance (leaf-symptom expression) was attributed to differences in boron concentration in plant tissues and, therefore, was proposed to be due to differences in passive boron uptake (Nable 1988, 1991). However, Mahalakshmi et al. (1995) found that some barley genotypes showed low leaf symptoms yet accumulated high concentrations of boron in plant tissues. In this study, several Clipper × Sahara doubled-haploid lines were found to accumulate high concentrations of boron in whole shoots yet produced low levels of leaf symptom. Significant ($P < 0.001$) correlations between symptom development, tissue concentration, dry weight production and relative root length were found but the coefficient values were relatively low (Table 1).

This contrasted with the high level of correlation (correlation coefficients ranged from 0.66–0.82, $P < 0.01$) between root length, symptom score, shoot concentration and dry weight for 14 wheat cultivars reported by Chantachume et al. (1995). The strong correlation found in the wheat study could have been due to the relatively small number of genotypes and the preselection of genotypes for extremes in leaf-symptom expression, dry matter production and/or grain yield. In addition to discrepancies between leaf-symptom score and tissue concentration, poor relationships between grain- yield response and leaf symptom expression have also been reported in both wheat and barley (Paull et al. 1988 b; Jenkin 1993; Riley and Robson 1994).

A major locus on chromosome 2H, associated with leaf-symptom score, was not associated with other tolerance parameters. In contrast, the chromosome-4H locus was significantly associated with all four tolerance parameters (Figs. 3, 4, 5). The chromosome-3H locus was associated with relative root length only. These findings provide evidence that the observed poor relationships between leaf-symptom development, tissue concentration (Makalakshmi 1995) and grain yield (Jenkin 1993; Riley and Robson 1994), and the relatively low correlation coefficients between tolerance parameters, are due to differences in genetic control.

The chromosome-2H locus may be involved in the translocation of boron in leaf tissue. It has been shown that there is no difference between roots and shoots in the accumulation of boron (Nable 1991) yet the concentration in leaves increases from young to old leaves and from base to tips (Nable et al. 1990). A similar pattern is followed in the development of leaf symptoms in barley.

Regions on chromosomes 4H and 6H appear to be involved in a boron exclusion mechanism which determine the relative accumulation of boron in shoots. The exclusion mechanism is, in turn, likely to reduce the effect of toxic levels of boron on root growth (RRL) and dry matter production. Evidence for this was found in the association of RRL (Fig. 2) and whole-shoot dry weight (Fig. 4) with the chromosome-4H region involved in the control of boron uptake.

The chromosome-3H locus was not associated with whole-shoot boron concentration but was involved in the RRL response. This locus could be involved in internal physiological compensation resulting in superior root growth under toxic situations.

Although a boron exclusion mechanism plays a major role in overall boron tolerance, since it has an effect on the expression of other tolerance traits, other mechanisms have also been shown to play a role. These observations support the conclusions of Cartwright et al. (1987) that a genetic exclusion mechanism is important but that other mechanisms are also likely to be involved.

Gene effects

The chromosome-2H locus appears to be the most important in controlling leaf-symptom expression while the chromosome 4H locus appears to be the most important in controlling boron uptake, root-length response and dry matter production. Only one chromosome region was associated with the response to dry matter production. It is possible that the region on chromosome 6H, associated with the control of boron uptake, and the chromosome-3H, region, associated with the control of RRL, also have an effect but, possibly due to the high error variance in the whole-shoot dry weight assay, no additional statistically significant relationships were established.

Further investigations on the role and relative contribution of each region and/or gene, particularly in relation to grain-yield response, and the transferability of these regions to a different genetic background, are required before an efficient selection strategy can be devised.

Evolution of boron tolerance genes in wheat and barley

Wheat chromosomes 4A (Paull et al. 1988), 7B and 7D (Paull 1990) have all been implicated in the control of boron tolerance in bread wheat. A strong association was identified between boron tolerance, as measured by the root-length assay, and an RFLP marker *xKsuG10* on chromosome 4A of wheat (Paull et al. 1993). Its location was detected close to the break point between 4AL-7BS on the modern 4AL chromosome (Devos et al. 1995; Mickelson-Young et al. 1995). In the present study, a region on chromosome 4H of barley was associated with all four boron tolerance traits measured. On the basis of the consistency in interval-analysis outputs from all four traits, a major gene, or cluster of genes, is located between RFLP markers *xWG116* and *xKsuG10* on chromosome 4H of barley. It is possible that wheat and barley may possess a common gene on chromosomes 4A and 4H respectively.

Marker-assisted selection for boron tolerance

No single glasshouse or laboratory boron tolerance bioassay currently available will successfully identify individuals carrying all four QTLs. The relative root-length and leaf-symptom assays are both time-consuming. The assay for whole-shoot boron concentration is expensive and destructive. The leaf-symptom assay is highly subjective and also time-consuming. Field-based grain-yield comparisons are prone to experimental error due to within-site variation and errors are compounded by genotype by environment interaction for characters other than boron tolerance.

RFLP markers closely linked to genes of agronomic importance have been demonstrated to be useful tools for indirect selection in a barley breeding program (Jefferies et al. 1997). RFLP marker-assisted selection is time efficient, non-destructive and, depending on linkage relationships, is characterised by a low selection error. In addition, RFLP markers can detect heterozygous individuals and genotypes can be screened for a number of marker-linked traits almost simultaneously. Marker-assisted selection for boron tolerance will provide the plant breeder with an efficient selection tool offering more flexibility than the current bioassay systems. Experiments to validate these markers in different genetic backgrounds are currently underway.

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