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ORIGINAL PAPER

Boron toxicity in rice (*Oryza sativa* L.). I. Quantitative trait locus (QTL) analysis of tolerance to boron toxicity

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Abstract Boron toxicity tolerance of rice plants was studied. Modern *japonica* subspecies such as Koshihikari, Nipponbare, and Sasanishiki were tolerant, whereas *indica* subspecies such as Kasalath and IR36 were intolerant to excessive application of boron (B), even though their shoot B contents under B toxicity were not significantly different. Recombinant inbred lines (RILs) of *japonica* Nekken-1 and *indica* IR36 were used for quantitative trait locus (QTL) analysis to identify the gene responsible for B toxicity tolerance. A major QTL that could explain 45% of the phenotypic variation was detected in chromosome 4. The QTL was confirmed using a population derived from a recombinant inbred line which is heterogenic at the QTL region. The QTL was also confirmed in other chromosome segment substitution lines (CSSLs).

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Introduction

Boron (B) is an essential micronutrient for higher plants, but in excess amounts it inhibits growth. The critical tissue concentration between B deficiency and B toxicity is very small (Blamey et al. 1997), that is, B is toxic to many plants at levels only slightly above that required for normal growth (Mengel and Kirkby 2001). There is considerable genetic variation in response to high B over a wide range of plant species (Gupta 1983). Differences in tolerance to B toxicity among cultivars has been studied extensively in barley (Nable et al. 1997), wheat (Nable 1988; Paull et al. 1988), and oilseed rape (Kaur et al. 2006). Reduced shoot and root elongation, chlorosis, and necrosis in leaves are characteristic of B toxicity. Boric acid interacts spontaneously with diol groups in the *cis* position (Power and Woods 1997); therefore, metabolic reactions involving *cis* diol compounds such as ATP, NADH, and RNA are regarded as candidate injury sites. Reid et al. (2004) studied the effects of excessive B on such processes in detail and concluded that impaired growth under high B levels was not due to the reduced energy supply nor the inhibition of protein synthesis. Roessener et al. (2006) conducted a comprehensive metabolite comparison of B-tolerant and intolerant cultivars of barley (*Hordeum vulgare* L.) and reported that none of the analyzed metabolites was sufficient to explain the cellular tolerance mechanism in the tolerant cultivar. In barley plants, B toxicity tolerance is related to a lower accumulation of B in both shoots and roots due to an active efflux of B from the roots (Hayes and Reid 2004), although the molecular mechanism is not known.

Rice (*Oryza sativa* L.) is one of the most important cereal crops in the humid tropics and subtropics. The possibility of B toxicity of rice on soils irrigated with high B water and on coastal saline soils has been pointed out

(Ponnamperuma and Yuan 1966; Ponnamperuma et al. 1981), and recognized in the Philippines (Cayton 1985). In Japan, waste water with high B content caused B toxicity in rice in Okayama Prefecture in 1997. Studies on B toxicity in rice, however, are very limited (Tokuoka and Morooka 1936; Ishizuka and Tanaka 1962), and reports are available for only *indica* rice (Paliwal and Mehta 1973; Cayton 1985).

The identification and isolation of gene(s) that are responsible for B tolerance is another approach towards understanding the mechanism of B toxicity. Using a quantitative trait locus (QTL) analysis, chromosomal regions associated with B tolerance were identified in barley (Jefferies et al. 1999) and wheat (Jefferies et al. 2000). In wheat (*Triticum aestivum*), B tolerance is controlled by a small number of major genes (Paull et al. 1991). Completion of the genomic sequence has facilitated cloning of QTLs in rice. The smallest genome size of rice among cereal crops is also a major advantage for QTL analysis in rice. In this report, we first studied the sensitivity of rice cultivars to B toxicity, and then performed a QTL analysis of B toxicity tolerance using a population derived from a cross between a B-tolerant *japonica* and a B-sensitive *indica* rice cultivar, as the first step towards understanding the mechanism of B toxicity in rice.

Materials and methods

Plant materials

A population of recombinant inbred lines (RILs) derived from a cross between a *japonica* line Nekken-1 and an *indica* cultivar IR36 was produced using the single seed descent method at the Laboratory of Plant Breeding, Kyoto University. An F_9 population of 83 lines was used in the present experiment. The parent cultivars of Nekken-1 are *japonica* Akihikari, Nihonmasari, and Ketan Nangka. Nekken-1 possesses the *japonica* background and the wide compatibility gene that prevents F_1 sterility, which is common in hybrids resulting from *indica*/*japonica* crosses (Yanagihara et al. 1995).

Three populations of chromosome segment substitution lines (CSSLs) were obtained from the Rice Genome Resource Center (RGRC), Tsukuba, Japan. CSSLs are derived from crosses of *japonica* Koshihikari and *indica* Kasalath, *japonica* Nipponbare and *indica* Kasalath, and *japonica* Sasanishiki and *indica*-*japonica* Habataki, and developed by backcrosses and marker assisted selections. They are carrying chromosomal segments of *indica* or *indica*-*japonica* cultivar in a genetic background of *japonica* cultivar. The substituted chromosome segments are covering most of the 12 chromosomes in 39 (Koshihikari/Kasalath and Sasanishiki/Habataki) or 54 (Nipponbare/Kasalath) lines (Ebitani et al. 2005). The genotype data of CSSLs are available at the RGRC web site (http://www.rgrc.dna.affrc.go.jp/). In this study, a line of Koshihikari/Kasalath CSSLs (SL209), four lines of Nipponbare/Koshihikari CSSLs (SL16, 18, 44 and 51), and a line of Sasanishiki/Habataki CSSLs (SL414) were used.

Hydroponic culture

A batch of 20 seeds of a cultivar or line was soaked for 3 days at 30°C in distilled water supplemented with a fungicide (3% w/v, TORIFUMIN; Nippon Soda Co., Ltd., Tokyo, Japan). Ten seeds were sown on a nylon mesh (18 mesh, 24 × 36 mm) stretched on a plastic frame. Eight meshes were floated on 2 L of a culture solution in a plastic container. The other batch of ten seeds of the same line or cultivar was raised similarly, but the culture solution was supplemented with high B levels. The plants on the mesh were raised for 7 days in a growth chamber (NS-280 FHW; Takayama Seisakusyo, Kyoto, Japan) under the following conditions: temperature 30°C, relative humidity 80%, photo period 12 h, and light intensity 350 μmol m^{-2} s^{-1}. The culture solution contained 0.5 mol m^{-3} $(NH_4)_2SO_4$, 0.13 mol m^{-3} KH_2PO_4, 0.25 mol m^{-3} KCl, 0.25 mol m^{-3} $CaCl_2$, and 0.25 mol m^{-3} $MgCl_2$, pH 6.0. Iron was supplied at 2.5 g Fe m^{-3} as FeNa-EDTA. Micronutrients were supplied according to Arnon's formula (Hewitt 1966) at half strength. Boron was supplied as boric acid at 0.5 g B m^{-3} for the controls and at 40, 54 or 60 g B m^{-3} as high B treatment, depending on the rice cultivars. That is, to Koshihikari, Kasalath and their CSSLs (SL209), B was applied at 40 g B m^{-3}, and to Nekken-1, IR36 and their RILs at 54 or 60 g B m^{-3}, and to Nipponbare, Kasalath and their CSSLs (SL16, 18, 44 and 51) and Sasanishiki, Habataki and SL414, at 60 g B m^{-3}.

At sampling, roots were rinsed in distilled water for at least 10 minutes and the seedling height and root length were determined. The plants were separated into roots and shoots, and the parts were bulked and dried in an oven at 70°C and weighed.

Soil culture

Three rice cultivars, Koshihikari, Nekken-1, and IR36, were cultivated in soil until maturity under various B concentrations. Koshihikari and Nekken-1 are *japonica* subspecies, and IR36 is an *indica* subspecies. Imbibed seeds were germinated and raised for 18 days in a fertilized granulated soil (Ryujo-Baido, Kureha, Tokyo, Japan) that is used for preparation of the seedlings for a transplanting machine.

A 3-kg batch of air-dried and sieved loamy-sand paddy soil taken from the Experimental Farm (Kitashirakawa,

Kyoto University) was put into a 4-littre plastic pot together with 1 g N, 1 g P_2O_5, and 1 g K_2O as $(NH_4)_2SO_4$, NaH_2PO_4 and KCl. One week before transplanting, two pots each were put into a plastic container filled with 0.02 m^3 tap water supplemented with B (0, 3, 10 or 30 g B m^{-3}) as boric acid. The water level was just below the rim of the pots so the B solution would not flow into the pot surface water, but the B solution could enter the pots through a hole (20 mm in diameter) on the side wall at the bottom. To keep the water level and the B concentration constant, tap water was supplemented every day. The B concentration of the tap water ranged from 4.6 to 6.0 mg B m^{-3}.

Three 18-day-old seedlings were transplanted to each pot on 10 May 2005 and 6 May 2006. Each treatment had four (Koshihikari) or two replicates (Nekken-1 and IR36). The pots were kept in a greenhouse until maturity. The B-containing outer water was changed three times during the culture period.

Just prior to harvesting, the plant height and the number of panicles were recorded. The panicles and flag leaves were sampled at 130 days (Nekken-1 and IR36) or 155 days (Koshihikari) after sowing. The shoots were bulked per pot and dried in an oven for two days at 70°C. Three batches of 100 filled grains were weighed for the 1,000-grain weight determination. Boron was quantified using the chromotropic acid method (Matoh et al. 1997). Statistic analyses were carried out by the two-way ANOVA and Tukey test.

Molecular marker analysis

Shoots of plants grown under the control condition (0.5 g B m^{-3}) were used for DNA extraction (CTAB method) and the extracted DNA was used for genotyping by polymerase chain reaction analysis. A total of 104 markers covering all 12 chromosomes were used; 95 of which were simple sequence repeat (SSR) markers (McCouch et al. 2002) and 9 of which were sequence tagged site markers (http://www.rgp.dna.affrc.go.jp). The linkage of two adjacent markers were significant ($P < 0.05$) and the largest genetic distance was 36 cM in the Kosambi function between RM3773 and RM6673 on chromosome 10.

To confirm the putative QTL, a marker Cht4 (Nakazki and Ikehashi 1998) and a newly developed marker P-4 were employed. The forward primer of P-4 was 5′-GGAGGACACATACATGCCACT-3′, and the reverse primer was 5′-GGTGCCACCTCTTAGTACCTT G-3′.

QTL analysis

Construction of a genetic linkage map and a QTL analysis was performed with a computer program Map Manager QTX (Manly et al. 2001) and Q gene (Nelson 1997). The simple interval mapping method was taken and the log-likelihood (LOD) score was utilized to hypothesize the QTL for traits. Based on permutation tests with 1,000 permutations, a suggestive LOD threshold of 1.6 ($P < 0.63$) and a significant LOD threshold of 2.9 ($P < 0.05$) were applied to the QTL detection.

Results

Boron toxicity in water-cultured rice seedlings

Germination of the Nekken-1 and IR36 seeds was not affected by B in culture solutions with up to 54 g B m^{-3} in the water culture experiments. Shoot elongation was inhibited according to the increase in the media B concentration, but IR36 was more sensitive to B toxicity and the plants died 7 days after sowing (Fig. 1a). Root elongation was more tolerant than shoot elongation, and there were no differences between the cultivars (Fig. 1b). Similarly, dry matter production of the shoot was more severely inhibited in IR36 (Fig. 1c), even though the root dry matter was not different significantly even under 54 g B m^{-3} (Fig. 1d). Boron concentrations in the shoots and roots increased linearly with increasing B concentrations (Fig. 1e, f), but there were no significant differences between the two cultivars with up to 27 g B m^{-3}. At 54 g B m^{-3}, not enough IR36 samples were obtained, and therefore B concentration could not be determined. Leaf tip burn was significant at 27 and 54 g B m^{-3} in both cultivars. Thus, Nekken-1 was more tolerant than IR36 to B toxicity at the seedling stage under water culture.

Boron toxicity in soil-cultured rice plants

Boron application (10 g B m^{-3}) to the irrigation water induced visible B toxicity symptoms such as necrotic spots in the tip and margin of leaf blades, and black spots on the grains, which were most severe in IR36 (Fig. 2). The growth parameters, such as plant height, panicle number, spikelet number per panicle, filled spikelet percentage, 1,000-grain weight and grain yield per hill, were presented in Table 1. At 10 g B m^{-3}, B toxicity did not affect plant height in any of the cultivars, but decreased the panicle number per hill in all the cultivars, spikelet number per panicle in IR36, filled spikelet percentage in Nekken-1, and increased 1,000-grain weight in Koshihikari (Table 1). The grain yield per hill reduced to 83% of the control plants in Koshihikari, 54% in Nekken-1, and 30% in IR36. The flag leaves of the 10 g B m^{-3} plants contained 830 ± 59 (Koshihikari), $1{,}300 \pm 28$ (Nekken-1), and $1{,}200 \pm 64$ (IR36) mg B kg^{-1} dried material at harvest. At 30 g B m^{-3}, visible symptoms and growth reduction became the most severe in all the cultivars (Fig. 2; Table 1). The grain yield

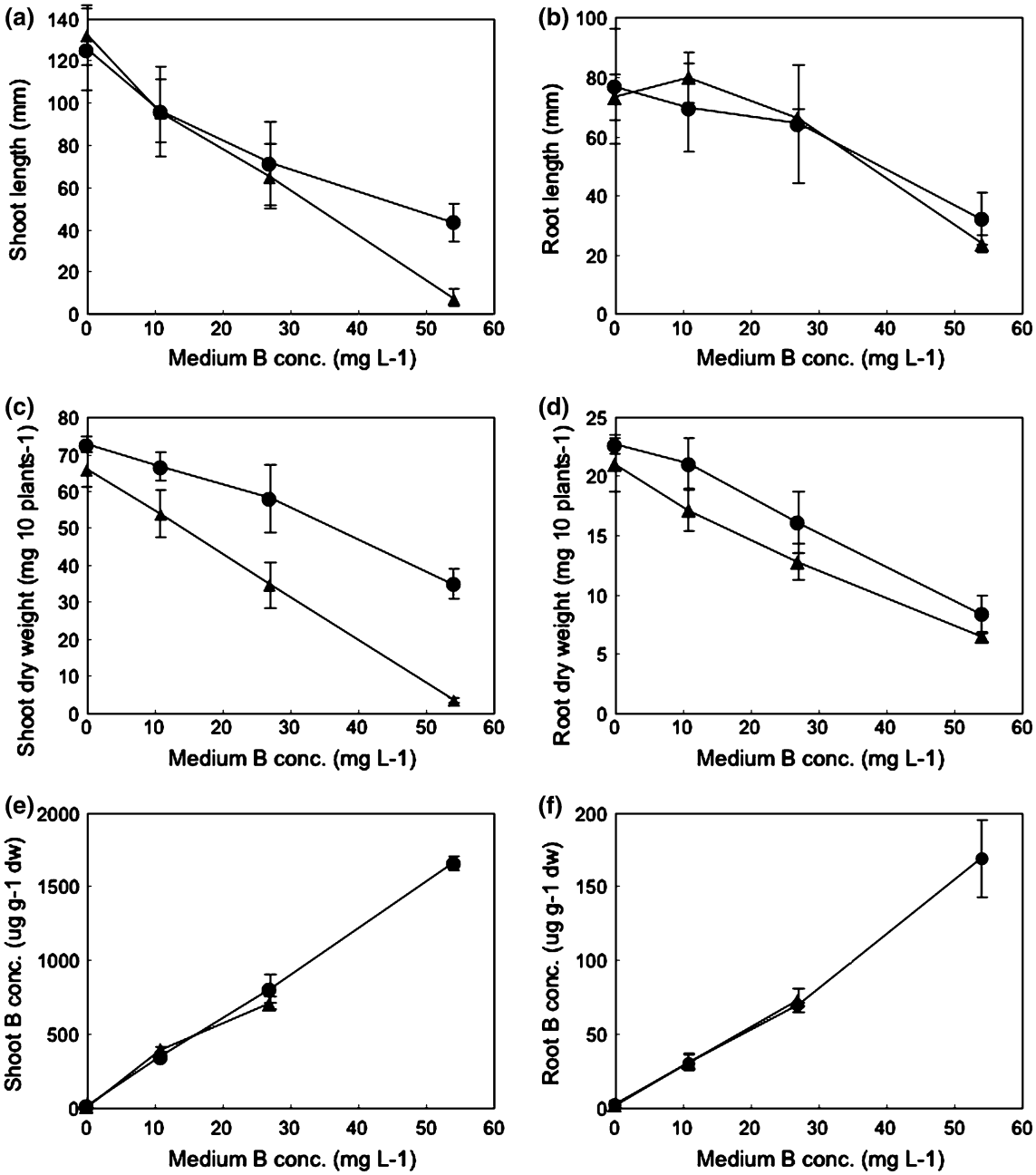

Fig. 1 Relationship between the B concentration in the rooting solutions and the shoot length (**a**), the root length (**b**), shoot dry weight (**c**), root dry weight (**d**), the boron concentration of shoot (**e**), and root (**f**) in the seedlings of Nekken-1 (*circle*) and IR36 (*triangle*). Imbibed seeds were sown and grown for 7 days in a culture solution supplemented with B at 0, 11, 27, or 54 g B m^{-3}. Values are the mean of 20 seedlings ± SD in **a** and **b** or the mean of two batches of ten seedlings ± SD in **c**, **d**, **e** and **f**

per hill decreased to 4% of control plants in Koshihikari, 5% in Nekken-1, and 0.2% in IR36. An exceptional increase in panicle number in IR36 was due to the generation of new ratoons, but this did not increase the grain yield. Statistic analyses on the growth parameters revealed significant ($P < 0.01$) interaction effects between the B concentrations and the cultivars, which demonstrates that the three cultivars respond to B toxicity differently in terms of the grain yield.

The heading date of Koshihikari, Nekken-1, and IR36 under the control condition was 100, 79, and 97 days after sowing. High B concentration (10 g B m^{-3}), delayed the heading date to 104 days (Koshihikari) and 83 days (Nekken-1), which is consistent with the effect of high B levels in barley and wheat (Paull et al. 1988) and rice (cv. Taichung65) (Tokuoka and Dyo 1938).

At 3 g B m^{-3}, there were no visible symptoms of B toxicity in the Koshihikari plants (Fig. 2). Rather, spikelet

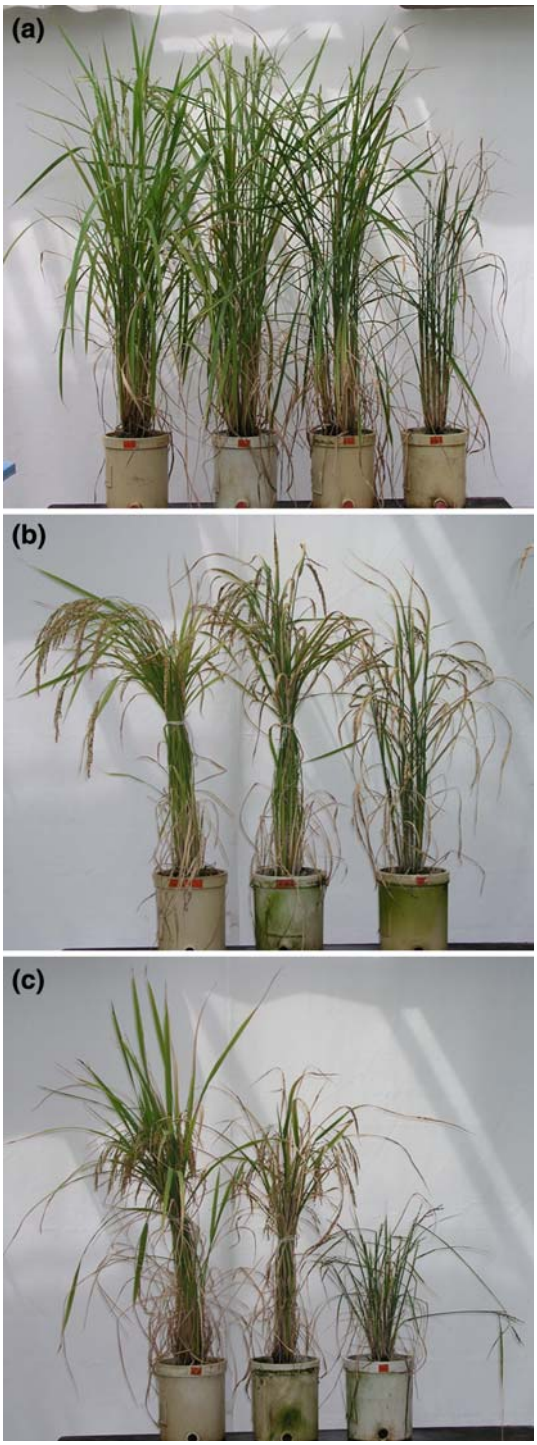

Fig. 2 Boron toxicity in soil-cultured rice plants at harvest. The rice plants were cultivated and treated with excessive B. Photographs were taken 125 days after sowing. **a** Koshihikari, the B concentration in the irrigation water was 0, 3, 10, and 30 g B m^{-3}, from *left* to *right*. **b** Nekken-1 and **c** IR36. For Nekken-1 and IR36, the B concentration in the irrigation water was 0, 10, and 30 g m^{-3}, from *left* to *right*

number per panicle and filled spikelet percentage increased, resulting in increased grain yield. Similar effects of 3 g B m^{-3} occurred in the 2005 and 2006 experiments, suggesting that the B requirement of the Koshihikari plant is not met by the current cultivation system.

QTLs for Boron tolerance

To evaluate tolerance to B toxicity of rice RILs, the reduction in shoot length under B toxicity in water culture was used as an index. There was genetic variation in shoot length under the standard B concentration (0.5 g B m^{-3}, Fig. 3a) and excessive B (60 g B m^{-3}, Fig. 3b) in the RIL population. To eliminate the genetic variation in shoot length under the standard B concentration, relative shoot length (RSL: shoot length at 60 g B m^{-3} divided by the shoot length at 0.5 g B m^{-3}) was used for evaluation. The RSL values of the RILs ranged from 0.06 to 0.76 and the distribution showed bimodality (Fig. 3c).

QTL analysis was performed using 104 markers and a major QTL (LOD = 10.92) for the RSL was detected on chromosome 4 (Fig. 4). Based on a single marker regression analysis, the locus RM3839, the nearest marker of the QTL, explained 45% of the total phenotypic variation in the population. The Nekken-1 allele contributed to the high RSL. Four minor suggestive QTLs were detected on chromosomes 2 (LOD = 1.95), 3 (LOD = 1.78), 8 (LOD = 1.67), and 11 (LOD = 1.90) (Fig. 4), which explained 11, 10, 8, and 10%, respectively, of the variation. The Nekken-1 allele contributed to the high RSL in all four loci. Three minor QTLs for shoot length under the control conditions were detected on chromosomes 7 (LOD = 1.83, RM5847), 9 (LOD = 1.67, E1828), and 11 (LOD = 1.67, RM286). Each of these loci explained 9% of the total phenotypic variation.

Confirmation of the putative QTL

Genotypic analysis revealed that one of the RIL had a heterozygous genotype at the marker locus RM3839, while the other 103 marker loci were homozygous. In this line, the genotype at the two marker loci P-4 and Cht4, flanking to RM3839, were homozygous. Based on the position on the physical map (Genbank AP008210.1) for the P-4 (23.3 Mbp) and the Cht4 (24.7 Mbp), the length of the heterozygous region was estimated as approximately 1,400 kbp.

Segregation of the genotype and the B tolerance in the next generation of the line was evaluated using 90 seeds. The marker locus RM3839 was derived from Nekken-1 in 17 plants and from IR36 in 19 plants. The region was heterozygous in the other 54 plants. There was a significant difference ($P < 0.01$) in the RSL among these genotypes at 60 g B m^{-3}; RSL of the IR36 type plants was 43% that of the Nekken-1 type plants (Fig. 5). Therefore, the genes responsible for B tolerance locate in this

Table 1 Growth responses of rice plants to excessive B supply

Rice cultivar	Boron addition (g m^{-3})	Plant height (cm)	Panicle number (per hill)	Spikelet number (per panicle)	Filled spikelet (%)	1,000-Grain weight (g)	Grain yield (g/hill)
Koshihikari	0	107.3[a] (100)	24.5[a] (100)	60.5[a] (100)	55.4[a] (100)	22.6[a] (100)	18.7[a] (100)
	3	107.9[a] (101)	20.5[a,b] (84)	66.8[a] (110)	67.4[a] (122)	23.3[a] (104)	21.1[a] (113)
	10	102.0[a] (95)	18.8[b] (77)	58.5[a] (97)	52.0[a] (94)	26.7[b] (118)	15.5[a] (83)
	30	69.8[b] (65)	10.3[c] (42)	30.3[b] (50)	10.4[b] (19)	19.1[c] (85)	0.8[b] (4)
Nekken-1	0	101.1[a] (100)	22.5[a] (100)	71.5[a] (100)	84.4[a] (100)	27.8[a] (100)	37.5[a](100)
	10	101.7[a] (101)	15.5[b] (67)	69.0[a] (97)	67.4[b] (80)	28.4[a] (102)	20.4[b] (54)
	30	78.8[b] (78)	9.5[c] (42)	57.0[a] (80)	20.9[c] (25)	17.7[b] (64)	2.0[c] (5)
IR36	0	80.6[a] (100)	26.5[a] (100)	83.5[a] (100)	82.2[a] (100)	24.2[a] (100)	44.0[a] (100)
	10	75.7[a] (94)	12.5[b] (47)	55.0[b] (66)	69.2[a] (84)	26.8[a] (111)	13.4[b] (30)
	30	51.3[b] (63)	25.0[a] (94)	22.0[c] (26)	2.9[b] (4)	15.0[b] (62)	0.2[b] (0.5)
F value of two-way ANOVA							
Boron		58.4**	49.2**	122**	161**	32.1**	784**
Cultivar		31.9**	9.2**	25.5**	15.5**	0.12[NS]	71.0**
Boron × Cultivar		1.07[NS]	15.5**	21.7**	5.47**	4.39*	118**

Values are the mean of four (Koshihikari) or two (Nekken-1 and IR36) replicates. Values in the parentheses are the relative to the control plants. Different letters following the values show the significant difference between the treatments by the Tukey test ($P < 0.05$). Two-way ANOVA was carried out on the data at 0, 10, 30 g B m^{-3} treatments using the B concentrations and the cultivars

NS not significant

* Significant at 5% level

** Significant at 1% level

1,400 kbp region and the characteristic is incompletely dominant.

QTLs in other populations

Response to B toxicity of the CSSLs between Nipponpare/Kasalath, Sasanishiki/Habataki, and Koshihikari/Kasalath, together with the parent cultivars under water culture are shown in Fig. 6. In Nipponpare/Kasalath CSSL populations, using Kasalath as a donor parent, SL16, SL18, SL44, and SL51 were carrying the chromosome segment of Kasalath containing the QTL region on chromosome 4. The RSL values of Nipponbare and Kasalath under 60 g B m^{-3} were 0.40 and 0.25 respectively, and the RSL of the four CSSLs were much lower than that of their genetic background Nipponbare or that of Kasalath. The RSL value of the SL414 that carrying Habataki segment on the QTL region in the background of Sasanishiki was also lower than that of Sasanishiki. The responses of the Koshihikari/Kasalath CSSLs together with the parent cultivars were carried out in the presence of 40 g B m^{-3}, which is the reason for the higher RSL of Kasarath compared to that under 60 g B m^{-3}. The SL209 was clearly sensitive to B toxicity compared to the parents. This line also carries the chromosome segment of Kasalath that containing the QTL region on chromosome 4. These results confirm that the QTL on chromosome 4 is important for tolerance to B toxicity in rice.

Discussion

Lower grain yield under mild B toxicity in rice was due to a reduced number of panicles, not a reduction in spikelet formation or grain-filling. This indicates that B toxicity particularly inhibits tillering at the vegetative stage. At a higher concentration of B (30 g B m^{-3}), all the yield components were seriously damaged. Garg et al. (1979) reported that 5 g B m^{-3} in a nutrient solution adversely affects the vitality of pollen grains, thereby inducing sterility in rice. In earlier reports, the critical limits for the appearance of toxicity symptoms in rice plants were >5 mg kg^{-1} hot water extractable soil B and >2.5 mg B L^{-1} in the irrigation water (Ponnamperuma et al. 1981; Cayton 1985), and >5 mg B L^{-1} for water culture solution (Ishizuka and Tanaka 1962). The resulting Koshihikari yield under current experimental conditions suggest that the critical concentration of B in the irrigation water is in the same range.

There are many criteria used to evaluate B tolerance among species and cultivars. Jefferies et al. (1999, 2000) successfully used root elongation under B toxicity as a criterion for QTL analyses of B tolerance in wheat and barley. In these crop plants, root elongation rates were highly correlated with B concentration in the shoots, leaf symptoms, and shoot dry weight of plants grown on soil containing high B levels (Chantachume et al. 1995). In rice plants, the increase in severity of visible symptoms was significant in

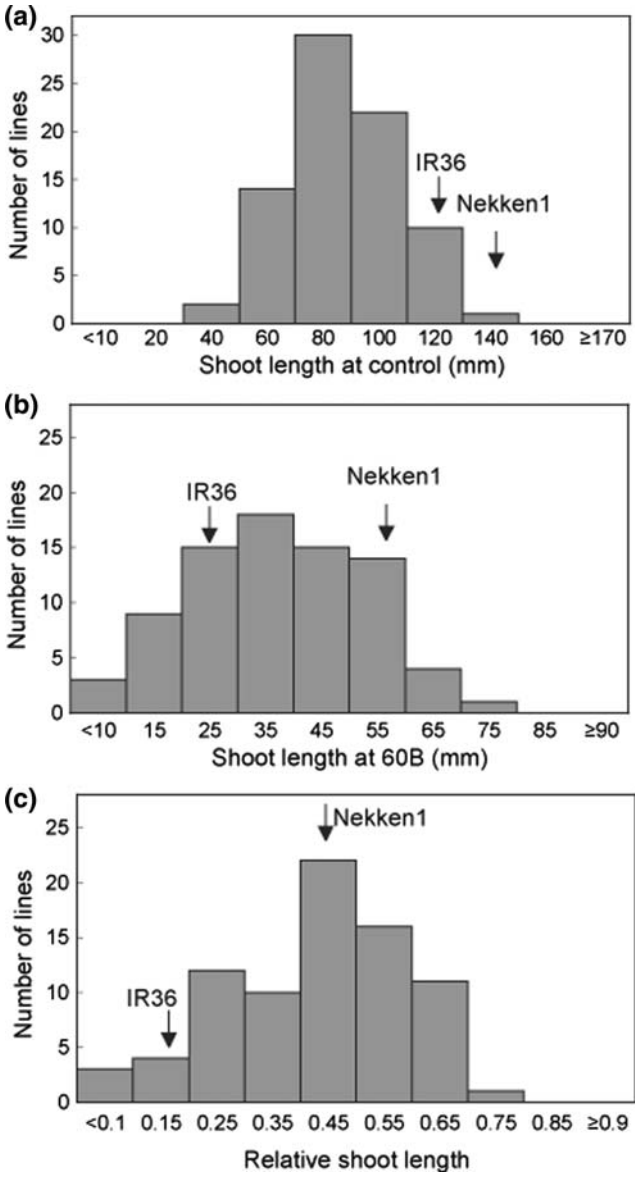

Fig. 3 Frequency distributions of the shoot length of the 7-day-old seedlings of the RIL populations between Nekken-1 and IR36. **a** The shoot length at 0.5 g B m^{-3}, (**b**) the shoot length at 60 g B m^{-3} and (**c**) the relative shoot length (shoot length at 60 g B m^{-3} was divided by that at 0.5 g B m^{-3}). Values are the means of ten plants of each RIL

the most susceptible cultivar IR36 (Fig. 2), however, the different sensitivity for producing visible symptoms was not so evident among cultivars at the seedling stage.

Tissue B concentrations are also reported to be effective criteria for evaluating B tolerance (Nable 1988). A major QTL for root elongation corresponds to the QTL region for shoot B content (Jefferies et al. 1999, 2000). In barley, root efflux mediates the lower B content (Hayes and Reid 2004). In rice, however, tissue B concentrations did not differ significantly between the tolerant Nekken-1 and sensitive IR36 cultivars at the seedling stage under 27 g B m^{-3} (Fig. 1c). When Koshihikari and the B-sensitive CSSL SL209 were subjected to moderately high B concentration (20 g B m^{-3}), their shoot B concentrations of the 7-day-old plants were 606 ± 52.0 and 531 ± 37.2 g B kg^{-1} dry weight and the difference was insignificant ($n = 5$). The flag leaves of Koshihikari, Nekken-1 and IR36 at the grain-filling stage contained similar levels of B, even though their sensitivities to B toxicity were different. On the other hand, the shoot elongation rate under B toxicity was more severely inhibited in *indica* subspecies IR36, Kasalath and Habataki than in *japonica* subspecies Nekken-1, Koshihikari, Nipponbare and Sasanishiki (Figs. 1a, 6). Examination using Nekken-1 and IR36 indicates that the difference in the shoot elongation rate was reflected in the shoot dry matter production (Fig. 1c). While the sensitive IR36 died within 7 days under 60 g B m^{-3}, the tolerant cultivar Nekken-1, Koshihikari, Nipponbare, and Sasanishiki were able to keep growing at least further two weeks (data not shown). It is likely that such a tolerant behavior to excessive B at the seedling stage may result in higher grain yield under excessive B at the grain-filling stage. Therefore, the shoot elongation rate at the seedling stage was used as a criterion to evaluate B tolerance in RILs. A QTL for the RSL on chromosome 4 was also detected in other mapping populations and the generality indicates the importance of the QTL for B tolerance in rice. Further studies are needed to confirm that the detected QTLs are also functional at the grain-filling stage in the *indica* rice.

Wheat and barley are far more tolerant to excessive B than rice, suggesting that different mechanisms for B tolerance may operate in these plants. In wheat and barley, B tolerance is related to lowering the accumulation of B (Nable 1988; Paull et al. 1988), and a major QTL for root elongation corresponds to the QTL region for the shoot B content (Jefferies et al. 1999, 2000). In barley, root efflux mediates the lower B content of the plants (Hayes and Reid 2004), therefore, the gene product of the QTL might be an efflux pump for B. Recently, the B toxicity tolerance gene *Bot1* on the chromosome 4H was identified as a B efflux transporter, the most similar ortholog of *Bot1* resides on rice chromosome 1 (Sutton et al. 2007). In rice plants, however, there was no difference in the shoot and root B contents. Therefore, the difference in cellular tolerance to B toxicity may determine the B toxicity tolerance among rice cultivars. At seedling stage, the *japonica* allele of the QTL on the long arm region of the chromosome 4 would act to maintain vigor under B toxicity. Continuous growth also confers the benefit of B dilution. The long arm region of rice chromosome 4 has a synteny with the long arms of wheat group chromosome 2 (Conley et al. 2004) and with barley chromosome 2H (Stein et al. 2007). Therefore, the QTL for the leaf B toxicity symptom in barley on the chromosome 2H (Jefferies et al. 1999) may relate to the QTL detected here in rice. In wheat, a major QTL region was

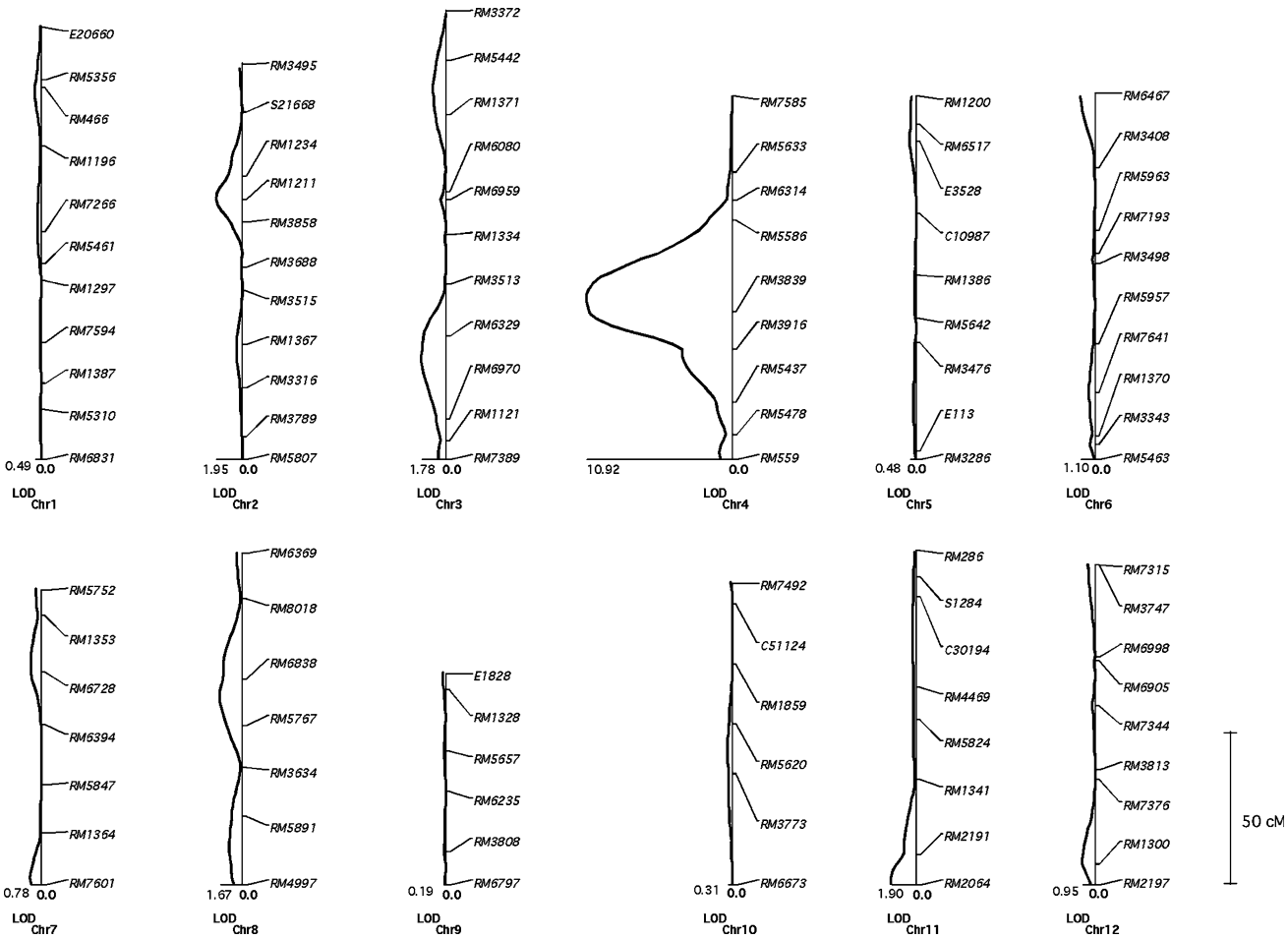

Fig. 4 Chromosomal locations of QTLs for relative shoot length under 60 g B m^{-3} B toxicity, based on an RIL population from a cross between Nekken-1 and IR36

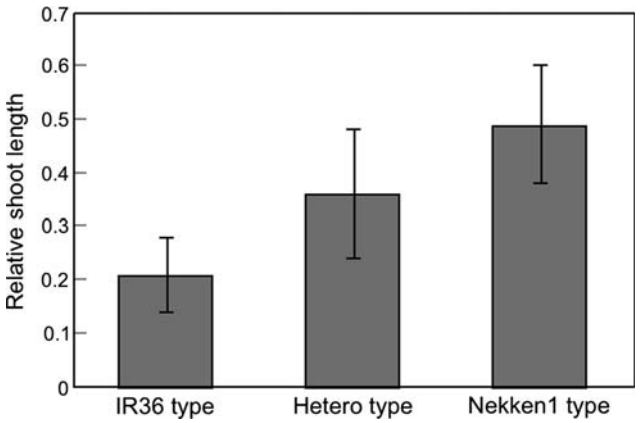

Fig. 5 Relative shoot length of 7-day-old near isogenic lines under 60 g B m^{-3} toxicity. Genotype at the RM3839 locus is indicated on the *X*-axis. Mean values ± SD of 19 (IR36 type), 54 (heterozygous), and 17 (Nekken-1 type) plants are shown. The differences were significant ($P < 0.01$)

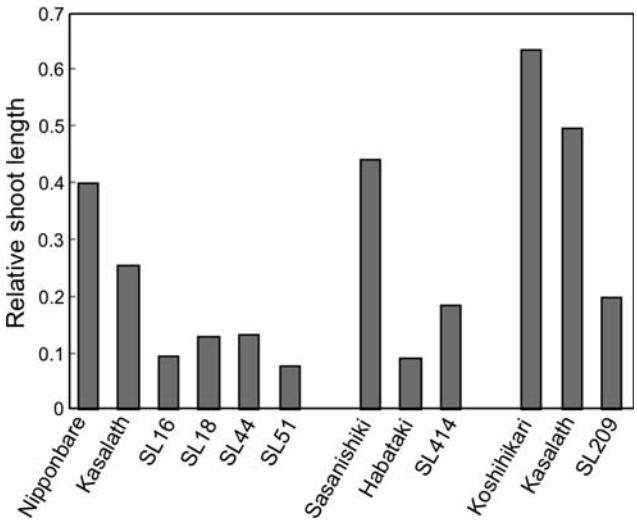

Fig. 6 Relative shoot length of 7-day-old seedlings of CLLSs and their parent cultivars under B toxicity. Supplemented B concentrations were 60 g B m^{-3} for Nipponbare, Kasalath, and thier CSSLs (SL16, SL18, SL44, and SL51), and Sasanishiki, Habataki and thier CSSL (SL414), or 40 g B m^{-3} for Koshishikari, Kasalath, and their CSSL (SL209). Values are the means of ten seedlings

identified on the chromosome 7B (Jefferies et al. 2000), which corresponds to a colinear 227 kb region on rice chromosome 6 (Schnurbusch et al. 2007), even though a signifi-

cant QTL could not be detected in the corresponding region in the present experiment. Cloning of the rice gene responsible for the observed QTL would provide useful information for the mechanism of B toxicity in rice and further studies are in progress.

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References

Blamey FPC, Asher CJ, Edwards DG (1997) Boron deficiency in sunflower. In: Bell RW, Rerkasem B (eds) Boron in Soils and Plants. Kluwer, Dordrecht, pp 145–149

Cayton MTC (1985) Boron toxicity in rice. IRRI Res Pap Ser 113:2–10

Chantachume Y, Smith D, Hollamby GJ, Paull JG, Rathjen AJ (1995) Screening for boron tolerance in wheat (*T. aestivum*) by solution culture in filter paper. Plant Soil 177:249–254

Conley EJ, Nduati V, Gonzalez-Hernandez JL, Mesfin A, Trudeau-Spanjers M, Chao S, Lazo GR, Hummel DD, Anderson OD, Qi LL, Gill BS, Echalier B, Linkiewicz AM, Dubcovsky J, Akhunov ED, Dvorrák J, Peng JH, Lapitan NLV, Pathan MS, Nguyen HT, Ma XF, Miftahudin, Kalavacharla V, Kianian SF, Sidhu D, Dilbirligi M, Gill KS, Choi DW, Fenton RD, Close TJ, McGuire PE, Qualset CO, Anderson JA (2004) A 2600-locus chromosome bin map of wheat homoeologous group 2 reveal interstitial gene-rich islands and colinearity with rice. Genetics 168: 625–637

Ebitani T, Takeuchi T, Nonoue Y, Yamamoto T, Takeuchi K, Yano M (2005) Construction and evaluation of chromosome segment substitution lines carrying overlapping chromosome segments of *indica* rice cultivar Kasalath in a genetic background of *japonica* elite cultivar Koshihikari. Breed Sci 55:65–73

Garg OK, Sharma AN, Kona GR (1979) Effect of boron on the pollen vitality and yield of rice plants. Plant Soil 52:591–594

Gupta UC (1983) Boron deficiency and toxicity symptoms for several crops as related to tissue boron levels. J Plant Nutr 6:387–396

Hayes JE, Reid RJ (2004) Boron tolerance in barley is mediated by efflux of boron from the roots. Plant Physiol 136:3376–3382

Hewitt EJ (1966) The composition of the nutrient solution. In: Sand and Water Culture Methods Used in the Study of Plant Nutrition. Farnham Royal Bucks, Commonwealth Agricultural Bureaux, England, pp 190

Ishizuka Y, Tanaka A (1962) Inorganic nutrition of the rice plant. 7. Effect of boron, zinc and molybdenum level in culture solution on yields and chemical composition of the plant. Jpn J Soil Sci Plant Nutr 33:93–96

Jefferies SP, Barr AR, Karakousis A, Kretschmer JM, Manning S, Chalmers KJ, Nelson JC, Islam AKMR, Langridge P (1999) Mapping of chromosome regions conferring boron toxicity tolerance in barley (*Hordeum vulgare* L.). Theor Appl Genet 98:1293–1303

Jefferies SP, Pallotta MA, Paull JG, Karakousis A, Kretschmer JM, Manning S, Islam AKMR, Langridge P, Chalmers KJ (2000) Mapping and validation of chromosome regions conferring boron toxicity tolerance in wheat (*Triticum aestivum*). Theor Appl Genet 101:767–777

Kaur S, Nicolas ME, Ford R, Norton RM, Taylor PWJ (2006) Selection of *Brassica rapa* genotypes for tolerance to boron toxicity. Plant Soil 285:115–123

Manly KF, Cudmore RH Jr, Meer JM (2001) Map Manager QTX, cross platform software for genetic mapping. Mamm Genome 12:930–932

Matoh T, Akaike R, Kobayashi M (1997) A sensitive and convenient assay for boron in plant using chromotropic acid and HPLC. Plant Soil 192:115–118

McCouch SR, Teytelman L, Xu Y, Lobos KB, Clare K, Walton M, Fu B, Maghirang R, Li Z, Xing Y, Zhang Q, Kono I, Yano M, Fjellstrom R, DeClerck G, Shneider D, Cartinhour S, Ware D, Stein L (2002) Development and Mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). DNA Res 9:199–207

Mengel K, Kirkby EA (2001) Boron. In: Principles of Plant Nutrition, 5th edn. Kluwer, Dordrecht, pp 621–638

Nable RO (1988) Resistance to boron toxicity amongst several barley and wheat cultivars: A preliminary examination of the resistance mechanism. Plant Soil 112:45–52

Nable RO, Bañuelos GS, Paull JG (1997) Boron toxicity. Plant Soil 193:181–198

Nakazki T, Ikehashi H (1998) Genomic sequence and polymorphisms of a rice chitinase gene, *Cht4*. Breed Sci 48:371–376

Nelson JC (1997) QGENE: software for marker-based genomic analysis and breeding. Mol Breed 3:239–245

Paliwal KV, Mehta KK (1973) Interactive effect of salinity, SAR and boron on the germination and growth of seedlings of some paddy (*Oryza sativa*) varieties. Plant Soil 39:603–609

Paull JG, Cartwright B, Rathjen AJ (1988) Responses of wheat and barley genotypes to toxic concentrations of soil boron. Euphytica 39:137–144

Paull JG, Rathjen AJ, Cartwright B (1991) Major gene control of tolerance of bread wheat (*Triticum aestivum* L.) to high concentrations of soil boron. Euphytica 55:217–228

Ponnamperuma FN, Yuan WL (1966) Toxicity of boron to rice. Nature 211:780–781

Ponnamperuma FN, Cayton MT, Lantin RS (1981) Dilute hydrochloric acid as an extractant for available zinc, copper and boron in rice soils. Plant Soil 61:297–310

Power PP, Woods WG (1997) The chemistry of boron and its speciation in plants. Plant Soil 193:1–13

Reid RJ, Hayes JE, Post A, Stangoulis JCR, Graham RD (2004) A critical analysis of the causes of boron toxicity in plants. Plant Cell Environ 25:1405–1414

Roessener U, Patterson JH, Forbes MG, Fincher GB, Langridge P, Bacic A (2006) An investigation of boron toxicity in barley using metabolomics. Plant Physiol 142:1087–1101

Schnurbusch T, Collins NC, Eastwood RF, Sutton T, Jefferies SP, Langridge P (2007) Fine mapping and targeted SNP survey using rice-wheat gene colinearity in the region of the *Bo1* boron toxicity tolerance locus of bread wheat. Theor Appl Genet 115:451–461

Stein N, Prasad M, Scholz U, Thiel T, Zhang H, Wolf M, Kota R, Varshney RK, Perovic D, Grosse I, Graner A (2007) A 1, 000-loci transcript map of the barley genome: new anchoring points for integrative grass genomics. Theor Appl Genet 114:823–839

Sutton T, Baumann U, Hayes J, Collins NC, Shi BJ, Schnurbusch T, Hay A, Mayo G, Pallotta M, Tester M, Langridge P (2007) Boron-toxicity tolerance in barley arising from efflux transporter amplification. Science 318:1446–1449

Tokuoka M, Dyo S (1938) Über den einfluss des bors auf das wachstum der reispflanze. III. Nettai Nougaku Kwaishi 10:151–157

Tokuoka M, Morooka H (1936) Über den einfluss des bors auf das wachstum der reispflanze. I. Jpn J Soil Sci Plant Nutr 10:189–199

Yanagihara S, McCouch SR, Ishikawa K, Ogi Y, Maruyama K, Ikehashi H (1995) Molecular analysis of the inheritance of the *S-5* locus, conferring wide compatibility in Indica/Japonica hybrids of rice (*O. sativa* L.). Theor Appl Genet 90:182–188