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Oilseed rape (*Brassica napus* L.) genotypic variation in response to boron deficiency

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Abstract: Boron efficiency of 16 oilseed rape genotypes was tested using both hydroponic and pot-soil growing techniques. From the nutrient solution experiment (0.1 and 10 μM B), 4 representative contrasting genotypes were selected based on relative root and shoot growth. These were then grown in pots with low-B soil (0.25 mg kg^{-1}). From the nutrient solution experiment, 2 genotypes selected as B-inefficient did not show any growth disorders, and the concentration of B in the shoots was above critical. Furthermore, 4 contrasting genotypes were subjected to the stable ^{11}B isotope-enriched uptake solution for 6 h to verify possible low B-induced active uptake by roots and xylem loading of B. The concentration of ^{11}B in either root cell sap or xylem exudate was higher than in the external nutrient solution, which indicated the presence of low B-induced active uptake for all tested genotypes, and, to some extent, their efficiency with low B. In conclusion, a combination of different growing techniques under controlled environmental conditions together with different parameters including relative root and shoot weight, shoot B concentration, and B uptake provided reliable B efficiency results in oilseed rape genotypes.

Key words: Boron deficiency, boron uptake, genotypic differences, oilseed rape, screening methods

Introduction

Low-boron soils (water soluble B < 0.25 mg kg^{-1}) are present in more than 80 countries throughout the world (Shorrocks 1997). B requirements are usually higher in dicots and particularly in oilseed rape and cruciferous plants, which are considered sensitive to B deficiency. Over the past decade, numerous studies of uptake and translocation of B in plants have been carried out, and they suggest a metabolically active B transport in the root cortex and xylem parenchyma (Dannel et al. 2002; Takano et al. 2008).

Genotypic variation in response to a lack of available B in soil has been found among numerous plant species (Rerkasem and Jamjod 1997). B efficiency is defined as the ability of a cultivar to grow and produce a good yield in B-deficient soil (Graham 1984). Different growing techniques have been used to determine B efficiency. Hence, B-efficient oilseed rape genotypes have been identified in field experiments (Yang et al. 1993; Xue et al. 1998; Stangoulis et al. 2000a) with clear differences in yield and shoot B concentrations. However, a clear explanation of the

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mechanisms involved in B efficiency is still lacking. Du et al. (2002) found differences in B utilization efficacy between 2 contrasting rapeseed genotypes and their F_1 hybrid grown in a pot-soil experiment under glasshouse conditions. To date, studies on both deficiency and toxicity of B have been conducted in oilseed rape grown hydroponically in order to identify B-efficient germplasm (Chantachume et al. 1995; Stangoulis et al. 2000b). Furthermore, Stangoulis et al. (2000a, 2000b) studied B efficiency in oilseed rape by comparing the results obtained from field, pot, and water culture experiments.

The objective of this study was to compare different screening methods (water culture and soil pot experiments) to determine the response of oilseed rape genotypes to B deficiency. In addition, 4 representative contrasting oilseed rape genotypes from the screening experiments were subjected to stable ^{11}B isotope-enriched uptake solution to verify a possible low B-induced active uptake by roots and xylem loading of B.

Materials and methods

Growth conditions, treatments, and plant material

Experiment 1: Screening of 16 oilseed rape genotypes grown in nutrient solution for B deficiency

Plants of 16 oilseed rape genotypes of different origins and quality were grown as follows. Seeds were germinated under controlled conditions at 25 °C on filter paper moistened with saturated $CaSO_4$ solution to provide good seed germination. After 4 days, seedlings were transferred to continuously aerated nutrient solutions containing (in mM) 0.7 K_2SO_4, 0.1 KCl, 2.0 $Ca(NO_3)_2$, 0.5 $MgSO_4$, and 0.1 KH_2PO_4, and also containing (in µM) 0.5 MnSO4, 0.5 ZnSO4, 0.5 $CuSO_4$, 0.01 $(NH_4)_6Mo_7O_{24}$, and 10 FeEDTA. Boron was added as H_3BO_3 at 2 levels of external supply, 0.1 µM (deficient) and 10 µM (adequate), designated as B0.1 and B10. Plants were grown (4 plants per 2-L pot) in a greenhouse with a light/dark regime of 16/8 h, light intensity of 400 µmol m^{-2} s^{-1} at plant height, air temperature of 25 °C during the day and 20 °C during the night, and relative humidity of 60%-70%. The experiment had 4 replicates in a randomized block design; 1 pot represented a replication. Only distilled water was used in making the nutrient solutions, which were renewed 2 times weekly. Plants were harvested 4 weeks after transfer to the nutrient solution. Roots were washed for a few seconds with distilled water to remove adherent nutrient solution and then blotted dry between 2 layers of filter paper. Fresh plant material was dried at 80 °C, and dry weight was determined.

Experiment 2: Response to B deficiency in 4 oilseed rape genotypes grown in pots with soil

Four genotypes were selected from Experiment 1. Based on plant growth (root and shoot dry weight), 2 genotypes (Navajo and Panther) were considered to be more tolerant to B deficiency while the other 2 genotypes (NS-L-7 and Pronto) were selected because they showed the strongest growth inhibition. Plants were grown under glasshouse conditions as described in Experiment 1 with 2 plants per pot in 2-L pots filled with air-dry soil. The basic soil properties were: pH (KCl) 4.30; available P (P_2O_5), 7.9 mg 100 g^{-1}; available K(K_2O) 13.5 mg 100 g^{-1}; and hot water-soluble B, 0.25 mg kg^{-1}. To avoid the possible effect of nutrient deficiency on plant growth, other nutrients were added, and the amounts were calculated for field conditions: N as urea (140 kg ha^{-1}), P as KH_2PO_4 (100 kg ha^{-1}), and K as K_2SO_4 water solution (120 kg ha^{-1}). Two B treatments were applied: low B at 0.25 mg kg^{-1} (hot water soluble soil B, denoted as B0) and 0.45 mg kg^{-1} B (0.20 mg kg^{-1} B added to soil as H_3BO_3 water solution and denoted as B+). Soil was thoroughly mixed with added fertilizer and water solutions and sieved. A few days after germination, plants were thinned to maintain 2 uniform plants in each pot. Leaves were harvested and roots washed from the soil 4 weeks after germination. Plant material was dried as described in Experiment 1, and B concentration was determined in root and shoot.

Experiment 3: Low B-induced active uptake by roots and xylem loading of 4 oilseed rape genotypes

Experiment 3 was conducted under controlled environmental conditions in a growth chamber with a light/dark regime of 16/8 h, a temperature regime of 24/20 °C, photon flux density of approximately 450 µmol m^{-2} s^{-1} at plant height, and relative humidity of about 70%. In this experiment, only deionized water (18.2 MW; Elga PURELAB Ultra Genetic, Germany) was used for preparation of the standard nutrient solutions (see Experiment 1). Experimental

design was the same as in the previously described experiments, with 4 plants per 3-L pot. To provide good growth, plants of 4 genotypes, Navajo, Panther, NS-L-7, and Pronto, were grown in nutrient solution containing 5 μM ^{10}B, supplied as labeled $H_3{}^{10}BO_3$ (99% atom ^{10}B; Aldrich, USA), for 3 weeks, and were then transferred to pots with low ^{10}B concentrations (0.5 μM). After 2 days of exposure to low B conditions, plants were exposed to different ^{11}B (99% atom ^{11}B; Aldrich) concentrations (2, 5, and 10 μM) for 6 h.

Root cell sap was obtained by following the methods described by Dannel et al. (1998). Roots were rinsed with deionized water, dried between 2 layers of filter paper, and immediately frozen at –18 °C. After thawing, defrosted tissues were centrifuged at 4000 × *g* for 10 min using Amicon® Ultra-15 tubes (Millipore, USA). Xylem exudates were collected for 1 h using a micropipette.

Analytical methods

For determination of B concentration in roots and shoots, dry material was ashed at 550 °C for 8 h. Ash was dissolved with 0.5 N H_2SO_4, and all samples were centrifuged at 1000 × *g* before determination of B concentration in roots and shoots by the azomethine-H method.

In root cell sap and xylem exudates, ^{11}B was determined by inductively coupled plasma-mass spectrometry (ICP-MS, ELAN 6000; PerkinElmer, Germany). These samples were diluted with HNO_3, and Be solution was added as an internal standard to yield final concentrations of 0.5 M HNO_3 and 10 μg L^{-1} Be.

Statistical analyses

Data obtained were tested by statistical analyses of variance and means were compared by the least significant difference (LSD) test.

Results

Experiment 1

After 4 days, typical B deficiency symptoms occurred in plants grown at B0.1. Cotyledons were shiny, while growth of lateral roots was inhibited. Later on, small necrotic lesions developed at the cotyledons, and small leaves were purplish in color.

The aim of this experiment was to screen 16 rapeseed genotypes for B deficiency. At both treatments (B0.1 and B10), genotypic variation in plant growth was significant ($P < 0.01$); up to 20-fold differences were found in shoot dry weight under B deficiency (Table 1). Initial seed B content was previously determined to be similar in all genotypes, and it was excluded as a possible cause of genotypic variation. Navaho, Panther, and Artus showed the highest plant dry weights under B deficiency, while growth inhibition was strongest in Pronto. Under adequate B supply, Tradition showed the highest plant dry weight and Express and NS-L-13 the lowest. The relative root and shoot growth (B0.1/B10) of a few genotypes was very low (Pronto, Rafaela, Banacanka, Tradition, and NS-L-7); in Navaho, which has a higher growth potential, it was only slightly reduced. On the other hand, Express and NS-L-13 appeared to have lower growth potential but higher relative growth.

As expected, the B concentration in plants was lower in all genotypes at B0.1, with significant 3- and 5-fold genotypic variation at B0.1 and B10, respectively (data not shown). Significant differences were found among genotypes in root and shoot B concentration, but there was no close relationship with plant growth.

Experiment 2

At both B treatments, plants of 4 genotypes had normal growth; there were no visual symptoms of B deficiency.

Root dry weight was higher in genotypes Navaho and Panther, while there was no significant difference between B treatments (Table 2). Shoot dry weight was very similar within genotypes and B treatments, even slightly higher at B0. Relative plant growth differed within genotypes. Relative shoot weight was higher in Pronto and NS-L-7, which was not in agreement with the results of Experiment 1. As expected, B accumulation in roots and shoots was statistically higher at B+. With the B0 treatment, differences in the B concentration in roots and shoots were small, while higher accumulation of B in shoots was recorded in Navaho and Pronto in the B+ treatment.

Table 1. Root and shoot dry weight (g), relative root and shoot growth (%), and net uptake (mg B plant^{-1}) of 16 oilseed rape genotypes grown in nutrient solutions under deficient (0.1 μM; B0.1) and adequate (10 μM; B10) external B supplies. Values are means of 4 replicates.

Genotype	Root dry weight			Shoot dry weight			Net B uptake	
	B0.1	B10	B0.1/B10[b]	B0.1	B10	B0.1/B10[b]	B0.1	B10
NS-L-15	0.007	0.05	14	0.12	0.62	19	1.18	12.99
Slavica	0.005	0.04	13	0.06	0.41	15	0.30	11.78
Pronto	0.002	0.03	7	0.02	0.45	4	0.21	12.10
Lirajet	0.005	0.05	10	0.07	0.45	16	0.26	15.46
Rafaela	0.002	0.05	4	0.04	0.46	9	0.22	11.81
Express	0.003	0.03	10	0.07	0.27	26	0.62	15.18
Panther	0.009	0.10	9	0.15	0.93	16	0.93	22.17
Silvia	0.003	0.04	8	0.05	0.47	10	0.22	14.51
Zenith	0.003	0.04	8	0.10	0.48	20	0.83	30.87
Tradition	0.002	0.07	3	0.09	1.10	8	0.42	71.55
NS-L-7	0.002	0.04	5	0.06	0.69	9	0.66	39.88
Navajo	0.012	0.05	24	0.44	0.63	70	2.53	60.07
Banacanka	0.002	0.05	4	0.07	0.83	8	0.48	50.8
Artus	0.003	0.05	6	0.14	0.77	18	1.14	60.5
Capitol	0.006	0.06	10	0.08	0.68	12	0.44	47.49
NS-L-13	0.002	0.02	10	0.08	0.29	28	0.51	16.19
LSD[a]	0.001	0.015		0.22	0.17		0.49	12.7

[a]LSD: least significant difference indicating genotype main effect, $P < 0.01$.
[b]: Relative root and shoot growth, expressed as percentage of the value at B0 to that at B10.

Table 2. Root and shoot dry weight, relative root and shoot growth (%), and concentration of B in roots and shoots of 4 rapeseed genotypes grown in pots filled with soil without addition of B (B0) and with additional B (B+). Values are means of 4 replicates.

Genotype	Root					Shoot				
	Dry weight (g)		B0/B+[b] (%)	B concentration (μg g^{-1} DW)		Dry weight (g)		B0/B+[b] (%)	B concentration (μg g^{-1} DW)	
	B0	B+		B0	B+	B0	B+		B0	B+
Navajo	0.05	0.07	71	21.0	21.7	0.50	0.68	74	25.8	57.2
Panther	0.06	0.05	120	22.8	22.0	0.65	0.60	108	20.4	49.6
Pronto	0.03	0.04	75	19.4	22.5	0.61	0.53	115	23.2	37.2
NS-L-7	0.04	0.04	100	19.2	22.1	0.63	0.52	121	21.8	41.6
LSD[a], G, B	0.013, ns[a]			2.7, 2.4		ns, ns			10.3, 8.9	

[a]ns: not significant for LSD test, $P < 0.01$, LSD = lease significant difference, G and B indicate cultivar main effect and boron main effect (there was no significant interaction of boron treatment with genotype).
[b]: Relative root and shoot growth, expressed as percentage of the value at B0 to that at B+.

Experiment 3

Experiment with stable B isotopes (^{10}B and ^{11}B) was conducted in order to find possible differences in B uptake and xylem loading with low B supplies among the 4 chosen oilseed rape genotypes. To induce possible active uptake, plants were grown under conditions of a very low ^{10}B supply (0.5 μM) for 2 days and exposed to different ^{11}B concentrations in nutrient solutions for 6 h.

^{11}B concentration in the root cell sap and xylem exudates of 4 oilseed rape genotypes was higher than in the external nutrient solution (Tables 3 and 4). Differences within genotypes were statistically significant, although obtained values were very similar. Accumulation of ^{11}B in root cortex sap and xylem exudates indicates possible existence of low B-induced active uptake by cortical cells and xylem loading in 4 genotypes.

Discussion

For plant breeding purposes, short-term screening and growing techniques were proposed by Rerkasem and Jamjod (1997). Since B deficiency also reduces root growth, as described in numerous reviews (Shelp 1993; Brown et al. 2002; Dannel et al. 2002), different parameters were used for determination of B

Table 3. Concentration of ^{11}B in root cell saps (μM) of 4 oilseed rape genotypes. Plants were previously exposed to low ^{10}B supply (0.5 μM) for 2 days and then subjected to different ^{11}B concentrations for 6 h. Data shown are means ± SD (n = 4).

Genotype	Concentration of ^{11}B in nutrient solution (μM)			
	0.5	2	5	10
Navajo	3.0 ± 0.3	7.9 ± 0.7	11.8 ± 0.9	16.7 ± 0.9
Panther	2.1 ± 0.3	6.7 ± 0.8	10.1 ± 1.1	17.3 ± 1.0
Pronto	1.8 ± 0.2	5.5 ± 0.8	8.9 ± 1.0	17.4 ± 1.2
NS-L-7	3.2 ± 0.4	5.5 ± 0.7	9.3 ± 0.8	16.7 ± 1.0
LSD[a]	0.6	0.5	0.9	1.8

[a]LSD: least significant difference indicating genotype main effect, $P < 0.01$.

Table 4. Concentration of ^{11}B in root xylem exudates (μM) of 4 oilseed rape genotypes. Plants were previously exposed to low ^{10}B supply (0.5 μM) for 2 days and then subjected to different ^{11}B concentrations for 6 h. Data shown are means ± SD (n = 4).

Genotype	Concentration of ^{11}B in nutrient solution (μM)			
	0.5	2	5	10
Navajo	5.8 ± 0.4	14.5 ± 1.2	25.2 ± 2.2	36.3 ± 2.5
Panther	5.0 ± 0.5	15.8 ± 1.3	26.0 ± 2.2	31.8 ± 2.2
Pronto	4.0 ± 0.6	13.7 ± 1.5	24.9 ± 2.0	34.0 ± 2.3
NS-L-7	6.3 ± 0.7	13.8 ± 1.5	28.0 ± 2.5	32.3 ± 2.0
LSD[a]	1.2	ns [a]	3.2	4.8

[a]ns: not significant for LSD test, $P < 0.01$; LSD = least significant difference indicating genotype main effect with $P < 0.01$.

efficiency. Chantachume et al. (1995) used root length to find differences in B toxicity tolerance between wheat genotypes. Stangoulis et al. (2000b) showed that relative root length is a more reliable parameter for determination of B efficiency in 61 oilseed rape genotypes grown in nutrient solutions than either the root elongation rate or root dry weight. Furthermore, identification of B-efficient genotypes by Stangoulis et al. (2000b) was in accordance with previously published field conditions studies (Yang et al. 1993; Xue et al. 1998). Results from the study presented here also showed that root dry weight could not be used as a reliable criterion for B-efficiency identification in the nutrient solution experiment. However, the 2 genotypes (Pronto and NS-C-7) selected as B-inefficient because they had the lowest root and shoot biomass in the nutrient solution experiment (Experiment 1) did not show any growth disorders in the pot experiment with low-B soil (Experiment 2). These genotypes differed in the root dry weight in the B0.1 treatment, but these differences might not be pronounced under a gradually increasing low supply of B; they may show a less severe B deficiency than when grown at a B supply of 0.1 μM. Oilseed rape plants grown at a broad range of external B concentrations, from 0.04 to 0.3 μM, always showed typical B deficiency symptoms (Asad et al. 2002). B concentration in shoots (>20 μg g^{-1} DW) was above the critical concentration recorded in the youngest open leaf at either the vegetative growth stages of oilseed rape (10-14 μg g^{-1} DW; Huang et al. 1996) or the reproductive stage of canola (18 μg g^{-1} DW; Asad et al. 2002).

The existence of low B-induced active uptake by roots and xylem loading indicated that the tested oilseed rape genotypes from Experiment 3 are B-efficient despite the differences in root and shoot dry weight occurring when these genotypes were grown under a low B supply. A similar study by Pfeffer et al. (1997) showed higher B concentrations in the root cell sap of sunflower plants previously grown under a low B supply (1 μM) compared to those grown at a high supply (100 μM). Absence of low B-induced active uptake by roots and xylem loading was also shown in a B-inefficient tomato mutant (Savic et al. 2007). On the other hand, Du et al. (2002) showed that B efficiency in oilseed rape might also be determined by B-utilization efficiency, since B reacts with sugars. This indicates that different shoot B concentrations in the present study could be a consequence of genotypic differences in cell wall chemical composition.

In conclusion, all 16 oilseed rape genotypes tested in this study showed B efficiency to some extent. A combination of different growing techniques under controlled environmental conditions together with different parameters including root and shoot dry weight, shoot B concentration, and B uptake provided reliable results regarding B efficiency in oilseed rape genotypes.

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References

Asad A, Blamey FPC, Edwards DG (2002) Dry matter production and boron concentration of vegetative and reproductive tissues of canola and sunflower plants grown in nutrient solution. Plant Soil 243: 243-252.

Brown PH, Bellaloui N, Wimmer MA, Bassil ES, Ruiz J, Hu H, Pfeffer H, Dannel F, Römheld V (2002) Boron in plant biology. Plant Biol 4: 205-223.

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

Dannel F, Pfeffer H, Römheld V (1998) Compartmentation of boron in roots and leaves of sunflower as affected by boron supply. Journal of Plant Physiology 26: 95-99.

Dannel F, Pfeffer H, Römheld V (2002) Update on boron in higher plants - uptake, primary translocation, and compartmentation. Plant Biol 4: 193-204.

Du CW, Wang YH, Xu FS, Yang YH, Wang HY (2002) Study on the physiological mechanism of boron utilization efficiency in rape cultivars. J Plant Nutr 25: 231-244.

Graham RD (1984) Breeding for nutritional characteristics in cereals. Adv Plant Nutr 1: 57-102.

Pfeffer H, Dannel F, Römheld V (1997) Compartmentation of boron in roots and its translocation to the shoot of sunflower as affected by short term changes in boron supply. In: Boron in Soils and Plants (Eds. RW Bell, B Rerkasem). Kluwer Academic Publishers. Dordrecht, the Netherlands, pp. 203-207.

Rerkasem B, Jamjod S (1997) Genotypic variation in plant response to low boron and implications for plant breeding. Plant Soil 193: 169-180.

Savic J, Nikolic M, Prodanovic S, Römheld V (2007) Boron uptake by the root cortex symplast of tomato and pea plants: evidence for low-boron induced active transport. Funct Plant Biol 34: 1130-1136.

Shelp BJ (1993) Physiology and chemistry of boron in higher plants. In: Boron and Its Role in Crop Production (Ed. UC Gupta). CRC Press. Boca Raton, Florida, USA, pp. 53-85.

Shorrocks V (1997) The occurrence and correction of boron deficiency. Plant Soil 193: 121-148.

Stangoulis JCR, Grewal HS, Bell R, Graham RD (2000a) Boron efficiency in oilseed rape: I. Genotypic variation demonstrated in field and pot grown *Brassica napus* L. and *Brassica juncea* L. Plant Soil 225: 243-251.

Stangoulis JCR, Webb MJ, Graham RD (2000b) Boron efficiency in oilseed rape: II. Development of a rapid lab-based screening technique. Plant Soil 225: 253-261.

Takano J, Miwa K, Fujiwara T (2008) Boron transport mechanisms: collaboration of channels and transporters. Trends Plant Sci 13: 451-457.

Xue J, Lin M, Bell R, Graham R, Yang X, Yang Y (1998) Differential response of oilseed rape (*Brassica napus* L.) cultivars to low boron supply. Plant Soil 204: 155-163.

Yang Y, Xue J, Ye Z, Wang K (1993) Responses of rape genotypes to boron application. Plant Soil 155/156: 321-324.