UDC 575:633
DOI: 10.2298/GENSR1302565S
Original scientific paper

OILSEED RAPE GENOTYPES RESPONSE TO BORON TOXICITY

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Savić J, A. Marijanović Jeromela, Đ. Glamočlija, and S. Prodanović (2013): *Oilseed rape genotypes response to boron toxicity.* Genetika, Vol 45, No. 2, 565-574

Response of 16 oilseed rape genotypes to B (boron) toxicity was analyzed by comparing the results of two experiments conducted in a glasshouse. In Experiment 1 plants were grown in standard nutrient solutions with 10 µM B (control) and 1000 µM B. Relative root and shoot growth varied from 20-120% and 31-117%, respectively. Variation in B concentration in shoots was also wide (206.5-441.7 µg B g^{-1} DW) as well as total B uptake by plant (62.3-281.2 µg B g^{-1}). Four selected genotypes were grown in Experiment 2 in pots filled with high B soil (8 kg ha^{-1} B; B8). Shoot growth was not affected by B8 treatment, while root and shoot B concentration was significantly increased compared to control. Genotypes Panther and Pronto which performed low relative root and shoot growth and high B accumulation in plants in Experiment 1, had good growth in B8 treatment. In Experiment 2 genotype NS-L-7 had significantly lower B concentration in shots under treatment B8, but also very high B accumulation in Experiment 1. In addition, cluster analyses classified genotypes in three groups according to traits contrasting in their significance for analyzing response to B toxicity. The first group included four varieties based on their shared characteristics that have small value for the relative growth of roots and shoots and large values of B concentration in shoot. In the second largest group were connected ten genotypes that are heterogeneous in traits and do not stand out on any characteristic. Genotypes NS-L-7 and Navajo were separated in the third group because they had big relative growth of root and shoot, but also a high concentration of B in the shoot, and high total B uptake. Results showed that none of tested genotypes could not be recommended for breeding process to tolerance for B toxicity.

Key words: B toxicity, oilseed rape, B concentration, nutrient solution, cluster analyses

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INTRODUCTION

Boron (B) toxicity affects crop production around the world, mostly in arid and semiarid regions (KEREN and BINGHAM, 1985). It has been reported that B toxicity decreases the yield of cereals (CARTWRIGHT *et al*., 1984; YAU and SAXENA, 1997) as well as production of oilseed Brassicas (NABLE and PAUL, 1991; CAMPBELL *et al*., 1998). Among all, high B concentrations have been reported in Australia (NUTALL *et al*., 2003) and Turkey (SILLANPAA, 1982). According to GEZGIN *et al.* (2002) nearly 10% of soils of Central Anadolia contain more than 5 mg of B kg^{-1} soil reaching critical concentrations for occurrence of B toxicity symptoms in crop plants. B toxicity is usually associated with low rainfall (CARTWRIGHT *et al*., 1984; PAULL *et al*., 1988), but source of high B concentration also can be irrigation water (NABLE *et al*., 1997). High B concentration in soil under irrigation with saline groundwater could be found in California (WIMMER *et al.*, 2003), the Negev region in Israel (YERMIYAHU *et al*., 2006), and the Lluta Valley in Chile (BASTIAS *et al*., 2004). Very often B soil toxicity to plants is related to salinity. Mechanism of relationships between them is still not clear but possible explanation could be interaction of B and CL YERMIYAHU *et al*., 2008).

Results of many studies showed that B tolerance differs between and within the plant species. Genotypic variation in response to B toxicity has been shown in wheat (*Triticum vulgare*) (PAULL *et al*., 1988), barley (NABLE, 1988; NABLE *et al*., 1990), pea (BAGHERI *et al*., 1992; AVCI and AKAR, 2005), tomato (GÜNES *et al*., 2000), *Brassica rapa* (KAUR *et al*. 2006). Sensitivity of leaf and root elongation to high B for barley was also demonstrated by REID *et al*. (2004). Screening method for testing B tolerance in wheat genotypes developed by CHANTACHUME *et al*. (1995) is used very often. This kind of screening can be used as an efficient tool for testing germplasm in breeding programs. Mechanism of B tolerance has been found in barley (HAYES and REID, 2004; SUTTON *et al*., 2007) and microorganisms as well (KAYA *et al*., 2009; MIWA *et al*., 2007).

Small number of studies on screening for B toxicity tolerance in oilseed rape genotypes has been carried out by now. ÖZTÜRK *et al*. (2010) performed field experiment with eight spring canola cultivars exposed to high B rates.

The objectives of this study were to test oilseed rape genotypes to B toxicity by growing plants in water culture and soil with toxic B supply with aim to obtain results that could be used in breeding process.

MATERIALS AND METHODS

Experiment 1

Quality and origin of 16 oilseed rape genotypes used in the study are presented in Table 1. Erucic acid content and glucosinolate level were previously measured by MARINKOVIĆ *et al*. (2003). Seed material was taken from oilseed rape collection of Institute for Field and Vegetable Crops, Novi Sad, Serbia. Seeds were germinated on filter paper at 25°C. Young seedlings were transferred to continuously aerated standard nutrient solution containing (mM): 0.7 K_2SO_4, 0.1 KCl, 2.0 $Ca(NO_3)_2$, 0.5 $MgSO_4$, 0.1 KH_2PO_4 and (µM) 0.5 $MnSO_4$, 0.5 $ZnSO_4$, 0.2 $CuSO_4$, 0.01 $(NH_4)_6Mo_7O_{24}$ and 40 Fe(III)-EDTA. B was added as H_3BO_3 at two levels of external supply: 10 µM (control) and 1000 µM (toxic). Four plants were grown in 2 l pots (one pot presented one replication) in the glasshouse under following environmental conditions: light/dark regime of

16/8 h, photon flux density of approximately 400 µmol m^{-2} s^{-1} at plant height and temperature around 25°C.

Plants were grown for four weeks and cut in hypocotyl, roots were rinsed with distilled water and dried between two layers of filter paper. Plant material was dried at 80°C and root and shoot dry weight was determined (g per $plant^{-1}$). B concentration in roots and shoots was determined colorimetrically by azomethine-H method, following procedure: dry material was ashed at 550°C for 8 h and Ash was dissolved with 0.5 N H_2SO_4 and all samples were centrifuged at 1000 *g*, before determination of B.

Table 1. Quality of 16 oilseed rape genotypes; glucosinolate and erucic acid content. In high- quality cultivars erucic acid content in oil is <2% and glucosinolate content <30 µM g^{-1} fresh weight

No.	Genotype	Glucosinolates (µM g^{-1} fresh weight)	Erucic acid (%)	Origin
1	Panther	22.1	0.1	Germany
2	Silvia	11.5	0.06	Germany
3	Artus	12.0	0	Germany
4	NS-L-15	18.0	2.19	Serbia
5	Slavica	18.2	0.09	Serbia
6	Zenith	0	0	Germany
7	Capitol	0	0	France
8	Lirajet	10.0	0.1	Germany
9	Pronto	10.0	0.1	Germany
10	Express	11.7	0	Germany
11	Rafaela	11.8	0	Germany
12	Tradition	21.5	0	France
13	NS-L-7	0	0	Serbia
14	Banaćanka	12.0	0	Serbia
15	NS-L-13	9.3	0	Serbia
16	Navajo	18.0	0	UK

Experiment 2

This experiment was conducted in the glasshouse. Four oilseed rape genotypes Navajo, Panther, Pronto and NS-L-7 were grown in pots filled with soil that was moderate low B pseudoglay with following properties: pH=4.30; total N 0.092%; available P_2O_5 7.9 mg 100 g^{-1}; available K_2O 13.5 mg 100 g^{-1} and hot water soluble boron 0.26 mg kg^{-1}. Air dried soil was sieved and basal fertilizers applied as urea (140 kg ha^{-1} N), 100 kg ha^{-1} P_2O_5 and 120 kg ha^{-1} K_2O as KH_2PO_4 and K_2SO_4 water solution, respectively (amounts calculated as for field conditions).

There were two B treatments: with no additional B (control) (B0) and 8 kg B ha^{-1} (B8), added as water solution of H_3BO_3.
Four weeks after germination, plants were harvested. Roots were washed with water to remove soil particles. Plant material was dried and B concentration in roots and shoots were determined as described in Experiment 1.

Statistical analysis

SPSS software package was used for performing the cluster analyses and analyses of variance. Analyses of variance was performed on growth parameters and B concentration (n=4). Means were compared by LSD test.

RESULTS AND DISCUSSION

Results from Experiment 1 regarding plant growth, root and shoot relative growth (1000 µM B/10 µM B, %), B concentration in roots and shoots and total B uptake by plant (µg B per $plant^{-1}$) of each genotype have been previously presented by SAVIC and NIKOLIC (2012). In this study, results on descriptive statistic are presented in Table 2. Selection of four oilseed rape genotypes (Navajo, Panther, Pronto and NS-L-7) used in Experiment 2 was based on results from Experiment 1. Selected genotypes were also used in another study by SAVIC *et al.* (2012) as contrasting ones in response to B deficiency.

Table 2. Descriptive statistics of relative root and relative shoot growth (1000 µM B/10 µM B, %), B concentration in plants and total B uptake per plant. Plants were grown in nutrient solutions under treatments of toxic B supply (1000 µM) and control (10 µM) for four weeks

	Relative root growth (%)*	Relative root growth (%)*	B concentration in roots (µg B g^{-1} DW)	B concentration in shoots (µg B g^{-1} DW)	Total B uptake (µg B per plant)
Minimum	20	31	21.0	206.5	62.3
Maximum	120	117	80.8	441.7	281.2
Mean	65.9	68.8	54.0	331.8	129.9
SD	29.4	25.9	18.3	70.4	66.6

*represents percentage of the value at 1000 µM to that at 10 µM

In Experiment 1, B toxicity symptoms were visible in all genotypes. Relative root and shoot growth varied from 20-120% and 31-117%, respectively (Table 2). Variation in B concentration in roots and shoots was also wide (21-80.8 µg B g^{-1} DW and 206.5-441.7 µg B g^{-1} DW, respectively) as well as total B uptake by plant (62.3-281.2 µg B g^{-1} DW), indicating that possible tolerance is present in genotypes with higher root and shoot growth and lower accumulation of B in the shoots. Cultivars Banaćanka and Slavica were recently tested to lead elevated levels *in vitro* by OREŠČANIN *et al*. (2012). Significant reduction of root lenght of plants treated with lead was observed in Slavica, while growth of Banaćanka was not affected, indicating that they may have differential response to various nutrient disorders, as it was shown for B in presented study.

The aim of performing cluster analyses was grouping the genotypes based on their response to B toxicity. Groups (clusters) were formed on the basis of the value of plants that

were subjected to screening to B toxicity in Experiment 1. Cluster analysis of relative root growth, relative shoot growth, B concentration in root, B concentration in shoot and total B uptake by plant of 16 oilseed rape genotypes under conditions of B toxicity resulted in a dendrogram of similarity, where genotypes are designated by numbers as in Table 1 (Fig. 1).

It can be seen that there are three groups (clusters) of genotypes (Fig. 1). The first and the third groups were smaller. The first group includes four varieties (4- NS-L-15, 5-Slavica, 6-Zenith and 10-Express), and the third includes two varieties (13 – NS-L-7 and 16 - Navajo). The second group is the largest, with 10 varieties. Clustering of genotypes in the first group is based on their shared characteristics that have small value for the relative growth of roots and shoots, as well as large values of B concentration in shoot (SAVIC and NIKOLIC, 2012) . In the second group are connected genotypes that are heterogeneous in traits and do not stand out on any characteristic. Genotypes NS-L-7 and Navajo were separated in the third group because they have big relative growth of root and shoot, but also a high concentration of B in the shoot, and high total B uptake. MARJANOVIĆ-JEROMELA *et al*. (2009) performed a phenotypic and molecular evaluation of thirty oilseed rape genotypes of different origin. By comparing dendrogram obtained by using RAPD markers and dendrogram obtained by analyzing ten quantitative traits, it was found that genotypes were clustered according to geographic origin. In presented study, genotypes originate from four countries and such grouping was not found.

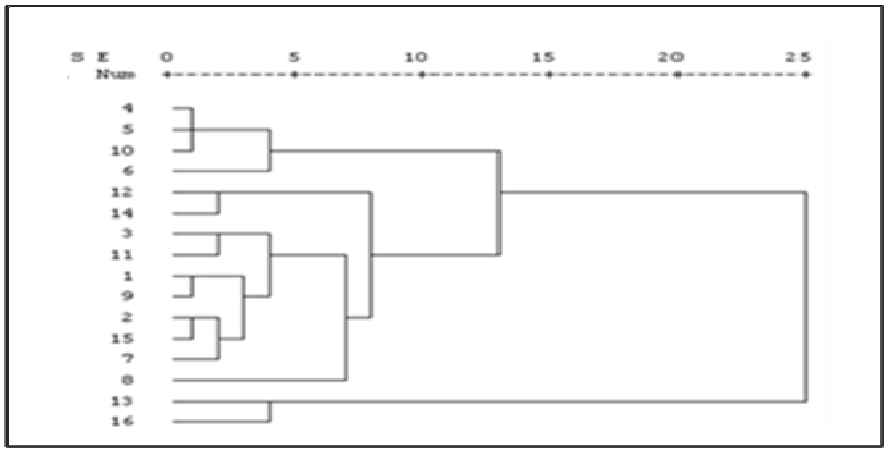

Figure 1. Dendrogram of similarity for16 oilseed rape genotypes grown under conditions of B toxicity using the hierarchical cluster analysis

In Experiment 2, there were no visible B toxicity symptoms at plants, as in Experiment 1. Contrasting genotypes grown in nutrient solutions were selected prior to cluster analysis. Root and shoot dry weight of four genotypes was raging from 0.03 - 0.07 g plant^{-1} and 0.50 – 0.73 g plant^{-1}, respectively (Table 3). Root dry weight was differing significantly between genotypes within B treatments ($P<0.05$), but values were similar. Differences in shoot dry weight between genotypes and B treatments were not statistically significant, indicating that plant growth of four genotypes was not strongly affected by B toxicity. Although, in Navajo both root

and shoot dry weight were higher at B8 treatment compared to B0. KAUR *et al*., (2006) used reduction of tap root length of *Brassica rapa* plants grown in nutrient solutions as criteria for determination of B tolerance to B toxicity, which corresponded with reduction of shoot biomass of plants from their soil experiment.

Table 3. Dry weight of roots and shoots and B concentration in plants of oilseed rape genotypes grown in pots with two B treatments: soil with no B added (B0) and soil with 8 kg B ha^{-1} (B8)

Genotype (A)	Boron treatments (B)											
	Root dry weight (g plant^{-1})			Shoot dry weight (g plant^{-1})			Root B concentration (mg B kg^{-1} DW)			Shoot B concentration (mg B kg^{-1} DW)		
	B0	B8	Mean	B0	B8	Mean	B0	B8	Mean	B0	B8	Mean
Navajo	0.05	0.07	0.06	0.50	0.73	0.62	21.0	31.9	26.5	25.8	92.6	59.2
Panther	0.06	0.06	0.06	0.65	0.64	0.65	22.8	26.9	24.9	20.4	91.6	56.0
Pronto	0.03	0.04	0.04	0.61	0.62	0.62	19.4	22.4	20.9	23.2	88.8	56.0
NS-L-7	0.04	0.05	0.05	0.63	0.62	0.63	19.2	23.3	21.3	21.8	68.1	45.0
Mean	0.05	0.06	0.06	0.59	0.65	0.62	20.6	26.1	23.4	22.8	85.3	54.0
F-*test*	A	B	AB^{ns}	A^{ns}	B^{ns}	AB^{ns}	A	B	AB	A	B	AB
LSD	0.009	0.008					2.0	1.8	1.1	7.7	6.6	

[a] lsd - least significant difference indicating genotype main effect, **P<0.05

In presented study, we measured root dry weight and according to low relative root growth of Panther and Pronto in Experiment 1 (SAVIC and NIKOLIC, 2012) and absence of shoot growth reduction in Experiment 2 (Table 3), seems that root length reduction is much better criteria for determination of B toxicity. In addition, cluster analyses showed that these two genotypes were heterogeneous in measured traits (Fig. 1), indicating they could not be considered as sensitive to B toxicity. On the other hand, TORUN *et al*. (2006) recorded tendency for an increase in growth of wheat plants grown in high B soil, which was the case in our study as shoot growth of Navajo increased at B8 (Table 3).

As it was expected, B root and shoot concentrations were increased by B8 treatment, with bigger differences in shoots, ranging from 68.1 – 92.6 (Table 3). Range of critical toxicity concentrations (mg kg^{-1} DW) in leaves are in the wide, from 100 in maize, over 400 in cucumber, to 1000 in squash and between 100 and 270 in wheat genotypes (PAULL *et al*., 1992). Critical B concentrations in leaves should be interpreted with reservation due to steep gradient in B concentration within a leaf blade (MARSCHNER, 2012). In our study, B concentration in shoots in Navaho, Panther and Pronto were close to 100 mg kg^{-1} DW and could be considered as toxic. At NS-L-7 shoot B concentration was significantly lower in comparison to other three genotypes (Table 3). In contrast to this, in Experiment 1, genotype NS-L-7 had high relative root and shoot growth, but very high accumulation of B in shoots (>400 mg kg^{-1} DW), while there were genotypes with <250 mg B kg^{-1} DW (SAVIC and NIKOLIC, 2012). ÖZTÜRK *et al*. (2010) also obtained similar values of increasing of B concentration in spring canola genotypes after application of 15 kg B ha^{-1}. Although, in genotypes with lower B accumulation, concentration in leaves was much less - 30 mg B kg^{-1} DW. Clear differences in shoot B concentration between *Brassica rapa* genotypes grown in soil with 29 mg B kg^{-1} soil for five weeks were obtained by KAUR *et al*., (2006), but in tolerant genotypes it was also much lower (30 and 23 mg kg^{-1} DW) in

comparison with results obtained in presented study. The obtained results suggest that NS-L-7 cannot be considered as genotype typically tolerant to B toxicity despite lower B accumulation in shoots. As WIMMER *et al*. (2005) suggested B tolerance is genotype specific with mechanisms including reduced uptake rates and differential translocation and allocation within plants, which support results obtained in presented study.

CONCLUSION

All above mentioned and results presented indicate that under conditions of B toxicity root length reduction is much better indicator for determination of tolerance to B toxicity. We did not found connection between results obtained by growing oilseed rape plants nutrient solutions containing toxic B concentrations and high B soil. Cluster analyses divided genotypes in three groups according to traits contrasting in their significance for analyzing response to B toxicity, i.e. high relative growth vs. high accumulation of B in plants. Despite the fact that oilseed rape genotypes tested to B toxicity differed in growth and B accumulation, no one showed response typical for both B toxicity sensitive and B toxicity tolerant genotypes.

ACKNOWLEDGEMENT

This work was supported by the Serbian Ministry of Education, Science and Technological Development (grant OI 173028 to JS).

Received March 07th, 2013
Accepted July 10th, 2013

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ODGOVOR GENOTIPOVA ULJANE REPICE NA TOKSIČNOST BORA

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Izvod

Odgovor 16 genotipova uljane repice na toksičnost B (bora) analizirana je poređenjem rezultata dva ogleda izvedenim u stakleniku. U Ogledu 1, biljke su gajene u hranljivim rastvorima sa dva tretmana B: 10 μM B (kontrola) i 1000 μM B (toksična koncentracija). Vrednosti za relativni porast korena i nadzemnog dela varirale su od 20-120% i 31-117%, po redu. Koncentracija B je takođe značajno varirala (206.5-441.7 μg B g^{-1} SM) kao i ukupno usvojena količina B po biljci. Na osnovu rezultata dobijenih u Ogledu 1 odabrana su četiri genotipa koja su u Ogledu 2 gajena u posudama sa zemljištem kome je dodat B preračunato za poljske uslove u količini od 8 kg ha^{-1} (B8) uz kontrolu. Toksične količine B nisu negativno uticale na porast biljaka, dok je koncentracija B u korenu i nadzemnoj masi značajno povećana u poređenju sa kontrolom. Iako su u Ogledu 1 zabeležene velike razlike u prastu biljaka i koncentraciji B u suvoj masi, rezultati dobijeni u Ogledu 2, nisu pokazali da je kod bilo kog genotipa prisutna tipična reakcija na osnovu koje bi bili svrstani u grupu osetljivih ili tolerantnih na tiksičnost B. Genotipovi Panther i Pronto kod kojih je u Ogledu 1 zabeležen mali relativni porast korena i nadzemnog dela, kao i velika koncentracija B u biljci, imali su dobar porast u tretmanu sa 8 kg ha^{-1}. U Ogledu 2, kod genotipa NS-L-7 zabeležena je značajno niža koncnetracija B u nadzemnom delu biljaka, ali i velika akumulacija B u Ogledu 1. Kluster analizom genotipovi su podeljeni u tri grupe na osnovu relativnog porasta korena i nadzemnog dela, koncentracije B u njima i ukupnom usvajanju B po biljci, ali tako da ne ukazuju na eventualno postojanje otpornosti na toksičnost B. Istraživanje je pokazalo da ni jedan od 16 testiranih genotipova ne može da bude preporučen za proces oplemenjivanja za otpornost na toksičnost B.

Primljeno 07. III 2013.
Odobreno 10. VII. 2013.