https://doi.org/10.17221/526/2018-PSE

Low phosphorus availability increases shoot boron concentration in canola and potato but not in wheat

Yanliang WANG*, Nicholas CLARKE, Anne Falk ØGAARD

Norwegian Institute of Bioeconomy Research, Ås, Norway
**Corresponding author: yanliang.wang@nibio.no*

ABSTRACT

Wang Y., Clarke N., Øgaard A.F. (2018): Low phosphorus availability increases shoot boron concentration in canola and potato but not in wheat. Plant Soil Environ., 64: 564–570.

A large proportion of global agricultural soils contain suboptimal available phosphorus (P) for the growth of many plant species. Boron (B) plays important roles in plant growth and development, but limited research has been conducted to study B uptake under low P availability. This study comprised a hydroponic and a mini-rhizobox experiment with canola (*Brassica napus* L.), potato (*Solanum tuberosum* L.) and wheat (*Triticum aestivum* L.) under P sufficient and deficient conditions. Boron concentrations, rhizosphere soil pH, and gene expression of *BnBOR1* in canola were determined. Shoot B concentrations were found significantly increased (11–149%) by low P availability in potato and canola but not in wheat. Reverse transcription polymerase chain reaction (RT-PCR) indicated that *BnBOR1;2a, BnBOR1;2c*, and *BnBOR1;3c* were up-regulated after seven days of low P treatment in canola roots. Our results indicate that plant shoot B concentration was dramatically influenced by P availability, and dicots and monocots showed a contrasting B concentration response to low P availability.

Keywords: macronutrient; deficiency; toxicity; nutrition; long-term experiment; boron transporter

Boron (B) plays an important role in plants since B is known to be essential for plant cell wall structure and function (Brown et al. 2002). Both B deficiencies and toxicities occur widely and affect agriculture significantly throughout the world (Shorrocks 1997, Camacho-Cristóbal et al. 2008, Varga et al. 2010). The B requirement of plants is generally low but differs among various species and cultivars, with monocots generally requiring less B for normal growth and development than dicots (Hu et al. 1996, Bellaloui and Brown 1998, Marschner 2011). In soil solution, B mainly exists as boric acid. Boron uptake by root cells is affected by various environmental factors such as soil pH, soil clay content and soil organic matter (Moraghan and Mascagni 1991, Hu and Brown 1997, Niaz et al. 2007, Ahmad et al. 2012).

Phosphorus (P) is an essential macronutrient for plant growth and development, and P deficiency significantly reduces plant biomass and crop yields (Chiou and Lin 2011, Wang et al. 2015, Lošák et al. 2016). A large proportion of global agricultural soils contain suboptimal available phosphorus for the growth of many plant species (Lynch 2011). Thus, annual crop production is often limited by P deficiency. This may in turn influence uptake of other nutrients. Previous studies suggested that P acquisition has significant interactions with uptake of N, Mg, Fe, Zn, and Mn (Fageria 2001). Despite the importance of both P and B, limited reports have been focused on B uptake in low P availability agricultural soils, in particular in recent years (Nusbaum 1947, Salinas et al. 1986, Günes and Alpaslan 2000).

The present study determined B concentrations in plant tissues of canola, potato and wheat under P sufficient and deficient conditions, under agricultural soil (rhizobox) and non-soil (hydroponic) conditions, to study plant B uptake under low P availability. Subsequently, the correlation between

Supported by the Norwegian Institute of Bioeconomy Research.

https://doi.org/10.17221/526/2018-PSE

rhizosphere soil pH and B concentrations, as well as *BnBOR1* expression, under P sufficient and deficient conditions was investigated. These results can be important for understanding the B uptake in response to low P availability in different crops.

MATERIAL AND METHODS

Plant materials and experimental design. Canola (*Brassica napus* cv. Marie), wheat (*Triticum aestivum* cv. Aino) and micropropagated seedlings of potato (*Solanum tuberosum* cv. Pimpernel) were selected for the present study. A hydroponic (Wang et al. 2015) and a mini-rhizobox experiment using agricultural soils (Wang et al. 2016) were employed in the greenhouse of the Norwegian University of Life Sciences.

For the hydroponic culture, plant seedlings (four replicates with one plant per replicate) were first grown in full-strength nutrient solution containing 1.5 mmol/L KCl, 2 mmol/L $Ca(NO_3)_2$, 1.0 mmol/L $MgSO_4$, 50 µmol/L KH_2PO_4, 1 µmol/L H_3BO_3,1 µmol/L $MnSO_4$,1 µmol/L $ZnSO_4$, 0.5 µmol/L $CuSO_4$, 0.37 µmol/L Na_2MoO_4 and 50 µmol/L Fe-EDTA (Wang et al. 2015). Five-day-old uniform seedlings without seed residues were then carefully transferred to the same medium supplemented with 50 (P50) or 1 (P1) µmol/L KH_2PO_4, and pH adjusted to 5.8 ± 0.2. Plants were grown under a photoperiod of 16 h light and 8 h dark at a light intensity of 200 ± 20 µmol/m^2/s and 50–75% relative humidity, with a temperature of 25°C/16°C (day/night). The nutrient solution was replaced every third day.

The mini-rhizobox experiment with four replicates with three plants per replicate was set up as described by Wang et al. (2016) under the same greenhouse conditions as for the hydroponic experiment. Briefly, each mini-rhizobox (0.2 m × 0.2 m × 0.01 m) was filled with ~0.5 kg fertilized homogenized agricultural soil, water was then added to achieve a soil moisture level of 25% (w/w). The water holding capacity was 45% (w/w) according to another study using the same soil (Brod et al. 2016). The soils were collected from the plough layer (0–0.2 m) of clay loam (26% clay, 38% silt, 36% sand) long-term fertilization field plots at Ås, Akershus county (59°39'N, 10°45'E), which had received either 48 (P high, soil HP) or 0 (P low, soil LP) kg P/ha/year as single superphosphate since 1966. The soils were analysed after passing through a 3.15 mm sieve in order to make an initial characterization and their selected properties are presented in Table 1. The differences in contents of C and N are probably due to soil variations within the field experiment from which the soil samples were collected. The pH was adjusted from 5.4 and 5.0 respectively to ~6.5 by adding moderate amounts of $CaCO_3$ before use (Wang et al. 2016). Macronutrients (except for P) and micronutrients (including 0.11 mg B/kg soil) were supplied. Three uniform seedlings were grown in each mini-rhizotron for five weeks, deionized water (15–40 mL) was given daily to keep the soil surface moist and the position of the mini-rhizobox was changed randomly every week. Rhizosphere soil water-soluble P (WSP) and plant available P, which was indicated by ammonium-lactate-extractable P, i.e., P_{AL}, were determined after the experiment and are listed by Wang et al. (2016). Briefly, P_{AL} of HP soil was 7–10 times higher than that in LP soil and WSP of HP soil was around 5 times higher than in LP soil after the experiment. Rhizosphere soil pH was measured and presented as described (Wang et al. 2016) and in the current manuscript.

Plant harvest and biomass measurements. In the hydroponic system, plants were harvested 4 weeks after being subjected to the P1 and P50 treatments and roots (which were washed by Milli-Q water before sampling) and shoots were sampled separately for subsequent analyses (Wang et al. 2015). In the mini-rhizobox experiment, the intact plants were carefully removed from the mini-rhizoboxes and divided into shoots and roots. The roots were then water cleaned after sampling of rhizosphere soil and rhizosphere extract as described by Wang et al. (2016). Shoots and roots were dried at 65°C for 48 h, and their dry weight (DW) was measured.

Table 1. Selected properties of the soils

Soil	HP	LP
pH_{H_2O}	5.4	5.0
Total carbon (%)	2.89	2.25
Total nitrogen (%)	0.28	0.24
AL extractable phosphorus (mg/kg soil)	150	37
AL extractable calcium (mg/kg soil)	1 700	1 300
Water soluble phosphorus (mg/kg soil)	10.64	0.81

AL – ammonium lactate; HP – high, LP – low phosphorus

https://doi.org/10.17221/526/2018-PSE

Determination of P and B in plant tissues. Elements including P and B in dry root and shoot tissues were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES, AtomComp 1100, Thermo Jarrell-Ash, USA) after digestion in a mixture of 65% (v/v) HNO_3/72% (v/v) $HClO_4$ (5:1, v/v) at 220°C in a microwave oven (Wang et al. 2015). The data on plant P concentrations were presented in two previous publications (Wang et al. 2015, 2016) and generally P concentrations decreased under low P availabilities.

RNA extraction and semi- quantitative RT-PCR. For RNA extraction, canola leaves and roots were sampled from the hydroponic system after two and seven days of the P1 and P50 treatments to assess the transcription levels of boron transporters using specific primers (Sun et al. 2012). Total RNA was extracted using a Spectrum™ Plant Total RNA Kit (Sigma-Aldrich, St. Louis, USA) and genomic DNA was removed using an On-column DNase I digest kit (Sigma-Aldrich, St. Louis, USA). RNA quantity and quality were assessed by a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, USA). First-strand cDNA was synthesized from 1.0 µg RNA using iScriptTM Adv cDNA kit for semi-quantitative RT-PCR. Two µL total first-strand cDNA of each sample was used as a template in a 20 µL standard *Taq* PCR reaction system. The PCRs were performed as described by Sun et al. (2012) and PCR products were electrophoresed and UV-visualized using the Bio-Rad Quantity one system (California, USA).

Data analysis. Student's *t*-test and *R* (version 3.2.3, Vienna, Austria) were used for data analyses. Two-way ANOVAs were used to study the main effects of soil, plant species, and their interaction on parameters involved in this study, followed by post hoc pair-wise Tukey's honest significant difference tests for multiple comparisons, along with the minimum significant difference at $P < 0.05$.

RESULTS

Boron concentration response. Generally, plant biomass was lower under low P availability compared to high P availability (Table 2). Shoot B concentrations were significantly higher in P1 hydroponic than in the P50 treatment for canola (93%) and potato (149%) but not for wheat (Figure 1a). Compared to the P50 treatment, P1 plants showed increased root B concentrations in potato (26%) but decreased in wheat (–25%, Figure 1b). Under rhizobox soil conditions, canola and potato grown in low P soil showed 11% and 57% higher shoot B concentrations respectively, compared with high P soil, but canola and wheat showed lower root B concentrations as shown in Figure 1c–d (–21% and –34%, respectively). ANOVA analysis suggested that shoot B concentration was significantly affected by soil P availability, plant species and their interactions ($F = 70.20$, $n = 24$, $P < 0.001$).

Rhizosphere soil pH. Rhizosphere soil pH was determined after harvest of plant samples in the rhizobox experiment. Significantly higher rhizosphere soil pH in low P soil than in high P soil was found in potato and wheat but not in canola (Figure 2). ANOVA analysis indicated that rhizosphere soil pH was significantly correlated with plant species, soils (LP vs. HP soil) and the interaction of soil and plant species ($F = 37.47$, $n = 24$, $P < 0.001$). There was no significant correlation between rhizosphere soil pH and shoot B concentration across all the tested three crops ($R^2 = 0.07$, $n = 24$, $P > 0.05$).

Table 2. Shoot and root dry weight (DW) of canola, potato and wheat grown in nutrient solutions at two levels of phosphorus (P) supply (P1 – 1 µmol P/L; P50 – 50 µmol P/L), and low P (LP) and high P (HP) clay loam soils

DW (g)	Canola		Potato		Wheat	
	shoots	roots	shoots	roots	shoots	roots
P1	0.20 ± 0.027	0.06 ± 0.003	0.13 ± 0.004	0.05 ± 0.004	0.29 ± 0.023	0.07 ± 0.003
P50	2.01 ± 0.134***	0.17 ± 0.008**	0.97 ± 0.083***	0.13 ± 0.011*	0.93 ± 0.089**	0.11 ± 0.011
LP	1.79 ± 0.101	0.83 ± 0.059	0.71 ± 0.023	0.37 ± 0.054	0.98 ± 0.093	0.68 ± 0.184
HP	2.81 ± 0.106***	1.01 ± 0.098	1.27 ± 0.047***	0.91 ± 0.068***	2.28 ± 0.134***	1.24 ± 0.346

Values are means ± standard error of $n = 4$. Letters indicate a significant difference (Student's *t*-test) within species between P1 and P50, or between LP and HP (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; no letter means no significant difference)

https://doi.org/10.17221/526/2018-PSE

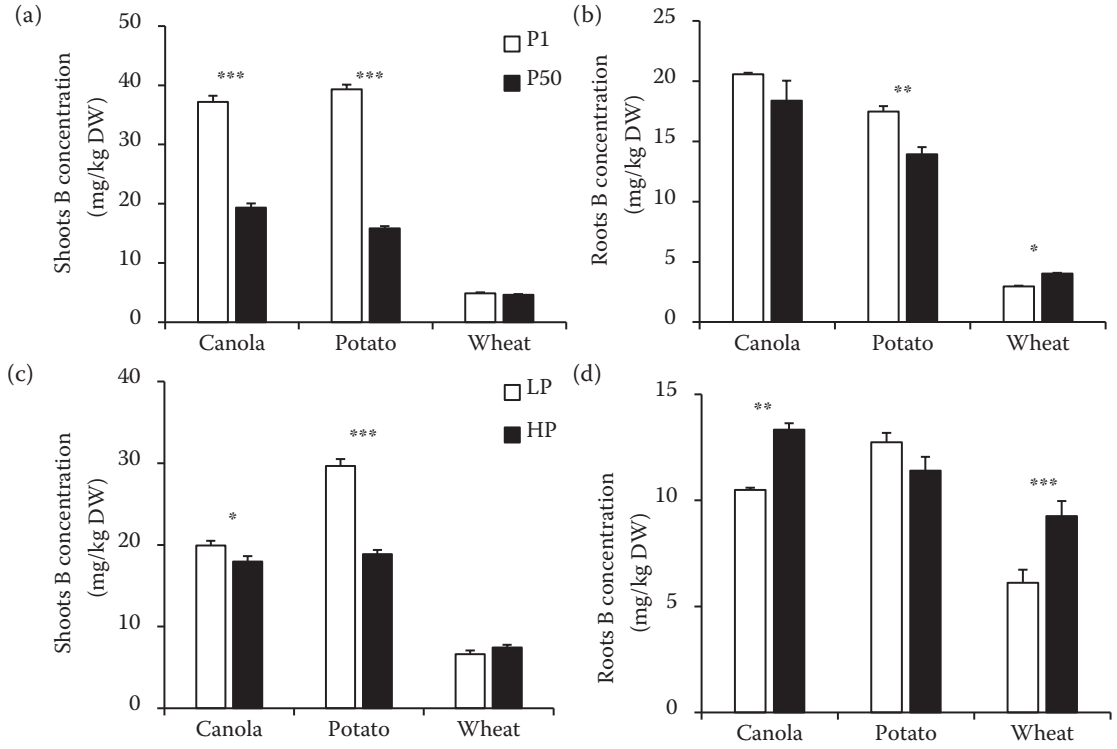

Figure 1. Shoot boron (B) concentration and root B concentration of canola, potato and wheat grown in nutrient solutions (a, b) at two levels of phosphorus (P) supply (P1 – 1 μmol P/L, P50 – 50 μmol P/L), and grown in low P (LP) and high P (HP) clay loam soils (c, d). Plants were harvested after four and five weeks' growth for hydroponic culture for the rhizobox experiment, respectively. Values are means ± standard error of $n = 4$. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; DW – dry weight

Gene expression of boron transporters in canola. Gene expression of *BnBOR1* including *BnBOR1;1a*, *BnBOR1;1c*, *BnBOR1;2a*, *BnBOR1;2c*, *BnBOR1;3a*, *BnBOR1;3c* was examined by semi-quantitative RT-PCR in canola roots and leaves grown hydroponically after two and seven days.

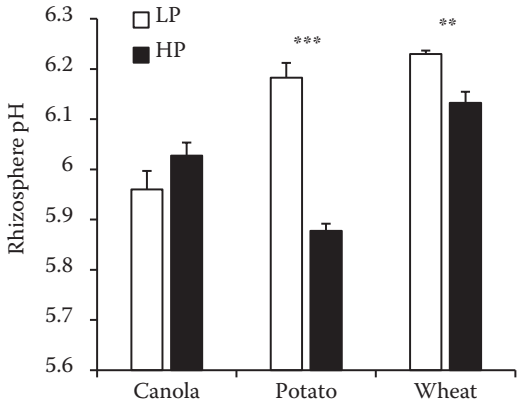

Figure 2. Rhizosphere soil pH of canola, potato and wheat grown in low phosphorus (LP) and high P (HP) soils. Error bars indicate standard error of $n = 4$. $^{**}P < 0.01$; $^{***}P < 0.001$

After two days of P deficiency, expression of *BnBOR1;2a* and *BnBOR1;3c* was not detected in canola leaves, while *BnBOR1;1a*, *BnBOR1;2a* and *BnBOR1;2c* were not expressed in roots. Generally, gene expression of those transporters showed no difference between the P1 and P50 treatments. After seven days of P deficiency, all the transcripts were detected in both leaves and roots, with *BnBOR1;2a*, *BnBOR1;2c*, and *BnBOR1;3c* up-regulated and *BnBOR1;1a* slightly down-regulated in canola roots, while *BnBOR1;3c* was down-regulated in canola leaves (Figure 3).

DISCUSSION

Plant B requirements vary among species and cultivars. Generally, dicots like cotton and leguminous plants have higher B requirement (20–70 mg/kg) than monocots such as the graminae family (5–10 mg/kg, Bergmann 1992, Marschner 2011). B was applied in both experiments in the current study and our data showed shoot B con-

https://doi.org/10.17221/526/2018-PSE

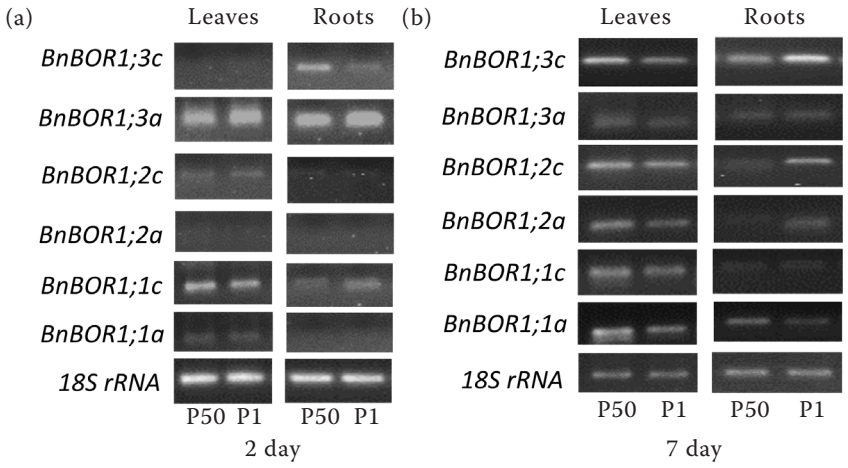

Figure 3. Semi-quantitative RT-PCR analysis of *BnBOR1* s in leaves and roots of canola subjected to P1 (1 µmol P/L) and P50 (50 µmol P/L) conditions in hydroponics after (a) two days and (b) seven days. 18 s rRNA was used as internal standard

centrations around 15.8–39.3 mg/kg in canola and potato, and B concentrations of 4.6–7.4 mg/kg in wheat, indicating that these plants did not suffer from B toxicity but might be slightly B deficient (particularly canola). Our study showed significant changes in B concentrations in canola and potato but not in wheat, which suggested that the B uptake of high B demand plant species was more sensitive to low P availability than low B requirement species. Canola is considered to have high B requirements, and deficiency of B at any growth stage in canola can severely affect its seed yield (Ahmad et al. 2012). Our study showed that potato also had a much higher shoot B concentration under P deficiency particularly under soil conditions, compared with plants grown in high P availability soil. Furthermore, our previous reports indicated that the high B demand plant species canola and potato tend to have higher quantities of other nutrients such as P (Wang et al. 2016) and that potato is less adaptive to low P availability than canola (Wang et al. 2015, 2016), which may in turn affect its B uptake and transport under low P availability.

Hydroponics and rhizoboxes are two very different systems for plant cultivation; the differences between the two systems would affect the nutrient sorption, plant root morphological and physiological responses etc. (Bengough et al. 2011). However, both systems showed an increased shoot B concentration after low P treatment in canola and potato but not in wheat. Interestingly, these two systems lead to completely different trends in canola root B concentration, for which the reason is unclear and further studies are needed. In addition, the differences in plant biomass of canola and potato were around 8–10 times between P1 and P50, and about 1.5–1.8 times between LP and HP; the differences in shoot B concentrations were about 1.9–2.5 times between P1 and P50, and around 1.2–1.6 times between LP and HP. The lower difference in shoot B concentrations with lower difference in biomass suggests a possible 'diluting' effect in shoot B concentrations due to greater biomass under P sufficient conditions.

Previous studies showed contrasting results on B uptake in response to P supply (Nusbaum 1947, Tanaka 1967, Yamanouchi 1980, Salinas et al. 1986, Chatterjee et al. 1990, Günes and Alpaslan 2000). The current study showed that the shoot B concentration under high P supply was lower than under P deficiency in canola and potato. An interaction between B and P for canola and potato has not been studied earlier to our knowledge, but our results corresponded well with studies where high P supply reduced tissue B concentrations in crops like maize and pea (Yamanouchi 1980, Salinas et al. 1986, Chatterjee et al. 1990, Günes and Alpaslan 2000). A recent study found a significant positive correlation between B uptake and P supply (most of the P applications are P sufficient) in barley (Mühlbachová et al. 2017) but it was found that wheat shoot B concentration was not affected by low P availability. Some reports indicated that P deficiency enhanced the severity of B deficiency in sweet potato, radish and sunflower (Nusbaum 1947, Tanaka 1967). All these results indicated that the interaction between B and P uptake was highly dependent on plant species (e.g. dicots vs monocots), plant growth medium and the degree of available P.

Less than 5–10% of soil B is in a form available to plants (Diana 2006). Soil pH is one of the most

https://doi.org/10.17221/526/2018-PSE

important factors affecting soil B availability and plant B uptake, usually higher soil pH leads to less available soil B (Ahmad et al. 2012), and B uptake by plants was noticeably higher where soil solution pH was lower (Wear and Patterson 1962). In the rhizobox experiment, the rhizosphere pH from low P soil was generally increased compared with bulk soil, possibly due to the application of NO_3^- as N fertilizer (Smiley 1974). No consistent relationship between changes in rhizosphere pH and shoot B concentrations was detected in the current study. Therefore, the observed higher shoot B concentration from low P soil in our study was mainly due to the lower P availability and probably the lower plant biomass. This is supported by the hydroponic experiment, where the P concentration in the growth medium was the key driver for decreased plant biomass and increased shoot B concentration.

There are a number of B transporters that have been identified, including NIPs, BORs and ATR (Dordas et al. 2000, Brown et al. 2002, Dannel et al. 2002, Sun et al. 2012). Among those, *AtBOR1* is the first identified B transporter and *AtBOR1* plays an important role in maintaining B homeostasis under B deficiency (Takano et al. 2005). Our hydroponic system contains 1 μmol/L B, which might be not sufficient for canola growth for a long period (as reflected by the B concentration data). Moreover, six orthologues of *AtBOR1* were identified and isolated in canola (Sun et al. 2012). Hence, the present study examined the expression of *BnBOR1* in canola. Sun et al. (2012) showed that the expressions of *BnBOR1;1c* and *BnBOR1;2a* were induced by B deficiency, whereas *BnBOR1;3a* and *BnBOR1;3c* showed an ubiquitous expression regardless of B supply at bloom stage under field conditions. In our study, after seven days of P1 and P50 treatments, all the six transcripts were detected in both canola leaves and roots under both P50 and P1, and found increased expression of *BnBOR1;2c* and *BnBOR1;3c* in roots but decreased expression of *BnBOR1;3c* in leaves under P1, providing valuable information for understanding molecular basis of B uptake under P deficiency.

In conclusion, by carrying out hydroponic and mini-rhizobox experiments, it was studied how B concentrations were affected by low P availability in canola, potato and wheat. It was found that low P availability was related to increased B concentrations in the aboveground tissues in canola and potato but not in wheat. In canola, *BnBOR1;2c* and *BnBOR1;3c* appeared to play important roles in stimulating B uptake under P deficiency. These findings provide insights into B uptake under low P availability in various crop species.

Acknowledgements

We thank Dr. Jihong Liu Clarke (NIBIO) for her various supports. We thank Jan Erik Jacobsen and Monica Fongen (NIBIO) for carrying out the ICP-AES analyses.

REFERENCES

Ahmad W., Zia M.H., Malhi S.S., Niaz A., Saifullah (2012): Boron deficiency in soils and crops: A review. In: Goyal A. (ed.): Crop Plant. Rijeka, InTech, 240.

Bellaloui N., Brown P.H. (1998): Cultivar differences in boron uptake and distribution in celery (*Apium graveolens*), tomato (*Lycopersicon esculentum*) and wheat (*Triticum aestivum*). Plant and Soil, 198: 153–158.

Bergmann W. (1992): Nutritional Disorders of Plants: Visual and Analytical Diagnosis. Jena, Stuttgart, New York, Gustav Fischer Verlag.

Bengough A.G., McKenzie B.M., Hallett P.D., Valentine T.A. (2011): Root elongation, water stress, and mechanical impedance: A review of limiting stresses and beneficial root tip traits. Journal of Experimental Botany, 62: 59–68.

Brod E., Øgaard A.F., Haraldsen T.K., Krogstad T., Frossard E., Oberson A. (2016): Drivers of phosphorus uptake by barley following secondary resource application. Frontiers in Nutrition, 3: 12.

Brown P.H., Bellaloui N., Wimmer M.A., Bassil E.S., Ruiz J., Hu H., Pfeffer H., Dannel F., Römheld V. (2002): Boron in plant biology. Plant Biology, 4: 205–223.

Camacho-Cristóbal J.J., Rexach J., González-Fontes A. (2008): Boron in plants: Deficiency and toxicity. Journal of Integrative Plant Biology, 50: 1247–1255.

Chatterjee C., Sinha P., Agarwala S.C. (1990): Interactive effect of boron and phosphorus on growth and metabolism of maize grown in refined sand. Canadian Journal of Plant Science, 70: 455–460.

Chiou T.J., Lin S.I. (2011): Signaling network in sensing phosphate availability in plants. Annual Review of Plant Biology, 62: 185–206.

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

Diana G. (2006): Boron in the soil, from deficit to toxicity. Informatore Agrario, 62: 54–58.

https://doi.org/10.17221/526/2018-PSE

Dordas C., Chrispeels M.J., Brown P.H. (2000): Permeability and channel-mediated transport of boric acid across membrane vesicles isolated from squash roots. Plant Physiology, 124: 1349–1362.

Fageria V.D. (2001): Nutrient interactions in crop plants. Journal of Plant Nutrition, 24: 1269–1290.

Günes A., Alpaslan M. (2000): Boron uptake and toxicity in maize genotypes in relation to boron and phosphorus supply. Journal of Plant Nutrition, 23: 541–550.

Hu H.N., Brown P.H. (1997): Absorption of boron by plant roots. Plant and Soil, 193: 49–58.

Hu H.N., Brown P.H., Labavitch J.M. (1996): Species variability in boron requirement is correlated with cell wall pectin. Journal of Experimental Botany, 47: 227–232.

Lošák T., Hlušek J., Lampartová I., Elbl J., Mühlbachová G., Čermák P., Antonkiewicz J. (2016): Changes in the content of soil phosphorus after its application into chernozem and haplic luvisol and the effect on yields of barley biomass. Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis, 64: 1603–1608.

Lynch J.P. (2011): Root phenes for enhanced soil exploration and phosphorus acquisition: Tools for future crops. Plant Physiology, 156: 1041–1049.

Marschner H. (2011): Marschner's Mineral Nutrition of Higher Plants. London, Academic Press.

Moraghan J.T., Mascagni H.J.Jr. (1991): Environmental and soil factors affecting micronutrient deficiencies and toxicities. In: Mortvedt J.J. (ed.): Micronutrients in Agriculture. Madison, Soil Science Society of America, 371–425.

Mühlbachová G., Čermák P., Vavera R., Káš M., Pechová M., Marková K., Kusá H., Růžek P., Hlušek J., Lošák T. (2017): Boron availability and uptake under increasing phosphorus rates in a pot experiment. Plant, Soil and Environment, 63: 483–490.

Niaz A., Ranjha A.M., Rahmatullah A.H., Waqas M. (2007): Boron status of soils as affected by different soil characteristics – pH, $CaCO_3$, organic matter and clay contents. Pakistan Journal of Agricultural Sciences, 44: 428–435.

Nusbaum C. (1947): Studies of boron deficiency in sweet potatoes. Phytopathology, 37: 435–436.

Salinas R., Cerdá A., Martinez V. (1986): The interactive effects of boron and macronutrients (P, K, Ca and Mg) on pod yield and chemical composition of pea (*Pisum sativum*). Journal of Horticultural Science, 61: 343–347.

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

Smiley R.W. (1974): Rhizosphere pH as influenced by plants, soils, and nitrogen fertilizers. Soil Science Society of America Journal, 38: 795–799.

Sun J., Shi L., Zhang C., Xu F. (2012): Cloning and characterization of boron transporters in *Brassica napus*. Molecular Biology Reports, 39: 1963–1973.

Takano J., Miwa K., Yuan L.X., von Wire´n N., Fujiwara T. (2005): Endocytosis and degradation of BOR1, a boron transporter of *Arabidopsis thaliana*, regulated by boron availability. Proceedings of the National Academy of Sciences of the United States of America, 102: 12276–12281.

Tanaka H. (1967): Boron absorption by crop plants as affected by other nutrients of the medium. Soil Science and Plant Nutrition, 13: 41–44.

Varga L., Ložek O., Ducsay L., Kováčik P., Lošák T., Hlušek J. (2010): Effect of topdressing with nitrogen and boron on the yield and quality of rapeseed. Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis, 58: 391–398.

Wang Y., Krogstad T., Clarke J.L., Hallama M., Øgaard A.F., Eich-Greatorex S., Kandeler E., Clarke N. (2016): Rhizosphere organic anions play a minor role in improving crop species' ability to take up residual phosphorus (P) in agricultural soils low in P availability. Frontiers in Plant Science, 7: 1664.

Wang Y.L., Almvik M., Clarke N., Eich-Greatorex S., Øgaard A.F., Krogstad T., Lambers H., Clarke J.L. (2015): Contrasting responses of root morphology and root-exuded organic acids to low phosphorus availability in three important food crops with divergent root traits. AoB Plants, 7: plv097.

Wear J.I., Patterson R.M. (1962): Effect of soil pH and texture on the availability of water-soluble boron in the soil. Soil Science Society of America Journal, 26: 344–346.

Yamanouchi M. (1980): The effects of phosphorus, potassium, calcium, magnesium and iron on the absorption and translocation of boron in several crops grown at a high concentration of boron. Journal of the Science of Soil and Manure, 51: 126–130.

Received on August 15, 2018
Accepted on October 2, 2018
Published online on October 22, 2018