

Institute of Plant Nutrition and Soil Science  
Kiel University

**Boron Uptake, Translocation and Distribution in Oilseed rape  
(*Brassica napus* L.): Effect of Application Methods,  
Boron Status of Plants and Nitrogen Forms**

**Dissertation**

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# **Chapter 1**

## **General Introduction**

## Chapter 1. General Introduction

### 1.1. Boron nutrition, uptake and transport

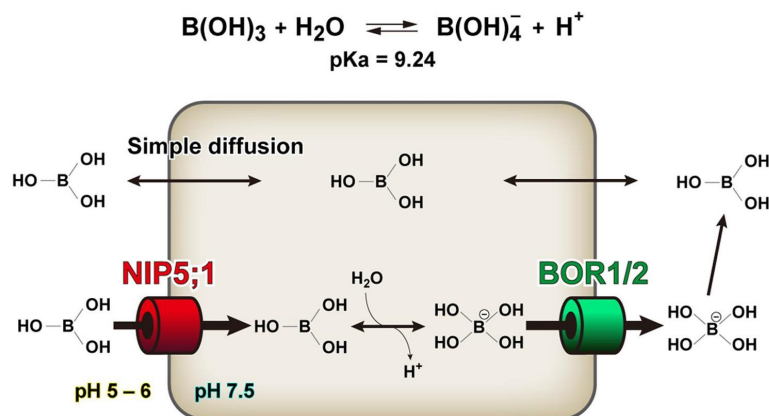
Boron (B) is a unique element since it has chemical and physical properties that lie between metals and non-metals. Boron has different isotopes and the most abundant among these are  $^{11}\text{B}$  (80.1 atom%) and  $^{10}\text{B}$  (19.9 atom%) (Uluisik et al., 2018). Boron in the plants and in the soil exists mostly as an uncharged boric acid ( $\text{B}(\text{OH})_3$ ) and only a small fraction occurs as borate anion ( $\text{B}(\text{OH})_4^-$ ) (Broadley M, 2012). Boric acid is a weak monobasic acid, which acts as Lewis acid with the borate anion form, their relative proportions depend upon the pH, with a pKa value of 9.24 according to the following equation:  $\text{B}(\text{OH})_3 + \text{H}_2\text{O} \leftrightarrow \text{B}(\text{OH})_4^- + \text{H}^+$ . The predominant form of B depends upon the pH, at an acidic or neutral pH value, the equilibrium is obviously shifted greatly towards boric acid. While the formation of the anion borate is only possible and geared towards soils with higher pH. This means that B adsorption is mainly pH-dependent, and boric acid is known to be the major chemical form of B taken up by plants (Broadley M, 2012).

The functions of B in the vascular plants have been established such as maintenance of the plasma membrane integrity, cell walls constitution, involvement in the biosynthesis of metabolomics, stimulation of the reproductive organs and eventually improvement in the quality of seeds (Cakmak et al., 1995; Goldbach & Wimmer, 2007; Broadley M, 2012; Wimmer et al., 2015; Wimmer et al., 2019). In addition, B is also involved in the synthesis of nucleic acid, phenolic metabolism, carbohydrate biosynthesis and translocation, pollen tube development, and root elongation (Brown et al., 2002; Landi et al., 2019; Wimmer et al., 2019). The main symptoms of B deficiency in the plants are reduced leaf expansion and inhibited roots elongation, and finally reduced yield and quality of crops (Gupta, 1980; Brown et al., 2002; Goldbach et al., 2007).

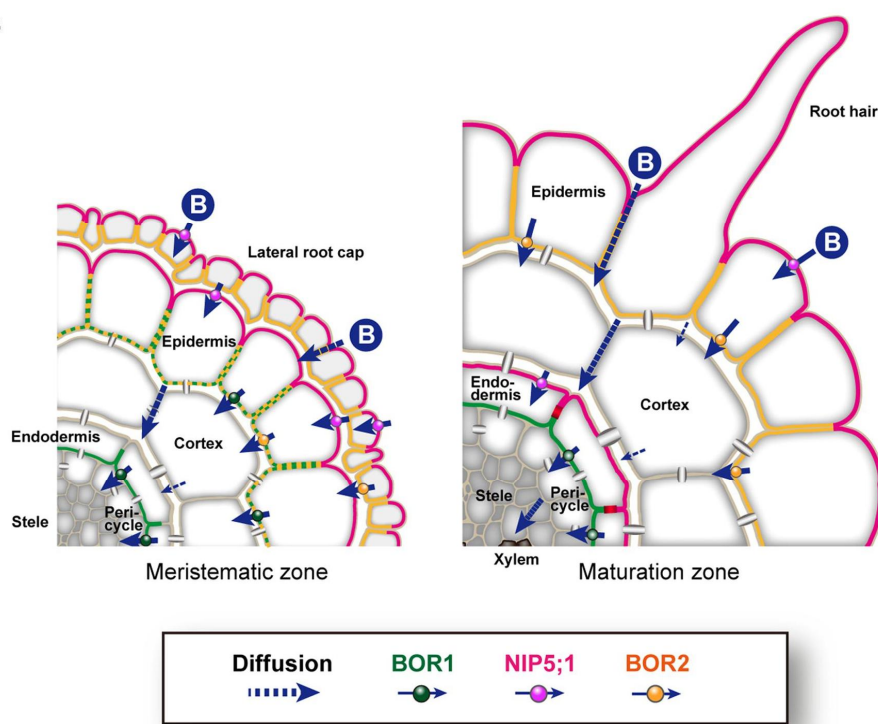
Boron is taken up by plant roots from the soil as boric acid, which is a small and uncharged molecule (Marschner, 2012). There are three uptake pathways associated with B uptake by plants namely (Fig. 1): passive diffusion, facilitated uptake via channels and active uptake via boron transporters (Brown et al., 2002; Miwa & Fujiwara, 2010; Yoshinari & Takano, 2017). It is evident that under sufficient or high B supply, the passive diffusion of boric acid is dominant for the uptake of B through plasma membrane. Several factors influence the passive uptake of boric acid into the plants such as the transpiration rate, the permeability of plasma membrane and the concentration of boric acid in the soil solution (Dordas & Brown, 2000;

Brown et al., 2002; Wimmer et al., 2015). Plants B requirements can be compensated under limited B supply via facilitated uptake through channels. These channels belong to the Major Intrinsic Proteins (MIPs) and have been discovered as a major component for facilitating B uptake in plants. The facilitated uptake is known to occur under conditions of low B supply (Dordas & Brown, 2000). Several boric acid channels involved in B uptake under limited B supply have been discovered recently. One of these channels is the NIP5;1, which facilitates B influx into roots cells of *Arabidopsis* (Takano et al., 2006). Takano et al. (2008) identified yet another channel namely NIP6;1 which is responsible for the xylem-phloem transfer of B and B distribution within the shoot. In addition, the plants use BOR1 exporter for the export of borate into the xylem under limited B supply as discovered by Takano et al. (2002). It is known that the main function of BOR1, which is located predominantly in the root pericycle cells, is B loading in the form of borate into the xylem and its transport against the concentration gradient (Takano et al., 2002; Yoshinari & Takano, 2017). Takano et al. (2002) found that the B concentration in xylem saps of the wild type plant was higher than the mutant and attributed this fact to the relatively higher expression of BOR1 in former plant species. The same group of authors (Takano et al. 2005) established that the expression of BOR1 in *A. thaliana* is also post-transcriptionally regulated. They also found that at higher concentrations of B, BOR1 is degraded, and this B concentration-dependent degradation of BOR1 is essential to prevent overaccumulation of B in shoots at higher B levels in soils. Boron uptake by roots and its translocation from roots to shoot via xylem are the two main processes that ensure the transport of B to the specific tissues and support the plant growth when the availability of B in the soil is limited (Takano et al., 2006; Miwa & Fujiwara, 2010; Reid, 2014). Recently, some isoforms of BOR1 and NIP5;1 have been identified in the crops, including *BnaBOR1*'s and *BnaNIP5;1* in *Brassica napus* which are involved in B absorption and transport of B into roots and shoots (Yang et al., 2013; Hua et al., 2016; Wang et al., 2017; Diehn et al., 2019).

A



B



**Fig. 1.** Three B transport mechanisms in plants - A model. **(A)** 1) Simple diffusion of boric acid, 2) Facilitated diffusion of boric acid via NIP5;1, 3) Borate export via BOR1/2. **(B)** Under low-B conditions, cell-type-specific expression patterns of NIP5;1, BOR1, and BOR2 in *Arabidopsis* roots (Yoshinari & Takano, 2017).

Boron, like many other elements, is not uniformly distributed within the various organs of the plants. The concentration of B in different organs of plants also depend on the age of the species and organ of the plants. There is clear evidence of B mobility within plant tissues (Brown & Shelp, 1997). On one hand, higher B concentration was also observed in younger leaves in comparison with older leaves, and this phenomenon was an indication of B mobility in some species. On the other hand, B concentration in mature leaves has been found to be higher than that of younger leaves, and this has been suggested as possible evidence of B immobility in

plants (Brown & Shelp, 1997). The phloem mobility of B is highly variable among the plant species and depend on the binding of B with sugar alcohols such as sorbitol or mannitol (Brown & Shelp, 1997; Will et al., 2011). In fact, in the species having the high translocation of sugar alcohols in the phloem, borate can bind with the sugar alcohols and can easily remobilize from older leaves to the young leaves (Brown & Hu, 1996; Eichert & Goldbach, 2010). Moderate B mobility in the phloem of oilseed rape and wheat is also related to the translocation of sucrose in the phloem (Stangoulis et al., 2010).

### 1.2. Boron demand of oilseed rape (*Brassica napus* L.)

Oilseed rape is one of the most cultivated worldwide oilseed crops. It requires a high amount of B supply for growth and is highly sensitive to B deficiency and most responsive to external B supply (Shorrocks, 1997). However, oilseed rape is often grown in soils with low B availability such as alkaline soils or sandy soils under rainfall conditions. The first response of oilseed rape to B deficiency is reduced growth, which finally ends at a significant decrease in the yield (Gupta, 1980). Oilseed rape has a high demand of B for growth, which depends on the growth stages. In general, under limited B supply, roots and shoots growth is interrupted through inhibition of the cell division and cell enlargement (Dell & Huang, 1997). Moreover, B deficiency in oilseed rape led to “flowering without seed setting” and finally yield losses (Zhang et al., 2014; Eggert & von Wiren, 2016). Boron requirement for most of the *B. napus* cultivars is higher than 0.5 mg B kg<sup>-1</sup> soil, which is more than the concentrations commonly existing in many agricultural soils (Shorrocks, 1997).



**Fig. 2.** The symptoms of B deficiency in oilseed rape

### 1.3. Foliar B fertilizer

Oilseed rape plants growing in the soil with high soil pH, low B availability, or in drought periods during the growth stages are particularly at risk of obtaining insufficient B supply. The B deficiency in plants can be alleviated by the application of B fertilizers, as soil fertilization or foliar spray (Shorrocks, 1997; Fageria et al., 2009; Fernández et al., 2013). Foliar applications may sometimes be more effective than applying boron to the soil. Studies have



shown that the foliar application of B fertilizers increase crop yields (Noreen et al., 2018) through improving B concentration in leaves and fruiting parts of the plants (Eichert & Goldbach, 2010). In some restricted condition, foliar application of B can be more effective and feasible than soil application, since foliar-applied B can be directly taken up by the leaves (Shorrocks, 1997). The field trials with *Brassica oleracea* have confirmed that foliar B fertilized plants produced significantly higher yield than soil B fertilized plants under B deficiency (Shelp et al., 1996). The authors assumed that foliar-applied B has greater mobility in plants, possibly due to access to a transport route within the plant inaccessible for soil-applied B under unfertilized conditions. Shorrocks (1997) have reported that B requirement for the growth of oilseed rape is 320 g ha<sup>-1</sup>. Three-year field experiments in Germany with *B. napus* receiving foliar application 250 g B ha<sup>-1</sup> together with Epsom salt twice annually showed 3-7% higher yield (Orlovius, 2001). At any specific development stage, the major advantage of the foliar application is that it can be performed very quickly following diagnosis of B deficiency (Fernández & Eichert, 2009). Jankowski et al. (2016) reported beneficial effects of the foliar application of B at 150-300 g ha<sup>-1</sup> on the nutritional value of winter oilseed rape seeds.

A narrow range between the deficient and toxic concentration of B implies that specialized soil- and crop-specific knowledge is required to use B fertilizers. A danger of toxic effects of B are associated with repeated fertilization for several years, application of B to seeds or placement of fertilizer near the seeds and for this reason, these treatments are not recommended (Shorrocks, 1997). The B content of irrigation water more than 10 mg B L<sup>-1</sup> can lead to B toxicity in semiarid and arid areas (Broadley M, 2012). Since oilseed rape is relatively tolerant to B toxicity, other crops of the rotation may show toxicity at a specific fertilizer application rate which is not toxic to oilseed rape. Chlorosis at leaf tips and margins, followed by the gradual development of necrotic spots on the entire leaves, are the main symptoms of B toxicity in oilseed rape (Orlovius, 2003; Savic et al., 2013).

The chemical forms of B used for foliar fertilizer, such as boric acid and Solubor (Na borate), are highly soluble and effective (Wimmer et al., 2015). These both forms of B are uncharged and have a small molecule, and hence can pass easily through cuticular membranes. However, depending upon crop type, differences may exist in the relative absorption efficiency of these two B forms. Cotton receiving foliar applications of boric acid and sodium borate showed different concentration of B in tissues, but soybean did not show such fertilizer-specific differences (Guertal et al., 1996). Hence, foliar application is an appropriate approach for correcting its deficiency.

#### **1.4 Uptake of B in relation to nitrogen and soil pH**

Several factors are known to affect B uptake in plants, e.g. soil pH, organic matter, texture and salinity. Among these, soil pH is the most important factor affecting the availability and uptake of B by plants. This is because the equilibrium between boric acid and borate in the media (soil) is pH-dependent, with a pKa of 9.24 (Wimmer et al., 2015). It has been established that at soil pH value below 7, more than 99% of the B present in the form of boric acid, which is readily available for plant uptake. Boron occurs as borate anion in alkaline soil, and is known to be adsorbed on organic matter, clay minerals and oxides (Wimmer et al., 2015). Tsadilas et al. (2005) reported that soil liming increased soil pH and significantly reduced B availability with a strong negative correlation with the soil pH. However, these authors observed non-significant effect of the N-forms on pH of the soil. Reynolds et al. (1987) reported that N forms had minimal but very essential effect on final soil pH and that the total amount of B in mature leaves could be attributed to the pH of the soil, which reduced the B concentration about five-folds between pH range of 7.0-7.4.

As elaborated earlier, the cationic and anionic properties of nitrogen (N-forms) determines how the pH of nutrient solution would be, and this might have an opposite effect on B uptake under low or adequate/high B supply. Since N supply in the form of  $\text{NO}_3^-$  leads to a pH increase in the rhizosphere, one could expect enhanced B uptake under conditions of low or limited B supply, where active B uptake as borate is relevant. On the other hand, N supply in the form of  $\text{NH}_4^+$  results in a reduction of rhizosphere pH, and therefore B uptake could be enhanced under adequate or high B supply, when passive and facilitated uptake pathways of boric acid are more important.

Although, B can solely be taken up by plants but its uptake by plants has known to be enhanced by other macronutrients especially nitrogen. Gupta (1980) reported that the uptake of B was markedly affected by the presence of other nutrients. It was established that the addition or supply of some nutrients such as nitrogen reduces the toxicity effects of high B levels. It has also been proposed by (Gupta, 1980) that among the macronutrients, N has been known to be the most vital in its effects on B uptake by plants. These authors observed that the degree of benefits gained in barley and wheat was related to the N rates and type of plant species. They further showed in cereals that increasing rates of N supplied to an initially deficient soil, significantly decreased B concentration of boot stage tissues in a greenhouse experiment. Irrespective of the N effects on B uptake, N supply has been found to alleviate B toxicity in crops. It has been evident that certain plant nutrients especially N could reduce the harmful effects of high B levels if not totally eradicated (Alt & Schwarz, 1973). Koohkan and Maftoun

(2016) found out that shoot B concentration was markedly affected by B and addition of N to the soil. They also found that N and B interaction was significant for shoot B concentration. The question whether pH and N-forms have direct effect on the uptake of B by plants has been highlighted by McCrimmon and Karnok (1992). They reported a positive relationship between pH and B concentration when different N-forms were supplied to cut, uncut and verdure Creeping bentgrass. The changes in pH observed in the different N-forms reflected in the micronutrient (e.g. B) concentrations of root and verdure tissues.

### **1.5 Objective**

In the first part of this study, we investigated the growth and distribution of B in oilseed rape (*Brassica napus*, cv. Alpaga) grown in the nutrient solution. The correlation between shoot B concentration and B in the nutrient solution was developed and the levels of external B concentration below and above which growth is limited were determined (Chapter 2).

Although the practice of foliar B application has been used worldwide, little research has been carried out on the comparative physiological and growth responses of B-deficient *B. napus* plants resupplied with B via roots and leaves. Therefore, in the second part of the thesis, we investigated the effect of continuous B resupply via roots and one-time foliar application on the growth, B concentration in different tissues and physiological behaviour of B-deficient brassica plants under hydroponics condition. Different levels of foliar-applied B were compared for their effectiveness and any possible toxicity. Moreover, we also investigated the expression levels of boric acid channel (NIP5;1), which controls B uptake and transport under limited B condition via facilitated diffusion of B to the roots cells, and of borate transporter BOR1 which control active uptake and transport of B through the xylem in roots and leaves of the plants (Chapter 3).

Having known that the dominant B uptake pathway under optimum B supply is the passive diffusion of boric acid, while under low B supply, B requirements can be met through facilitated and/or active uptake of boric acid or borate, we hypothesised that N forms might affect B uptake by plants depending on the B supply level. More specifically, in the third part of this study, we investigated if the nitrate-induced increase in pH would reduce B uptake especially at optimum B supply, where passive diffusion dominates. Additionally, we tested the question whether  $\text{NH}_4^+$  and  $\text{NO}_3^-$  have opposite effects on B uptake at low B supply (Chapter 4).

## 1.6 References

- Alt, D., & Schwarz, W. (1973). Boron toxicity, boron uptake and boron distribution in young cucumber plants under influence of N-form *Plant and Soil*, 39(2), 277-283.
- Broadley M, B. P., Cakmak I, Rengel Z, Zhao F. (2012). Function of Nutrients: Micronutrients. In: Marschner's Mineral Nutrition of Higher Plants. In r. Edition (Ed.), (pp. 191 –248). Academic Press, San Diego.
- Brown, P. H., Bellaloui, N., Wimmer, M. A., Bassil, E. S., Ruiz, J., Hu, H., Pfeffer, H., Dannel, F., & Romheld, V. (2002). Boron in plant biology *Plant Biology*, 4(2), 205-223.
- Brown, P. H., & Hu, H. N. (1996). Phloem mobility of boron is species dependent: Evidence for phloem mobility in sorbitol-rich species *Annals of Botany*, 77(5), 497-505.
- Brown, P. H., & Shelp, B. J. (1997). Boron mobility in plants *Plant and soil*, 193(1), 85-101.
- Cakmak, I., Kurz, H., & Marschner, H. (1995). Short-term effects of boron, germanium and high light intensity on membrane permeability in boron deficient leaves of sunflower *Physiologia Plantarum*, 95(1), 11-18.
- Dell, B., & Huang, L. (1997). Physiological response of plants to low boron *Plant and Soil*, 193(1), 103-120.
- Diehn, T. A., Bienert, M. D., Pommerrenig, B., Liu, Z. J., Spitzer, C., Bernhardt, N., Fuge, J., Bieber, A., Richet, N., Chaumont, F., & Bienert, G. P. (2019). Boron demanding tissues of *Brassica napus* express specific sets of functional Nodulin26-like Intrinsic Proteins and BOR1 transporters *Plant Journal*, 100(1), 68-82.
- Dordas, C., & Brown, P. H. (2000). Permeability of boric acid across lipid bilayers and factors affecting it *J Membr Biol*, 175(2), 95-105.
- Eggert, K., & von Wiren, N. (2016). The role of boron nutrition in seed vigour of oilseed rape (*Brassica napus* L.) *Plant and Soil*, 402(1-2), 63-76.
- Eichert, T., & Goldbach, H. E. (2010). Transpiration rate affects the mobility of foliar-applied boron in *Ricinus communis* L. cv. Impala *Plant and soil*, 328(1), 165-174.
- Fageria, N. K., Filho, M. P. B., Moreira, A., & Guimarães, C. M. (2009). Foliar Fertilization of Crop Plants *Journal of Plant Nutrition*, 32(6), 1044-1064.
- Fernández, V., & Eichert, T. (2009). Uptake of hydrophilic solutes through plant leaves: current state of knowledge and perspectives of foliar fertilization *Critical Reviews in Plant Sciences*, 28(1-2), 36-68.
- Fernández, V., Sotiropoulos, T., & Brown, P. (2013). Foliar Fertilization - Scientific Principles and Field Practices. International Fertilizer Industry Association (IFA), Paris, France.
- Goldbach, H. E., Huang, L., & Wimmer, M. A. (2007). Boron Functions in Plants and Animals: Recent Advances in Boron Research and Open Questions. In *Advances in plant and animal boron nutrition* (pp. 3-25).

- Goldbach, H. E., & Wimmer, M. A. (2007). Boron in plants and animals: Is there a role beyond cell-wall structure? *Journal of Plant Nutrition and Soil Science*, 170(1), 39-48.
- Guertal, E., Abaye, A., Lippert, B., Miner, G., & Gascho, G. (1996). Sources of boron for foliar fertilization of cotton and soybean *Communications in soil science and plant analysis*, 27(15-17), 2815-2828.
- Gupta, U. C. (1980). Boron nutrition of crops *Advances in agronomy*, 31, 273-307.
- Hua, Y., Zhou, T., Ding, G., Yang, Q., Shi, L., & Xu, F. (2016). Physiological, genomic and transcriptional diversity in responses to boron deficiency in rapeseed genotypes *J Exp Bot*, 67(19), 5769-5784.
- Jankowski, K. J., Hulanicki, P. S., Krzebietke, S., Zarczynski, P., Hulanicki, P., & Sokolski, M. (2016). Yield and quality of winter oilseed rape in response to different systems of foliar fertilization *Journal of Elementology*, 21(4), 1017-1027.
- Koohkan, H., & Maftoun, M. (2016). Effect of nitrogen- boron interaction on plant growth and tissue nutrient concentration of canola (*Brassica napus* L.) *Journal of Plant Nutrition*, 39(7), 922-931.
- Landi, M., Margaritopoulou, T., Papadakis, I. E., & Araniti, F. (2019). Boron toxicity in higher plants: an update *Planta*, 250(4), 1011-1032.
- Marschner, P. (2012). *Marschner's Mineral Nutrition of Higher Plants*. Elsevier Academic Press Inc.,
- McCrimmon, J. N., & Karnok, K. J. (1992). Nitrogen form and the seasonal root and shoot response of creeping bentgrass *Communications in Soil Science and Plant Analysis*, 23(9-10), 1071-1088.
- Miwa, K., & Fujiwara, T. (2010). Boron transport in plants: co-ordinated regulation of transporters *Annals of Botany*, 105(7), 1103-1108.
- Noreen, S., Fatima, Z., Ahmad, S., & Ashraf, M. (2018). Foliar application of micronutrients in mitigating abiotic stress in crop plants. In *Plant nutrients and abiotic stress tolerance* (pp. 95-117). Springer.
- Orlovius, K. (2001). Effect of foliar fertilisation with magnesium, sulfur, manganese and boron to sugar beet, oilseed rape, and cereals. In *Plant Nutrition* (pp. 788-789). Springer.
- Orlovius, K. (2003). Oilseed rape *Fertilizing for High Yield and Quality, Bulletin*, 16.
- Reid, R. (2014). Understanding the boron transport network in plants *Plant and Soil*, 385(1-2), 1-13.
- Reynolds, S. B., Scaife, A., & Turner, M. K. (1987). Effect of nitrogen form on boron uptake by cauliflower *Communications in Soil Science and Plant Analysis*, 18(10), 1143-1154.
- Savic, J., Marjanovic-Jeromela, A., Glamoclija, D., & Prodanovic, S. (2013). Oilseed rape genotypes response to boron toxicity *Genetika-Belgrade*, 45(2), 565-574.

- Shelp, B. J., Vivekanandan, P., Vanderpool, R. A., & Kitheka, A. M. (1996). Translocation and effectiveness of foliar-fertilized boron in broccoli plants of varying boron status *Plant and Soil*, 183(2), 309-313.
- Shorrocks, V. M. (1997). The occurrence and correction of boron deficiency *Plant and soil*, 193(1-2), 121-148.
- Stangoulis, J., Tate, M., Graham, R., Bucknall, M., Palmer, L., Boughton, B., & Reid, R. (2010). The Mechanism of Boron Mobility in Wheat and Canola Phloem *Plant Physiology*, 153(2), 876-881.
- Takano, J., Miwa, K., & Fujiwara, T. (2008). Boron transport mechanisms: collaboration of channels and transporters *Trends in Plant Science*, 13(8), 451-457.
- Takano, J., Miwa, K., Yuan, L. X., von Wiren, 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(34), 12276-12281.
- Takano, J., Noguchi, K., Yasumori, M., Kobayashi, M., Gajdos, Z., Miwa, K., Hayashi, H., Yoneyama, T., & Fujiwara, T. (2002). Arabidopsis boron transporter for xylem loading *Nature*, 420(6913), 337-340.
- Takano, J., Wada, M., Ludewig, U., Schaaf, G., von Wiren, N., & Fujiwara, T. (2006). The Arabidopsis major intrinsic protein NIP5;1 is essential for efficient boron uptake and plant development under boron limitation *Plant Cell*, 18(6), 1498-1509.
- Tsadilas, C. D., Kassioti, T., & Mitsios, I. K. (2005). Influence of liming and nitrogen forms on boron uptake by tobacco *Communications in Soil Science and Plant Analysis*, 36(4-6), 701-708.
- Ulusik, I., Karakaya, H. C., & Koc, A. (2018). The importance of boron in biological systems *Journal of Trace Elements in Medicine and Biology*, 45, 156-162.
- Wang, S. L., Yoshinari, A., Shimada, T., Hara-Nishimura, I., Mitani-Ueno, N., Ma, J. F., Naito, S., & Takano, J. (2017). Polar Localization of the NIP5;1 Boric Acid Channel Is Maintained by Endocytosis and Facilitates Boron Transport in Arabidopsis Roots *Plant Cell*, 29(4), 824-842.
- Will, S., Eichert, T., Fernández, V., Möhring, J., Müller, T., & Römheld, V. (2011). Absorption and mobility of foliar-applied boron in soybean as affected by plant boron status and application as a polyol complex *Plant and soil*, 344(1), 283-293.
- Wimmer, M. A., Abreu, I., Bell, R. W., Bienert, M. D., Brown, P. H., Dell, B., Fujiwara, T., Goldbach, H. E., Lehto, T., Mock, H. P., von Wiren, N., Bassil, E., & Bienert, G. P. (2019). Boron: an essential element for vascular plants A comment on Lewis (2019) 'Boron: the essential element for vascular plants that never was' *New Phytologist*, 6.
- Wimmer, M. A., Goldberg, S., & Gupta, U. C. (2015). Boron. In *Barker A (Ed), Pilbeam D (Ed) Handbook of Plant Nutrition* (2<sup>nd</sup> ed., pp. 305-346). CRC Press.

- Yang, L., Zhang, Q., Dou, J. N., Li, L., Guo, L. F., Shi, L., & Xu, F. S. (2013). Characteristics of root boron nutrition confer high boron efficiency in Brassica napus cultivars *Plant and Soil*, 371(1-2), 95-104.
- Yoshinari, A., & Takano, J. (2017). Insights into the Mechanisms Underlying Boron Homeostasis in Plants *Frontiers in Plant Science*, 8, 8, Article 1951.
- Zhang, D. D., Zhao, H., Shi, L., & Xu, F. S. (2014). Physiological and genetic responses to boron deficiency in Brassica napus: A review *Soil Science and Plant Nutrition*, 60(3), 304-313.

## **Chapter 2**

### **Growth and distribution of boron in oilseed rape (*Brassica napus* L.) as affected by boron supply**

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to be submitted



**Growth and distribution of boron in oilseed rape  
(*Brassica napus* L.) as affected by boron supply**

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### **Abstract**

Oilseed rap (*Brassica napus* L.) is one of the most important oilseed crops and has relatively high B requirements for growth. In this study, the hydroponic experiment was performed to determine the critical B requirement and B distribution in *B. napus*. The plants were grown for four weeks at a range of B levels (0.25 to 1000  $\mu\text{M}$ ) supplied in the nutrient solution. Results showed significant differences in the root and shoot dry matters and B accumulation in these tissues among the supplied B levels. Severe visible symptoms of B deficiency were observed on the leaves at levels lower than 1  $\mu\text{M}$  B and of toxicity at 1000  $\mu\text{M}$  B in the nutrient solution. The maximum shoot and root dry matters were recorded at 25  $\mu\text{M}$  B in the nutrient solution. The plants supplied with the lowest and the highest B levels produced 35% and 37% lesser shoot dry matter than those supplied with 25  $\mu\text{M}$  B, while the corresponding decreases in the root dry matter were 48% and 36%, respectively. The critical concentration of B, the lowest concentration at which plants produced 90% of the maximum shoot dry matter, was proved to be 1  $\mu\text{M}$  B for oilseed rape. At this level of external B supply, B concentration in the shoot was 26.9  $\text{mg kg}^{-1}$  DM. It was found that with the increase in B level in the nutrient solution, the relative distribution of B between roots and shoots shifted in favour of shoots.

Keywords: Boron, *Brassica napus*, nutrient solution, translocation factor, B distribution

### **Abbreviations**

B, boron; DM, dry matter; ICP-MS, Inductively Coupled Plasma Mass Spectrometer

## Introduction

Boron (B) is a micronutrient for the growth and development of vascular plants (Broadley M, 2012). Boric acid ( $B(OH)_3$ ), an uncharged molecule, is known to be the major chemical form of B taken up by plants (Broadley M, 2012). About 96% of B in the soil as well as in plants exists in the form of boric acid ( $B(OH)_3$ ) and only a small fraction occurs as borate anion ( $B(OH)_4^-$ ) (Wimmer et al., 2015). Research has established that B is the only element that is taken up by plants as a neutral molecule. In acidic soil, the relative proportion of boric acid over borate anion will be higher than that in neutral or alkaline soil, and hence the proportional uptake of B will be higher in the former case. Subsequent to its uptake by roots, B is translocated to the shoots and reproductive structures by transpiration stream via vascular tissues (Brown & Shelp, 1997).

The minimum concentration of a nutrient in a specified plant part required to produce 90% of the maximum biomass or yield of a plant species is called “critical concentration” (Smith, 1986). The B requirements of plants generally depend on two main factors, the plant species and the stage of development (Broadley M, 2012). Among plants species, the oilseed plants have the highest B requirements, and B requirement at reproductive stages is higher than that at vegetative stages of growth. Dell and Huang (1997) suggested that the apparent higher B requirement during the reproductive stage of growth arise from the fact that low transpiration rate at this stage reduces B supply to the reproductive tissues which are usually located at the far-ends of plants.

A number of studies have diagonalized critical B concentration for oilseed rape, but these vary widely in value, and most of them are specific with respect to sampling time, plant part sampled and the fraction of B analysed in plants tissue. For example, Huang et al. (1996) estimated that the critical concentration for B deficiency is 10-14 mg B kg<sup>-1</sup> dry matter in the youngest opened leaf and 6-8 mg B kg<sup>-1</sup> dry matter in the youngest mature leaves. On the other hand, (Rashid et al., 1994) reported critical B concentration to be 32 mg kg<sup>-1</sup> in whole shoot and 38 mg kg<sup>-1</sup> in mature leaves for *B. napus* grown in calcareous soils. Bergmann (1992) observed the typical B deficiency symptoms on the leaves of *B. napus* grown in the nutrient solution containing less than 0.3 µM B. Similarly, Asad et al. (1997) used a chelating resin to keep constant B concentration in the nutrient solution and found that the plants showed severe decrease in the biomass at the nutrient solution B concentration of  $\leq 0.3$  µM.

Oilseed rape is the major global oilseed crops having high B demand for growth and seed production (Gupta, 1980). Nevertheless, oilseed rape is highly sensitive to B deficiency, which inhibits the growth of roots and shoots by limiting cell elongation and finally reduced yield

(Dell & Huang, 1997; Stangoulis et al., 2001). To achieve the maximum yield potential, the B requirement of oilseed rape is more than 0.5 mg B kg<sup>-1</sup> soil, whereas B toxicity symptoms occur at the soil B level of 5 mg kg<sup>-1</sup> soil (Shorrocks, 1997). In the present study, we investigated the growth and distribution of B in oilseed rape (*Brassica napus*, cv Alpaga) grown at a range of B levels (0.25 – 1000 µM B) in the nutrient solution. The relationship between the external B in the nutrient solution and B concentration in the shoot was developed. The levels of external B below and above which growth is limited were identified.

## 2. Material and Methods

### 2.1 Plant culture

The hydroponic experiment was conducted in the greenhouse of the Institute of Plant Nutrition and Soil Science, Kiel University, Germany. Greenhouse conditions were set as follows: 22 °C/18°C (day/night) temperature, 60% relative humidity, and 14 h d<sup>-1</sup> photoperiod from 6:00 to 20:00 with 250-320 µmol photon m<sup>-2</sup> s<sup>-1</sup> light intensity recorded by a lightmeter (Li-189, Lincoln, USA). Oilseed rape (*Brassica napus* L. cv. Alpaga) seeds, provided by Norddeutsche Pflanzenzucht Lembke (NPZ), Hohenlieth (Germany), were soaked in aerated 1 mM CaSO<sub>4</sub> solution for 24 h. Afterwards, the seeds were rinsed with distilled water and sandwiched between two layers of moist filter paper. Sandwiched seeds were transferred into a container containing 1 mM CaSO<sub>4</sub> solution and kept in dark for 4 days and later for 2 days in the light. Six days after germination, oilseed rape seedlings were transferred into plastic pots containing 4.5 L aerated quarter strength modified Hoagland nutrient solution. The concentration of the nutrient solution was increased to one-half strength on day 4 and to full strength on day 8 after transplanting. The composition of the full-strength nutrient solution was as follows: 2.0 mM Ca(NO<sub>3</sub>)<sub>2</sub>, 0.5 mM K<sub>2</sub>SO<sub>4</sub>, 0.25 mM KH<sub>2</sub>PO<sub>4</sub>, 0.325 mM MgSO<sub>4</sub>, 50 µM NaCl, 2 µM MnSO<sub>4</sub>, 0.4 µM ZnSO<sub>4</sub>, 0.4 µM CuSO<sub>4</sub>, 0.1 µM Na<sub>2</sub>MoO<sub>4</sub> and 40 µM Fe-EDTA. The B treatments, which were applied from the beginning, included a series of B levels viz. 0.25, 0.5, 1.0, 2.5, 5.0, 10.0, 25, 50, 100, 250, 500 and 1000 µM B (H<sub>3</sub>BO<sub>3</sub>) in the nutrient solution. The pH of the nutrient solution was adjusted to 6.0-6.5 daily with 0.1 M KOH or HCl throughout the growth period. Nutrient solutions were renewed every 3-4 days depending on the growth of the plants. Double deionized water (18.2 MΩ) was used for the preparation of nutrient solutions to avoid B contamination.

### 2.2. Harvesting and analytical methods

The experimental units having four replicates were arranged in a completely randomized design. The plants were harvested on day 28 after transplanting when they have got four to five fully expanded leaves. The roots and shoots were harvested separately, rinsed with deionized

water three times to remove the adhering nutrients on plant material and dried at 65°C until constant weight. The dry matters of the roots and shoots were recorded, and dried tissue samples were ground to a fine powder using a ball-mill grinder. Approximately 200 mg of dried material was digested in 10 mL of 69% HNO<sub>3</sub> (ROTIPURAN Supra for ICP, 69%) in a closed-vessel 1800 watts microwave digestion system (MARS 6 Xpress, CEM Corporation, Matthews, USA) with the settings as described in details by Jezek et al. (2015). Afterwards, the digested samples were diluted with 2% HNO<sub>3</sub> to 100 mL and stored at 4°C until further analysis. The concentration of B was determined by an inductively coupled plasma mass spectrometer (ICP-MS; Agilent 7700, Agilent Technologies Inc., USA). The translocation factor was calculated as the ratio of B concentration in the shoots to that in the roots (Wu et al., 2016).

### 2.3 Data analysis

Significant differences between treatments were determined by Duncan's test and One-way ANOVA at the level of 0.05 by using the SPSS 22.0 software. All the values in this study were means ± SE from four biological replicates. The graphs were made using Graphpad Prism 8.4.2 software.

## 3. RESULTS

### 3.1. Dry matter yield and B concentration in roots and shoots

Serve visible symptoms of B deficiency and toxicity, such as chlorosis at the leaf margin and leaf tips, were observed on *B. napus* leaves at lower and higher levels of B in nutrient solution (Fig. 1). Roots and shoots dry matter of *B. napus* significantly ( $p \leq 0.05$ ) depended upon the level of B supplied in nutrient solution (Fig. 2). Although nonsignificant differences in dry shoot matters were observed between 1.0 to 100 µM B treatments, the dry matters increased with each succeeding level till 25 µM B and started declining at and above 100 µM B in the nutrient solution. With the statistically nonsignificant difference from that obtained at 10, 50 and 100 µM B treatments, the maximum root dry matter was recorded for 25 µM B treated plants. As compared to 25 µM B, roots dry matter was reduced by 48%, 24%, 23%, and 18% at 0.25, 0.5, 1.0 and 2.5 µM B treatment, respectively. Similarly, as compared to 25 µM B, higher B levels viz. 250, 500 and 1000 µM reduced root dry matter by 21%, 22% and 36%, respectively.

Low or high B supply similarly affected shoot dry matter; however, the effect of the lowest or highest B supply on shoot dry matter was lesser severe than root dry matter. The plants supplied with the lowest and highest B levels produced 35% and 37% lesser shoot dry matter than that achieved with 25 µM B treatment (Fig. 2A). Although the shoot dry matter of the plants treated with 25 µM B tended to be higher than that produced at the preceding or succeeding levels, the

differences were statistically nonsignificant. Interestingly, the plant treated with 1  $\mu\text{M}$  B in the nutrient solution produced 90% of the maximum shoot dry matter as produced at 25  $\mu\text{M}$  B.

As expected, the increase of B supply followed a concomitant increase in B concentration in the roots and shoots of *B. napus* plants. The lowest B concentration in roots (15.3  $\text{mg kg}^{-1}$  DM) was recorded at 0.25  $\mu\text{M}$  B in the nutrient solution, whereas increasing B supply from 1.0 to 10  $\mu\text{M}$  B showed a nonsignificant increase ( $p \leq 0.05$ ) during 28 days experiment periods. However, increasing B supply from 25  $\mu\text{M}$  to 100  $\mu\text{M}$  (4-fold) and 1000  $\mu\text{M}$  (40-fold) increased root B concentration by 1.5 fold and 3.2 fold, respectively. Similarly, B concentration in the shoots increased significantly with B supply level in the nutrient solution. The plant supplied with the lowest B level in the nutrient solution, B concentration in the shoot was 12.1  $\text{mg kg}^{-1}$  DM. With the highest B supply, the concentration of B in the shoot was 4-fold higher as compared to 25  $\mu\text{M}$  B treated plants. Boron concentration in the shoots was higher than that in the roots in all B treatments, and increasing B supply further favoured this trend (Fig. 3).

Boron translocation from roots to shoots was not affected at low B supply levels (0.25 to 1  $\mu\text{M}$  B) in the nutrient solution (Table. 1). Interestingly, the translocation factor was increased by increasing the B concentration in the nutrient solution from 2.5 to 10  $\mu\text{M}$  B and then remained relatively constant till 250  $\mu\text{M}$  B. However, the plants growing under 500 and 1000  $\mu\text{M}$  B in the nutrient solution drastically increased the translocation factor, i.e. increased by 14% and 22% than the 25  $\mu\text{M}$  B treated plants. A highly positive linear relationship ( $R^2 = 0.97$ ) was found between shoot B concentration and B supply in the nutrient solution after 28 days of the experiment (Fig. 4). However, a less positive linear relationship ( $R^2 = 0.529$ ) was observed between roots B concentration and increasing B supply from 0.25 to 5  $\mu\text{M}$  B in the nutrient solution.

#### 4. Discussion

In this study, we clarified the response of *B. napus* grown with different B concentration ranging from deficiency to toxicity in the hydroponic. This is challenging because B has high mobility and it's a trace element with a very narrow range between deficiency, adequacy and toxicity. A few studies have been conducted to clarify the concentration which adequates for *B. napus* growth in the hydroponic by using B chelating resins (Asad et al., 1997; Stangoulis et al., 2001; Savic et al., 2013). With an optimistic expectation, covering a wide range of B supply from lowest (0.25  $\mu\text{M}$  B) to highest (1000  $\mu\text{M}$  B), the growth response of plants has been found to follow the optimum curve (Fig. 2). Nonetheless, visible symptoms of B toxicity on leaves of *B. napus* plants at highest B (1000  $\mu\text{M}$  B) appeared even before significant growth retardation was observed. It was found that B deficiency (0.25  $\mu\text{M}$  B) and B toxicity (1000  $\mu\text{M}$  B) severely

affected the biomass of *B. napus* (Fig. 2) compared to the optimum (25  $\mu\text{M}$ ) B supply. This finding is actually in accordance with what was reported above by Asad et al. (2002); Savic et al. (2013) was Yang et al. (2013). The maximum biomass performances of *B. napus* plants were obtained at 25  $\mu\text{M}$  B external concentrations (Fig.2). Interestingly, obtaining maximum biomass at vegetative growth in *B. napus* at this B level is slightly higher as compared with what has earlier been reported by Huang et al. (1996), Asad et al. (1997), and Stangoulis et al. (2001). The critical value indicates that *B. napus* plants supplied with different B concentrations responded very well at this concentration; it could be established that the critical B in the nutrient solution for four weeks old *B. napus* in the present work were found to be 1  $\mu\text{M}$  B. At this B level in the nutrient solution, B concentration in the shoot was 26.9  $\text{mg kg}^{-1}$  DM, which was lower than critical B concentration (32  $\text{mg kg}^{-1}$  DM ) in whole shoots of oilseed rape as determined by (Rashid et al., 1994). The reduction in growth and increase in B concentration in plant tissues as a consequence of B toxicity has been established in *B. napus* (Stangoulis et al., 2001).

As it was expected, roots and shoots B concentration were increased with the increasing level of B supply in the nutrient solution, with always higher B concentration in the shoots than in the roots (Fig. 3). After 28 days of the experiment, shoots B concentration at the lowest B supply level was 12.1  $\text{mg kg}^{-1}$  DM, which is below the critical concentration for B deficiency in oilseed rape as described by Savic et al. (2012) and Rashid et al. (1994).

The observation that roots B concentration did not significantly increase between the treatment treated with B ranging from 2.5 to 10  $\mu\text{M}$  B in the nutrient solution (Fig. 3), indicates that preferential B binding in the cell wall of the roots saturate the cell walls and decrease the ability of plant to further accumulate B in roots (Matoh, 1997; O'Neill et al., 2004). Nevertheless, shoots B concentration was increased with the increase of B supply from 2.5 to 10  $\mu\text{M}$  B, because only a part of B is bound in the cell walls of roots while the remaining free B is translocated to the shoot via transpiration stream (Matoh, 1997; Wimmer & Eichert, 2013; Funakawa & Miwa, 2015). Higher B supply levels favoured the partitioning of B into shoots, which accumulated 3-4 fold higher B than roots (Fig. 2). These findings are in agreement with Dannel et al. (1998) who showed that shoot B accumulated was higher than in the roots of sunflower.

The plants did not show any symptoms of B toxicity during the early stage of vegetative growth, the symptoms became apparent and more severe with time, when the plant kept on absorbing and accumulating more B in the leaves (Savic et al., 2013). The increase in roots and shoots dry matter of *B. napus* were accompanied by an increase in B concentration in the plant parts,

resulting in an increase in B content in the plant. Taken together, the results are consistent with the view that preferential B distribution in the shoot of *B. napus* plants is mainly influenced by transpiration, leading to higher B accumulation in the leaves via the transpiration stream, and finally resulting in B toxicity.

## **5. Conclusion**

In conclusion, this study showed that the critical concentration of B in the nutrient solution was 1  $\mu\text{M}$ , at this level, the *B. napus* produced 90% of shoots dry matter response as compared to the sufficient B supply (25  $\mu\text{M}$ ). The increasing B supplied in the nutrient solution lead to increased shoots and roots B concentration and increased B distribution in the shoots, less in the roots. The translocation factor did not affect by the moderate B supply to the nutrient solution. There was a close correlation between B concentration in the nutrient solution and B concentration in the shoots.

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## References

- Asad, A., Bell, R. W., Dell, B., & Huang, L. (1997). External boron requirements for Canola (*Brassica napus* L) in boron buffered solution culture *Annals of Botany*, 80(1), 65-73.
- Asad, A., Blamey, F. P. C., & Edwards, D. G. (2002). Dry matter production and boron concentrations of vegetative and reproductive tissues of canola and sunflower plants grown in nutrient solution *Plant and Soil*, 243(2), 243-252.
- Bergmann, W. (1992). Nutritional disorders of plants: visual and analytical diagnosis *Gustav Fischer Verlag Jena*.
- Broadley M, B. P., Cakmak I, Rengel Z, Zhao F. (2012). Function of Nutrients: Micronutrients. In: Marschner's Mineral Nutrition of Higher Plants. In r. Edition (Ed.), (pp. 191 –248). Academic Press, San Diego.
- Brown, P. H., & Shelp, B. J. (1997). Boron mobility in plants *Plant and soil*, 193(1), 85-101.
- 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*, 153(5), 615-622.
- Dell, B., & Huang, L. (1997). Physiological response of plants to low boron *Plant and Soil*, 193(1), 103-120.
- Funakawa, H., & Miwa, K. (2015). Synthesis of borate cross-linked rhamnogalacturonan II *Front Plant Sci*, 6, 223.
- Gupta, U. C. (1980). Boron nutrition of crops *Advances in agronomy*, 31, 273-307.
- Huang, L. B., Ye, Z. Q., & Bell, R. W. (1996). The importance of sampling immature leaves for the diagnosis of boron deficiency in oilseed rape (*Brassica napus* cv Eureka) *Plant and Soil*, 183(2), 187-198.
- Jezek, M., Geilfus, C. M., & Muhling, K. H. (2015). Glutamine synthetase activity in leaves of *Zea mays* L. as influenced by magnesium status *Planta*, 242(6), 1309-1319.
- Matoh, T. (1997). Boron in plant cell walls *Plant and Soil*, 193(1-2), 59-70.
- O'Neill, M. A., Ishii, T., Albersheim, P., & Darvill, A. G. (2004). Rhamnogalacturonan II: structure and function of a borate cross-linked cell wall pectic polysaccharide *Annu Rev Plant Biol*, 55, 109-139.
- Rashid, A., Rafique, E., & Bughio, N. (1994). Diagnosing boron deficiency in rapeseed and mustard by plant analysis and soil testing *Communications in Soil Science and Plant Analysis*, 25(17-18), 2883-2897.
- Savic, J., Marjanovic-Jeromela, A., Glamoclija, D., & Prodanovic, S. (2013). Oilseed rape genotypes response to boron toxicity *Genetika-Belgrade*, 45(2), 565-574.
- Savic, J., Romheld, V., & Nikolic, M. (2012). Oilseed rape (*Brassica napus* L.) genotypic variation in response to boron deficiency *Turkish Journal of Agriculture and Forestry*, 36(4), 408-414.

- Shorrocks, V. M. (1997). The occurrence and correction of boron deficiency *Plant and soil*, 193(1-2), 121-148.
- Smith, F. (1986). Interpretation of plant analysis; concepts and principles. In D J Reuter & J. B. Robinson (Eds.), *Plant Analysis. An Interpretation Manual* (pp. 1-12.). Inkata Press, Melbourne, Australia.
- Stangoulis, J. C., Brown, P. H., Bellaloui, N., Reid, R. J., & Graham, R. D. (2001). The efficiency of boron utilisation in canola *Functional Plant Biology*, 28(11), 1109-1114.
- Wimmer, M. A., & Eichert, T. (2013). Review: mechanisms for boron deficiency-mediated changes in plant water relations *Plant Sci*, 203-204, 25-32.
- Wimmer, M. A., Goldberg, S., & Gupta, U. C. (2015). Boron. In *Barker A (Ed), Pilbeam D (Ed) Handbook of Plant Nutrition* (2<sup>nd</sup> ed., pp. 305-346). CRC Press.
- Wu, J., Geilfus, C.-M., Pitann, B., & Mühling, K.-H. (2016). Silicon-enhanced oxalate exudation contributes to alleviation of cadmium toxicity in wheat *Environmental and Experimental Botany*, 131, 10-18.
- Yang, L., Zhang, Q., Dou, J. N., Li, L., Guo, L. F., Shi, L., & Xu, F. S. (2013). Characteristics of root boron nutrition confer high boron efficiency in Brassica napus cultivars *Plant and Soil*, 371(1-2), 95-104.

**Figure Legends:**

**Table 1.** The distribution of B, ratio shoot/root dry matter and translocation factor between shoots and roots of *B. napus* with B treatments.

**Fig. 1.** Growth performance of oilseed rape (*Brassica napus*, cv. Alpaga) grown in the hydroponic condition under low B supply for 10 days.

**Fig. 2.** Shoot (A) and root (B) dry matter yields and shoot biomass response (C) of *Brassica napus* grown with different B concentration in nutrient solution for 28 days. Dropbox represents the means ( $\pm$ SE) of four independent pot replicates. Different letters indicate significant differences between the treatments ( $P \leq 0.05$ ).

**Fig. 3.** Boron concentration in shoots (A) and roots (B), and relative distribution B in shoots and roots of *B. napus* grown with different B concentration in nutrient solution for 28 days. Bars represent means ( $\pm$ SE) of four independent pot replicates. Different letters indicate significant differences between the treatments ( $P \leq 0.05$ ).

**Fig. 4.** The linear regression of between B concentration in the nutrient solution and shoots B concentration (A), roots B concentration (B) in *Brassica napus* grown with different B concentration in nutrient solution for 28 days. Data points represent the means ( $\pm$ SE) of four independent replicates.

**Table 1.**

B in the nutrient solution ( $\mu\text{M}$ )	Shoot total B/root total B	Shoot DM/root DM	Translocation Factor (TF)
0.25	8.92	9.08	0.98
0.5	7.53	7.71	0.97
1	10.16	8.54	1.18
2.5	12.71	7.99	1.59
5	14.80	7.56	1.96
10	18.30	7.34	2.48
25	17.91	7.32	2.45
50	19.30	7.31	2.39
100	13.44	7.53	1.90
250	18.66	8.15	2.27
500	23.94	7.21	3.30
1000	30.12	7.64	3.92

**Fig. 1.**



Fig.2.

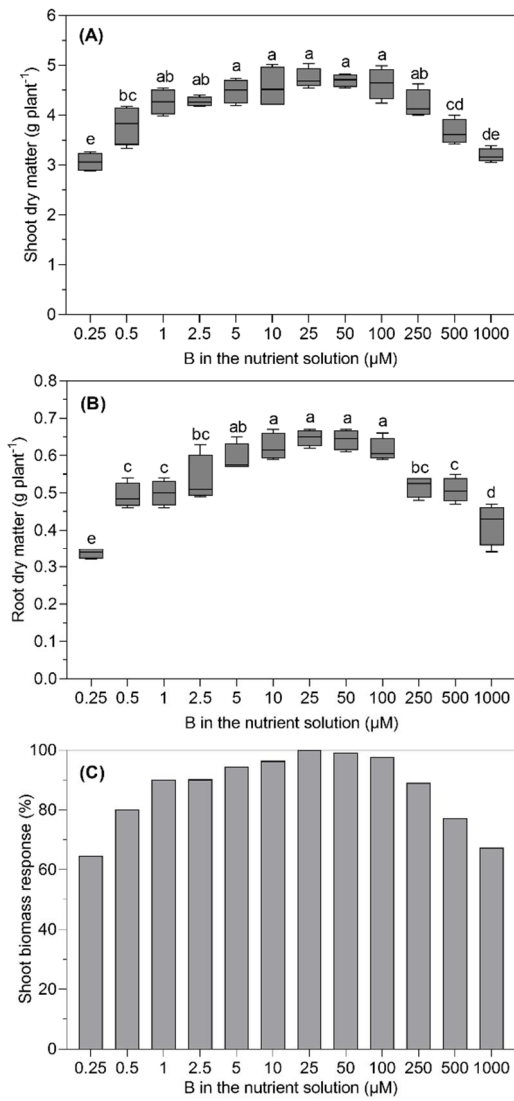


Fig.3.

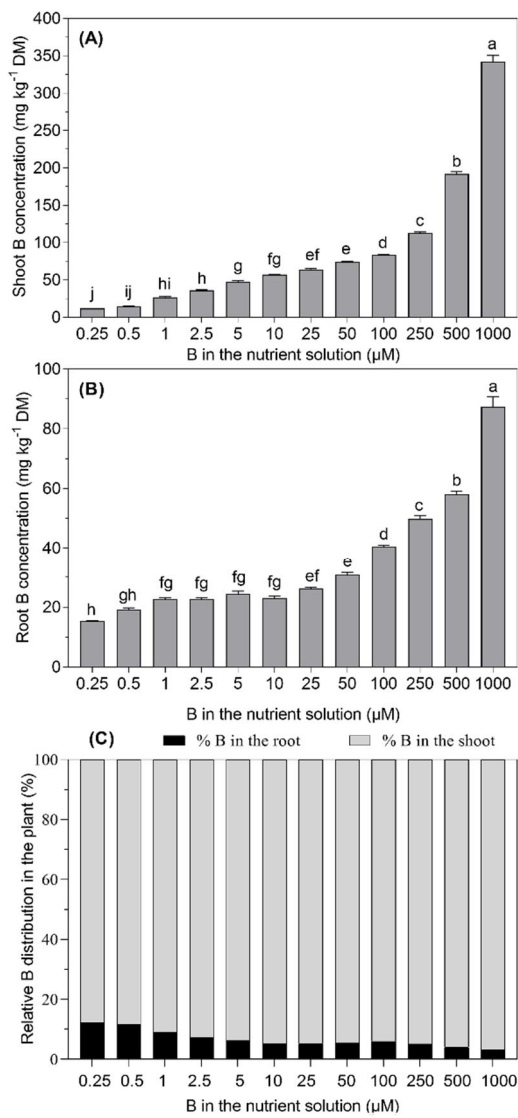
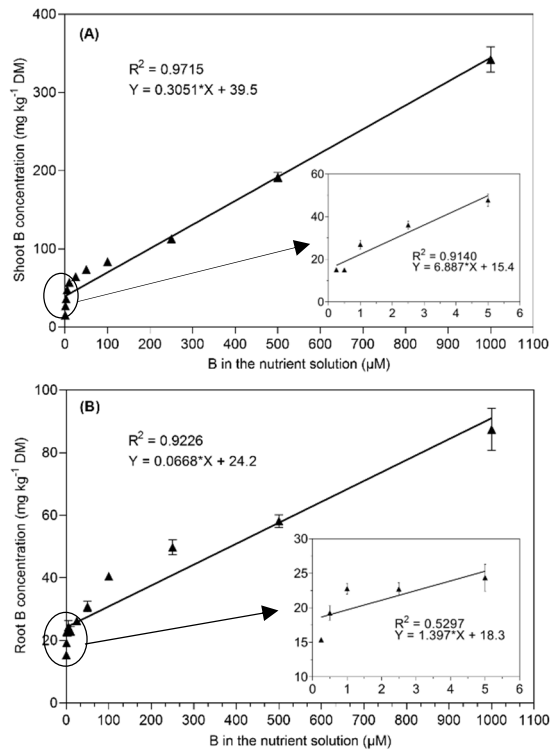


Fig. 4.





## **Chapter 3**

# **One-Time Foliar Application and Continuous Resupply via Roots Equally Improved the Growth and Physiological Response of B-Deficient Oilseed Rape**

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## **One-Time Foliar Application and Continuous Resupply via Roots Equally Improved the Growth and Physiological Response of B-Deficient Oilseed Rape**

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### Abstract

Oilseed rape (*Brassica napus* L.) is high boron (B) demanding crop, and initially, normal growing plants might show B deficiency at advanced growth stages on soils with marginal B availability. Hence, we compared the effects of B resupply via roots and leaves on growth and physiological response, and relative expression of B transporters in B-deficient oilseed rape plants. Four-week-old plants initially grown with inadequate B (1  $\mu\text{M}$  B for first two weeks and 0.25  $\mu\text{M}$  B for next two weeks) were later grown either as such with 0.25  $\mu\text{M}$  B, with 25  $\mu\text{M}$  B in nutrient solution or foliar sprayed with 7 mL of 30, 60 and 150 mM B solution  $\text{plant}^{-1}$  as boric acid. Plants grown with 25  $\mu\text{M}$  B in the nutrient solution from the beginning were included as adequate B treatment. Results showed that B resupply to B-deficient plants via roots and leaves (60 mM B) equally improved root and shoot dry matters, but not to the level of plants grown with adequate B supply. Foliar applied 150 mM B proved toxic, whereby causing leaf burn but not affecting dry matters. Resupply of B via roots increased B concentration in roots and leaves, while leaf-applied B did so only in leaves. Net carbon assimilation had a positive relationship with dry matters accumulation. Except for the highest foliar B level, B resupply via roots and leaves increased the accumulation of glucose, fructose and sucrose in leaves. Boron deficient plants showed significant upregulation of *BnaNIP5;1* in leaves and roots and of *BnaBOR1;2* in roots. Boron resupply via roots reversed the B deficiency-induced upregulation of *BnaNIP5;1* in roots, whereas the expression of *BnaBOR1;2* was reversed by both root and foliar B resupply. In leaves, B resupply by both the methods reversed the expression of *BnaNIP5;1* to the level of B adequate plants. It is concluded that B resupply to B-deficient plants via roots and leaves equally, but partially corrected B deficiency in *B. napus* grown in hydroponics.

Keywords: Boron, *Brassica napus*, B resupply, Foliar application, B transporters

## 1. Introduction

Oilseed rape (*Brassica napus* L.) is one of the major oilseed crops and used worldwide for animal and human nutrition. It is highly sensitive to B deficiency, having B requirements higher than 0.5 mg B kg<sup>-1</sup> soil [1], and shows a notable reduction in seed yield and quality under B deficient conditions [2,3]. Nevertheless, oilseed rape is often cultivated on soils with low or reduced B availability to plants by liming, high soil pH and drought periods during the main growth stages [4,5]. Therefore, a continuous B supply is important throughout the vegetative and reproductive stages for normal growth of plants. Boron deficiency appears often after hot, dry weather because less B can be absorbed by plants as the top soil dries out. The decreased availability probably results from B becoming less mobile in the mass flow to the roots and by polymerisation of boric acid [2]. Boron deficiency is considered to inhibit plant growth and reduce yield in oilseed rape [1,6,7]. Physiological responses of the plants to B deficiency relate to the instability of the cell wall and membrane, resulting in the inhibition of the root elongation and expansion of leaves [3,8]. In addition, B deficiency affects carbohydrate metabolism and nucleic acid synthesis, and thus could lead to complete inhibition of the absorption and transport of the nutrients in several cases [2,9]. The photosynthetic rate, transpiration rate, stomatal conductance, leaf gas exchange and intercellular CO<sub>2</sub> concentration are severely arrested under B deficiency [10].

The B-deficiency in plants can be alleviated by the application of B fertilizers, as soil fertilization or foliar spray [1,11,12]. Studies have shown that the foliar application of B fertilizers increase crop yields [13] through improving B concentration in leaves and fruiting parts of the plants [14]. A three-year field experiment in Germany showed that foliar application of 250 g B ha<sup>-1</sup> twice annually, on each occasion together with Epsom salt, led to increased yield of *B. napus* [15]. Jankowski, *et al.* [16] reported that leaf-application of B at 150-300 g ha<sup>-1</sup> to winter oilseed rape significantly improved the nutritional value of the seeds. Recently, Ma, *et al.* [17] reported that foliar application of 500 mg B ha<sup>-1</sup> during the early flowering stage of canola plants in the field trial showed elevated B concentration and accumulation in straw but this effect was not fully translated to seed yield.

Under conditions of restricted soil B availability, foliar application of B may be more effective than soil application because the foliar-applied B can be directly taken up via leaves and is not fixed in the soil as it happened with the soil application [1,12]. Moreover, for *Brassicaceae* and *Chenopodiaceae* species that have high B requirements than other crops, fertilization of soils with B can lead to toxicity of this micronutrient to the successive crops [18]. These come up with the assumption that foliar application of B under such conditions is a priority alternative to soil application. However, the efficiency of foliar-applied B to mitigate B deficiency depends

on B mobility through the phloem [14,19], which is linked to the possibility of B complexing with sugar alcohols and/or sucrose [20-23]. Boric acid is the main boron fertilizer, and being an uncharged molecule, it has high permeability through the plasma membranes [24]. The passive diffusion of boric acid is dominated when the available concentration of B is sufficient for the plants [25]. The uptake and transport of B in plants under limited B supply are ensured by NIP5;1, which play an important role in facilitating the diffusion of boric acid across the plasma membrane, and BOR1 which facilitate the transport of borate anion from roots to shoots via xylem loading [26-29]. Recently, Diehn, *et al.* [30], Zhang, *et al.* [31], and Yang, *et al.* [32] mentioned the functions of the specific genes *BnaNIP5;1* and *BnaBOR1;1* involved in the uptake and transport of B in *B. napus*. Although the practice of foliar B application has been used worldwide, little research has been carried out on the comparative physiological and growth responses of B-deficient *B. napus* plants resupplied with B via roots and leaves. Therefore, we investigated the effect B resupply via roots and foliage on the growth, B concentration in different tissues, physiological behaviour and the relative expression of specific genes involved in B uptake and translocation in B-deficient *B. napus* plants under hydroponics condition for two weeks. Different levels of foliar-applied B were compared for their effectiveness and any possible toxicity.

## 2. Results

### 2.1. Roots and shoots dry matters

Oilseed rape plants grown under B deficient condition showed the symptoms of B deficiency both in the foliar mature leaves (rolled down at the margins, remain green) and young leaves (stunted and curled upwards at the margins) (Fig. 1). The plants sprayed with 150 mM B solution showed a slight leaf burn at the margins. The growth of B deficient plants was substantially recovered following B resupply both via roots and leaves.

Roots dry matter of -B control plants was only 31% of that produced under +B control (Fig. 2A). Boron resupply via the roots to B deficient plants improved root dry matter by 20 % over the -B control (Fig. 2A). However, the effect of B resupply via leaves on root dry matter depended upon the B concentration in foliar spray: -B+LR<sub>30</sub> treatment did not significantly improve the root dry matter whereas -B+LR<sub>60</sub> and -B+LR<sub>150</sub> improved root dry matter by 27% and 21%, respectively. Shoots dry matter under -B control was 80% of that obtained under the +B control. Boron root resupply and leaf resupply treatments -B+LR<sub>30</sub> and -B+LR<sub>60</sub> significantly improved shoot dry matter and the magnitude of increases over -B control were 9%, 11% and 11%, respectively. Leaf resupply treatment -B+LR<sub>150</sub> did not affect shoot dry matter as compared to -B control (Fig. 2B).

### 2.2. Boron concentration

The highest B concentration in the roots ( $30 \text{ mg kg}^{-1} \text{ DM}$ ) was recorded for +B control plants, while in -B control plants, the root B concentration was only 32% of what was recorded for +B control (Fig. 3A). Boron resupply via roots (-B+RR) to B deficient plants increased B concentration up to 90% of what was recorded for +B control plants. Boron resupply via leaves did not affect the B concentration in roots. In mature leaves, both root and foliar resupply improved B concentration, and increase with the foliage resupply was dose-dependent and higher than the root resupply (Fig. 3B). Moreover, B concentration in the mature leaves with root resupply could not reach the level of +B control, which was achieved even with the lowest dose of foliage resupply. In young leaves, the B resupply treatments increased B concentration in the same manner as they did for mature leaves (Fig. 3C).

However, B concentration in young leaves reached the level or higher than +B control plants with only the highest dose (150 mM B) of foliage resupply. Boron root resupply and foliar resupply with 60 mM B had similar B concentrations in the young leaves, and these were 47 % higher than the -B control plants.

### 2.3. Photosynthetic and Transpiration Rates

The photosynthetic rate of the B-deficient plants showed a significant decline from 20 to  $13 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  involved two weeks after treatment application (Fig. 4A). However, during the same period, a negative change of only  $2 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  was recorded for +B control plants. Boron resupply to the B-deficient plants via roots, and via leaves with 30 and 60 mM B solution did not affect the photosynthetic rate of *B. napus* after 7 days of treatment application; however, the assimilation rate continued to decrease after 14 days of the treatment period. Photosynthetic rates with neither B resupply via the roots nor the leaves could reach the level of +B control plants.

Transpiration rates of the control plants did not differ between the treatments significantly throughout the whole measuring period (Fig. 4B). Boron resupply via roots or leaves with 60 mM B solution slightly increased the transpiration rate compared to the -B control. The plants resupplied via foliage with 30 and 150 mM B solution did not improve the transpiration rate during two-week of the treatment period.

### 2.4. Soluble Sugars

Concentrations of soluble sugars in both the mature leaves and young leaves were many folds higher than those recorded in the roots (Fig. 5). Boron deficiency (-B) led to a substantial increase in glucose and sucrose concentration in roots as compared to +B control plants and those resupplied via roots. In foliar B resupplied plants, glucose concentration remained the same as it was in -B control plants, but sucrose concentration decreased in the dose-dependent manner, reaching the level of +B plants with the highest dose of B foliar spray. In the mature

and young leaves, B deficiency led to a decline in the concentrations of all sugars, and the decline was more drastic in young leaves. Boron resupply via roots increased glucose and sucrose concentrations in the mature leaves while it improved the concentrations of all the soluble sugars in young leaves. In most of the cases, the improvement in the concentration of soluble sugars was maximum at the lowest rate of foliar B resupply, which progressively decreased with an increase in the rate of application and became nonsignificant or negative in some cases.

Both in mature leaves and young leaves, B resupply via foliage produced a higher increase in the concentration of fructose, whereas reverse response was recorded for sucrose concentration.

#### 2.5. Relative expression of *BnaBOR1;2* and *BnaNIP5;1* in roots and leaves

In order to study whether resupply B in the nutrient solution or foliar-applied affects the transport pathway of B under B deficiency, the expression of genes potentially involved in B uptake and transport in *B. napus* plants were investigated. As compared to B-sufficient (+B control) plants, B deficient plants (-B control) showed significant upregulation of *BnaNIP5;1* in the roots (3-fold) and leaves (1.5-fold), and of *BnaBOR1;2* in only roots (4.5-fold) (Fig. 6). In the roots, the B deficiency induced-upregulation of both *BnaBOR1;2* and *BnaNIP5;1* was significantly reversed by B resupply via roots, but the expression levels were still higher than the +B control plants. Similarly, B resupply via foliage reversed the B deficiency induced-upregulation of *BnaBOR1;2* in roots and the expression reached the level of +B control plants at the highest rate of foliar B application. However, B resupply via foliage could not reverse the B deficiency-induced upregulation of *BnaNIP5;1* in roots. In leaves, both the methods of B resupply reversed the B deficiency induced upregulation of *BnaNIP5;1* to the level of +B control plants. Interestingly, the relative expression of *BnaBOR1;2* in the leaves was neither affected by B deficiency nor by B resupply to the B deficient plants.

### 3. Discussion

In this study, we observed B deficiency symptoms (rolled down at the margins, remain green) in the leaves of B-deficient *B. napus* plants (Fig. 1). In accordance with this and as it was expected, B-deficient plants produced lower root and shoot dry matters than B-sufficient plants (Fig. 2). This is in agreement with the common fact that plants grown under B-deficient condition show reduced yield [1,16,33]. Boron concentration in the leaves of B-deficient plants was also close to or below the critical concentration of B for *B. napus* plants [34]. On the other hand, B resupply both via roots and foliage to the B-deficient plants increased root and shoot dry matters of *B. napus* (Fig. 2). Improvement in dry matter yield was concomitant with the increase in B concentration in the leaves and roots (Fig. 3) in the roots resupply treatment. Some previous studies showed that foliar B application could improve B availability in leaves and

increase the B concentration in leaves [14,17,33,35-37]. The fact that foliar B resupply increased the concentration of B in treated leaves as well as young leaves (Fig. 3B and C) implies that foliar-applied B can be transported from mature leaves to young leaves or the reproductive organs via the phloem [14,18,38-42]. According to Stangoulis, et al. [23], B has moderate phloem mobility in oilseed rape, since limited amounts of borate can be translocated to the young tissues through binding with sucrose. In this study, B resupply via roots and leaves to B-deficient plants increased shoots dry matter more than roots dry matter (Fig. 2), depicting that root growth is more sensitive to B deficiency than shoot growth. However, resupply of B via the leaves to B-deficient oilseed rape plants did not increase B concentration in the roots as compared to the -B control treatment. These results are in agreement with the results from Asad, et al. [33], who found that leaf application of B to B-deficient sunflower plants did not improve B concentration in the roots even at the highest B sprayed solution (1200 mM B). The possible reason for this could be the dilution effect since B content in the roots of the -B treatment was not significantly different as compared to foliage resupply treatments (data not shown) and the roots dry matter of foliage treatments increase together with the increasing of B resupply. The other reason could be the recycling of B from the roots to the shoot via xylem loading [14]. Our result also showed that B concentration in mature leaves of foliar B resupplied, B-deficient plants reached the level of +B control plants even with 30 mM B, and further increased with the increasing foliar-applied B level. However, B concentration in the young leaves of 30 mM B and 60 mM B treatments did not reach the level of +B control plants. Our findings are in agreement with the result from Orlovius [15] and Stangoulis, et al. [23], who demonstrated that B has moderate mobility in oilseed rape. Thus, it could be interpreted that obtaining a sufficient concentration of B in young leaves requires a relatively higher dose of B in the foliar spray. In this regard, the required level of B leaf application (150 mM B) showed a significant decrease in shoot dry matter, photosynthesis rate. The highest foliar B concentrations in the present experiment might have led to high free and unbound B in the leaves which became toxic for the plants. Boron concentration in leaves with this foliar treatment was close to the toxic levels (above 200  $\mu\text{g g}^{-1}$ ) of B in *B. napus* [43] which implies impracticability of using this B level for foliar B application. Based on these results, we might expect that in the leaves of the oilseed rape plants receiving high amount of B, which would have a larger leaves area and higher B concentration (30 – 150 mM B) the spray solution as compared to roots resupply (25  $\mu\text{M B}$ ), and the plants were harvested after two weeks of treatment. Therefore, B concentration in the mature leaves and young leaves were increased, this is more beneficially to prevent the symptoms of B deficiency in the young tissues under low B supply conditions.



In our study, B-deficient plants showed a significant decrease in photosynthesis whereas B resupply via roots (25  $\mu\text{M}$  B) and leaves (30 and 60 mM B solution) resulted in increased photosynthetic and transpiration rates (Fig. 3). Photosynthesis was improved by 15% with both B resupply via roots and foliar application of 60 mM B solution, which explains the improvement in roots and shoot dry matters under these treatments. Hossain, *et al.* [44] reported that the photosynthetic rate of oilseed rape plants was increased when soil-fertilized with 9 kg B  $\text{ha}^{-1}$  in the field experiment. This increase in transpiration rate by B resupply could have been due to the increase in the number and functioning of stomata under B-sufficient conditions [10,14,45]. Foliar spray of 150 mM B solution inhibited shoot growth, photosynthesis rate and transpiration rate as compared to the +B control treatment. In addition, we might speculated that resupply B via root or leaves, the recovered of photosynthesis might be due to the plant have more capability to expand the leaves and accumulate more chlorophyll in the leaves [46]. Boron deficiency and resupply had different effects on the concentration of soluble sugars in the plant tissues. Glucose, fructose and sucrose concentration showed a significant decrease in the leaves of *B. napus* in -B treatment as compared to +B control (Fig. 4). In line with the current study, Hegazi, *et al.* [47] and Zhao and Oosterhuis [48] reported that concentrations of sugars in the leaves were decreased under B deficiency. Resupply via the roots in nutrient solution and leaves as foliar spray (only 30 and 60 mM B solution) to B-deficient plants led to increased glucose, fructose and sucrose concentrations in leaves. This is well justified by the improved photosynthetic rate under these B resupply treatments. In this case, roots and leaf application of B to B-deficient plants had beneficial effects on shoot growth but it did not appear under the highest level of foliar B treatment (150 mM B solution).

Although it is well known that *BnaNIP5;1* and *BnaBOR1;2* genes are ubiquitously expressed and have a significant role in B absorption and translocation in *B. napus* [30,32,49,50], the effect of roots or leaf resupply on the expression of these genes in B-deficient plants was still lacking. We found that the relative expressions of both the genes in the roots of -B control plants were significantly upregulated (Fig. 6), confirming that the B in nutrient solution was not sufficient for plant growth under this treatment. Since B concentration in the roots and leaves was close to the critical level for B deficiency, *BnaNIP5;1* channel and *BnaBOR1;2* transporters were highly upregulated to enhance the absorption and transport of boric acid into the root cell and xylem to meet the B demands of plants [25,27]. Boron resupply via roots to B-deficient plants resulted in downregulation of *BnaNIP5;1* and *BnaBOR1;2* in the roots and reached close to the level of +B control plants (Fig. 6), suggesting that B resupply via roots is sufficient for passive diffusion of boric acid via the plasma membrane into the roots and transport to the shoot through transpiration stream. This agrees with the fact that resupply of B via the roots to the B-

deficient plants partially retrieved the roots and leaves B concentration, roots and shoots dry matters and physiological attributes as compared to the +B control plants. Interestingly, the relative expression of *BnaNIP5,1* in the roots of foliage treatments was upregulated even at the highest B sprayed (150 mM B), whereas the relative expression of *BnaBOR1;2* was not significantly different as compared to the +B control, except for the foliage treatment resupply with 150 mM B. In addition, the expression level of *BnaNIP5,1* and *BnaBOR1;2* were mainly upregulated in the roots of B-deficient *B. napus* plants [30,51]. The data from this study confirm the role of *BnaNIP5,1* and *BnaBOR1;2* in the uptake and distribution of B in response to B supply. However, a full reversal of the B-deficiency induced upregulation in the relative expression of *BnaNIP5,1* in leaves by B resupply via both the roots and leaves (Fig. 6) might be explained by high B concentration in the treated leaves, which was enough to support the phloem re-translocation needs of the plants by passive diffusion of B through the plasma membrane [14,22,52].

#### 4. Materials and Methods

##### 4.1. Plant cultivation and treatment application

Winter oilseed rape (*Brassica napus* L. cv. Alpage, Norddeutsche Pflanzenzucht Lembke (NPZ), Hohenlieth, Germany) was grown hydroponically in the greenhouse at day/night temperature 22 °C/18 °C and the photoperiod from 8:00 to 22:00 with light intensity of 350  $\mu\text{M photon m}^{-2} \text{s}^{-1}$  (recorded by a light meter, Li-198, Lincoln, USA). Seeds were soaked in aerated 1 mM  $\text{CaSO}_4$  solution for 24 h, germinated on a filter paper in darkness and then seedlings were exposed to light. Six-day-old, uniform-sized seedlings were transferred to plastic pots (2 plants per pot) containing 10 L of one quarter-strength nutrient solution. The strength of the nutrient solution was increased to one-half and full-strength on day 4 and 8 days of transplantation, respectively. From day 8 onward, only one plant was retained in each pot containing full strength nutrient solution. The composition of the full-strength nutrient solution was as follows: 2.0 mM  $\text{Ca}(\text{NO}_3)_2$ , 0.5 mM  $\text{K}_2\text{SO}_4$ , 0.25 mM  $\text{KH}_2\text{PO}_4$ , 0.325 mM  $\text{MgSO}_4$ , 50  $\mu\text{M}$  NaCl, 1  $\mu\text{M}$   $\text{H}_3\text{BO}_3$ , 2  $\mu\text{M}$   $\text{MnSO}_4$ , 0.4  $\mu\text{M}$   $\text{ZnSO}_4$ , 0.4  $\mu\text{M}$   $\text{CuSO}_4$ , 0.1  $\mu\text{M}$   $\text{Na}_2\text{MoO}_4$  and 40  $\mu\text{M}$  Fe-EDTA. The nutrient solution was replaced every 4<sup>th</sup> day and the pH of the nutrient solution was adjusted to  $6 \pm 0.2$  every second day using 0.1 M HCl. To avoid B contamination, double deionized water (18.2 M $\Omega$ ) was used to prepare the nutrient solution throughout the study.

For the first two weeks, the plants were grown at 1  $\mu\text{M}$  B (sufficient to support plant growth at this stage), and for the latter two weeks, B concentration in nutrient solution was reduced to 0.25  $\mu\text{M}$ . Plants showed severe B deficiency after the 4<sup>th</sup> week of growth. Boron deficient, four-week-old plants (each having seven leaves) were treated as follows: 1<sup>st</sup> group of plants was allowed to grow as such with 0.25  $\mu\text{M}$  B in the nutrient solution (negative control, -B), 2<sup>nd</sup>

group was resupplied with 25  $\mu\text{M}$  B (as  $\text{H}_3\text{BO}_3$ ) in the nutrient solution (-B+RR), while 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> groups were foliar sprayed with 7 mL of 30 (-B+LR<sub>30</sub>), 60 (-B+LR<sub>60</sub>) and 150 (-B+LR<sub>150</sub>) mM B (as  $\text{H}_3\text{BO}_3$ , equivalent to 2.25, 4.5 and 11.3 mg B plant<sup>-1</sup>, respectively). Each B spray solution was adjusted to the pH 5.3 using 0.1 N NaOH. A positive control (+B), supplied with 25  $\mu\text{M}$   $\text{H}_3\text{BO}_3$  in the nutrient solution from the beginning, was also included among the treatments. Each treatment had ten independent pot replications. Since the temperature of the greenhouse was low in the morning, foliar treatments were realized at this time to avoid immediate evaporation of the applied spray solution. Silwet® Gold (0.1%) was added as a wetting agent to enhance adherence of the spray solution to leaves. The pots were covered with polyethylene sheets while spraying the plants to avoid the contamination of the nutrient solution.

#### 4.2. Gas exchange measurements

Gas exchange measurements were carried out by a portable gas exchange system (LI-6400XT, LI-COR Biosciences, Lincoln, NE, USA). Photosynthetic photon flux density, provided by a red/blue LED light source, was adjusted to 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and ambient  $\text{CO}_2$  concentration to 400  $\mu\text{mol mol}^{-1}$  by  $\text{CO}_2$  injection. The first measurement was taken just before applying B treatments, and then every 4<sup>th</sup> day until the harvesting of plants. Measurements were taken from 10 am to 16 pm on a central 6 cm<sup>2</sup> leaf segment of the 6<sup>th</sup> leaf.

#### 4.3. Harvesting and sample preparation

Plants were harvested two weeks after applying B treatments, separated into mature leaves (lower seven leaves), young leaves (untreated leaves) and roots, and washed with deionized water. Plant samples from one set of five replicates were oven-dried at 65 °C for 72 h and used for recording dry matters and analyzing minerals and sugars. The 2<sup>nd</sup> set of five replicates was frozen in liquid N and stored at -80 °C for transporters' measurements. The dried tissue samples were ground to fine powder using a ball-mill grinder.

#### 4.4. Determination of Boron

The dried samples (approx. 200 mg each) were digested with 10 mL of 69%  $\text{HNO}_3$  (ROTIPURAN Supra for ICP, 69%) in a closed-vessels 1800 watts microwave digestion system (MARS 6 Xpress, CEM Corporation, Matthews, USA) adjusted to the following conditions: 2 min at 100 °C, 1 min at 120 °C, 20 min at 180 °C and 20 min cooling time. Afterwards, the digested samples were diluted to 100 mL and stored at 4 °C until further analysis. The concentration of B in the roots and shoots were determined by ICP-MS (Inductively Coupled Plasma Mass Spectroscopy, Agilent 7700, Agilent Technologies Inc., USA). To avoid B contamination from glassware, only plasticware were used throughout the experimentation and during B analysis.

#### 4.5. Determination of soluble sugars

Soluble sugars were extracted from 30 mg of dry material using 1.5 mL of double deionized water. The samples were placed in a shaking bath (Medingen SWB20, Dresden) preheated to 100 °C for 5 minutes. Thereafter, the samples were cooled on ice for 30 minutes and then centrifuged for 10 minutes at 12,000 rpm at 4 °C. The supernatants were collected and diluted 10 times. The samples were mixed one more time with the vortex (5 seconds) and centrifuged (12,000 rpm, 5 min). Samples were cleaned-up with chloroform and run over C18 columns (Strata®. 8B-S001-DAK, Phenomex, Torrance, CA, USA). Sucrose, glucose, and fructose concentrations were measured by anion exchange chromatography using an ICS-5000 system equipped with a Carbo Pac PA-100 column and an integrated amperometric detector (Dionex, Sunnyvale, CA, United States).

#### 4.6. Primer design and Sanger sequencing

To investigate the mRNA transcript level of *BnaBORI;2* and *BnaNIP5;1* transporters, primers were designed from sequences available on NCBI database for *BnaBORI;2* (ID GU827643) and *BnaNIP5;1* (KT899999) of *Brassica napus*. Primer3plus and primer-blast (<http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi/>, <https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi>) software were used for primer designing. Screened primer characteristics were checked and evaluated *in silico* by the online tool oligo calc (<http://biotools.nubic.northwestern.edu/OligoCalc.html>) and multiple primer analyzer tools provided by Thermo Fisher. All primer pairs were purchased from Eurofins Genomics (Ebersberg, Germany). The details of the primers are listed in Table S1. Furthermore, amplification of the correct gene was confirmed by the sequencing of the amplicon (Sanger sequencing, Institut für Klinische Molekularbiologie, Kiel).

#### 4.7. RNA extraction, reverse transcription and real-time quantitative PCR

Plant samples from the 2<sup>nd</sup> set of cultivation previously frozen in liquid nitrogen and stored at -80 °C were used for RNA extraction. Total RNA was isolated from the powdered shoot and root material with TRIZOL reagent (Invitrogen, CA, USA) according to the manufacturer's protocol. The amount of RNA was quantified using a NanoDrop spectrophotometer (ND1000, Thermo Fisher Scientific, USA), and purity was checked by gel electrophoresis. The cDNA was synthesized following the manufacturer's instructions in the Verso™ cDNA kit from Thermo Fisher Scientific as described in detail by Dinh, et al. [50].

Quantitative RT-PCR was conducted by PowerUp™ SYBR™ Green Master Mix (Applied Biosystems) with family-specific conserved primers (Table S1) on CFX96 Real-Time System (Bio-Rad Laboratories GmbH, München, Germany). For each reaction, total volume was 20 µl containing 100 nM of each primer and 2 µl of diluted cDNA templates and the sequence of

cycling conditions was as follows: 95 °C for 3 min, followed by 40 cycles of 95 °C for 10 s, 60 °C for 30 s, 72 °C for 30 s, followed by 95 °C for 10 min. Three biological replicates and two technical replicates were used for each treatment. Transcript levels of gene were normalized with endogenous control (*BnaActin* rapeseed actin gene, GenBank Accession No. AF111812) [53], and the fold expression changes of target mRNAs were determined using the  $2^{-\Delta\Delta C_t}$  method [54].

#### 4.8. Statistical analysis

The data were statistically analyzed using SPSS software version 22.0 (IBM Corp., Armonk, NY, USA). All of the data were tested for normality distribution with Shapiro-Wilk test before analysis. Reported data values in the figures are mean  $\pm$ SE of five independent pot replicates. Effects of the treatments were tested using oneway-ANOVA and following the Duncan's multiple range test of six different groups at  $p \leq 0.05$ . GraphPad Prism 8 was used for making graphs.

### 5. Conclusions

It is concluded that under controlled conditions, both root and foliar resupply of B to B-deficient oilseed rape plants can partially, retrieve the B deficiency-induced loss in dry matter production, physiological functioning and relative expression of specific B transporters. Spray solution with 60 mM B concentration can be used to get the optimum recovery response without any toxicity to the foliage of oilseed rape plants. One-time foliar resupply of B to B-deficient oilseed rape plants significantly increased B concentration in the leaves and physiological retrieval response as did by the continuous resupply via roots throughout the two-week of growth. However, a partial retrieval of the growth with individual root or foliar resupply to B-deficient plants suggest the need of dual resupply both via roots and foliage to achieve a complete growth recovery of the deficient plants.

**Supplementary Materials:** The following are available online at [www.mdpi.com/xxx/s1](http://www.mdpi.com/xxx/s1), Table S1: title,

**Author Contributions:** K. H. M. conceptualization and designed the experiments; A. Q. D. conducted the experiments; A. Q. D. and A. S. performed the qRT-PCR analysis; A. Q. D. writing original draft; A. N and K. H. M. writing, review and editing. All authors have read and agreed to the published version of the manuscript.

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## References

1. Shorrocks, V.M. The occurrence and correction of boron deficiency. *Plant and Soil* **1997**, *193*, 121-148.
2. Marschner, P. *Marschner's Mineral Nutrition of Higher Plants*; Elsevier Academic Press Inc.: 525 B Street, Suite 1900, San Diego, CA 92101-4495 USA, **2012**.
3. Dell, B.; Huang, L.B. Physiological response of plants to low boron. *Plant and Soil* **1997**, *193*, 103-120.
4. Wimmer, M.A.; Goldberg, S.; Gupta, U.C. Boron. In *Barker A (Ed), Pilbeam D (Ed) Handbook of Plant Nutrition*, 2<sup>nd</sup> ed.; CRC Press: **2015**; pp. 305-346.
5. Brown, P.H.; Shelp, B.J. Boron mobility in plants. *Plant and soil* **1997**, *193*, 85-101.
6. Zhang, D.; Zhao, H.; Shi, L.; Xu, F. Physiological and genetic responses to boron deficiency in Brassica napus: a review. *Soil Science and Plant Nutrition* **2014**, *60*, 304-313.
7. Eggert, K.; von Wiren, N. The role of boron nutrition in seed vigour of oilseed rape (Brassica napus L.). *Plant and Soil* **2016**, *402*, 63-76.
8. Matoh, T. Boron in plant cell walls. *Plant and Soil* **1997**, *193*, 59-70.
9. Brown, P.H.; Bellaloui, N.; Wimmer, M.A.; Bassil, E.S.; Ruiz, J.; Hu, H.; Pfeiffer, H.; Dannel, F.; Romheld, V. Boron in plant biology. *Plant Biol.* **2002**, *4*, 205-223.
10. Wimmer, M.A.; Eichert, T. Review: Mechanisms for boron deficiency-mediated changes in plant water relations. *Plant Science* **2013**, *203*, 25-32.
11. Fageria, N.K.; Filho, M.P.B.; Moreira, A.; Guimarães, C.M. Foliar Fertilization of Crop Plants. *Journal of Plant Nutrition* **2009**, *32*, 1044-1064.
12. Fernández, V.; Sotiropoulos, T.; Brown, P. Foliar Fertilization - Scientific Principles and Field Practices. International Fertilizer Industry Association (IFA), Paris, France. **2013**.
13. Noreen, S.; Fatima, Z.; Ahmad, S.; Ashraf, M. Foliar application of micronutrients in mitigating abiotic stress in crop plants. In *Plant nutrients and abiotic stress tolerance*, Springer: **2018**; pp. 95-117.
14. Eichert, T.; Goldbach, H.E. Transpiration rate affects the mobility of foliar-applied boron in *Ricinus communis* L. cv. Impala. *Plant and soil* **2010**, *328*, 165-174.
15. Orlovius, K. Effect of foliar fertilisation with magnesium, sulfur, manganese and boron to sugar beet, oilseed rape, and cereals. In *Plant Nutrition*, Springer: **2001**; pp. 788-789.

16. Jankowski, K.J.; Hulanicki, P.S.; Krzebietke, S.; Zarczynski, P.; Hulanicki, P.; Sokolski, M. Yield and quality of winter oilseed rape in response to different systems of foliar fertilization. *J. Elem.* **2016**, *21*, 1017-1027.
17. Ma, B.-L.; Zheng, Z.; Whalen, J.K.; Caldwell, C.; Vanasse, A.; Pageau, D.; Scott, P.; Earl, H.; Smith, D.L. Uptake and nutrient balance of nitrogen, sulfur, and boron for optimal canola production in eastern Canada. *Journal of Plant Nutrition and Soil Science* **2019**, *182*, 252-264.
18. Shelp, B.J.; Vivekanandan, P.; Vanderpool, R.A.; Kitheka, A.M. Translocation and effectiveness of foliar-fertilized boron in broccoli plants of varying boron status. *Plant and Soil* **1996**, *183*, 309-313.
19. Brown, P.H.; Bassil, E. Overview of the acquisition and utilization of boron, chlorine, copper, manganese, molybdenum, and nickel by plants and prospects for improvement of micronutrient use efficiency. *The molecular and physiological basis of nutrient use efficiency in crops* **2011**, 377-428.
20. Reid, R. Understanding the boron transport network in plants. *Plant and Soil* **2014**, *385*, 1-13.
21. Will, S.; Eichert, T.; Fernández, V.; Müller, T.; Römheld, V. Boron foliar fertilization of soybean and lychee: effects of side of application and formulation adjuvants. *Journal of Plant Nutrition and Soil Science* **2012**, *175*, 180-188.
22. Will, S.; Eichert, T.; Fernández, V.; Möhring, J.; Müller, T.; Römheld, V. Absorption and mobility of foliar-applied boron in soybean as affected by plant boron status and application as a polyol complex. *Plant and soil* **2011**, *344*, 283-293.
23. Stangoulis, J.; Tate, M.; Graham, R.; Bucknall, M.; Palmer, L.; Boughton, B.; Reid, R. The mechanism of boron mobility in wheat and canola phloem. *Plant physiology* **2010**, *153*, 876-881.
24. Dordas, C.; Brown, P.H. Permeability of boric acid across lipid bilayers and factors affecting it. *The Journal of membrane biology* **2000**, *175*, 95-105.
25. Miwa, K.; Fujiwara, T. Boron transport in plants: co-ordinated regulation of transporters. *Annals of botany* **2010**, *105*, 1103-1108.
26. Takano, J.; Wada, M.; Ludewig, U.; Schaaf, G.; von Wiren, N.; Fujiwara, T. The Arabidopsis major intrinsic protein NIP5;1 is essential for efficient boron uptake and plant development under boron limitation. *The Plant cell* **2006**, *18*, 1498-1509.
27. Takano, J.; Miwa, K.; Fujiwara, T. Boron transport mechanisms: collaboration of channels and transporters. *Trends Plant Sci.* **2008**, *13*, 451-457.

28. Takano, J.; Noguchi, K.; Yasumori, M.; Kobayashi, M.; Gajdos, Z.; Miwa, K.; Hayashi, H.; Yoneyama, T.; Fujiwara, T. Arabidopsis boron transporter for xylem loading. *Nature* **2002**, *420*, 337-340.
29. Yoshinari, A.; Takano, J. Insights into the Mechanisms Underlying Boron Homeostasis in Plants. *Frontiers in plant science* **2017**, *8*, 8.
30. Diehn, T.A.; Bienert, M.D.; Pommerrenig, B.; Liu, Z.J.; Spitzer, C.; Bernhardt, N.; Fuge, J.; Bieber, A.; Richet, N.; Chaumont, F., et al. Boron demanding tissues of Brassica napus express specific sets of functional Nodulin26-like Intrinsic Proteins and BOR1 transporters. *Plant J.* **2019**, *100*, 68-82.
31. Zhang, Q.; Chen, H.F.; He, M.L.; Zhao, Z.Q.; Cai, H.M.; Ding, G.D.; Shi, L.; Xu, F.S. The boron transporter BnaC4.BOR1;1c is critical for inflorescence development and fertility under boron limitation in Brassica napus. *Plant Cell and Environment* **2017**, *40*, 1819-1833.
32. Yang, L.; Zhang, Q.; Dou, J.N.; Li, L.; Guo, L.F.; Shi, L.; Xu, F.S. Characteristics of root boron nutrition confer high boron efficiency in Brassica napus cultivars. *Plant and Soil* **2013**, *371*, 95-104.
33. Asad, A.; Blamey, F.P.C.; Edwards, D.G. Effects of boron foliar applications on vegetative and reproductive growth of sunflower. *Annals of botany* **2003**, *92*, 565-570.
34. Huang, L.B.; Ye, Z.Q.; Bell, R.W. The importance of sampling immature leaves for the diagnosis of boron deficiency in oilseed rape (*Brassica napus* cv Eureka). *Plant and Soil* **1996**, *183*, 187-198.
35. Skarpa, P. Effect of boron foliar application at critical growth stages on sunflower (*helianthus annuus* L.) yield and quality. *J. Elem.* **2013**, *18*, 449-459.
36. Viçosi, K.A.; Carvalho, A.S.d.; Silva, D.C.; Almeida, F.P.; Ribeiro, D.; Flores, R.A. Foliar fertilization with boron on the growth, physiology, and yield of snap beans. *J. Soil Sci. Plant Nutr.* **2020**, 1-8.
37. Jankowski, K.J.; Sokolski, M.; Dubis, B.; Krzebietke, S.; Zarczynski, P.; Hulanicki, P.; Hulanicki, P.S. Yield and quality of winter oilseed rape (*Brassica napus* L.) seeds in response to foliar application of boron. *Agr. Food Sci.* **2016**, *25*, 164-176.
38. Huang, L.B.; Bell, R.W.; Dell, B. Evidence of phloem boron transport in response to interrupted boron supply in white lupin (*Lupinus albus* L. cv. Kiev Mutant) at the reproductive stage. *Journal of experimental botany* **2008**, *59*, 575-583.
39. Stangoulis, J.C.; Brown, P.H.; Bellaloui, N.; Reid, R.J.; Graham, R.D. The efficiency of boron utilisation in canola. *Functional Plant Biology* **2001**, *28*, 1109-1114.



40. Liu, G.-D.; Wang, R.-D.; Wu, L.-S.; Peng, S.-A.; Wang, Y.-H.; Jiang, C.-C. Boron distribution and mobility in navel orange grafted on citrange and trifoliolate orange. *Plant and soil* **2012**, *360*, 123-133.
41. Hajiboland, R.; Farhanghi, F. Remobilization of boron, photosynthesis, phenolic metabolism and anti-oxidant defense capacity in boron-deficient turnip (*Brassica rapa* L.) plants. *Soil Science & Plant Nutrition* **2010**, *56*, 427-437.
42. Konsaeng, S.; Dell, B.; Rerkasem, B. Boron mobility in peanut (*Arachis hypogaea* L.). *Plant and Soil* **2010**, *330*, 281-289.
43. Bergmann, W. Nutritional disorders of plants: visual and analytical diagnosis. *Gustav Fischer Verlag Jena* **1992**.
44. Hossain, M.F.; Pan, S.G.; Duan, M.Y.; Mo, Z.W.; Karbo, M.B.; Bano, A.; Tang, X.R. Photosynthesis and antioxidant response to winter rapeseed (*Brassica napus* L.) as affected by boron. *Pak. J. Bot.* **2015**, *47*, 675-684.
45. Fernández, V.; Eichert, T. Uptake of hydrophilic solutes through plant leaves: current state of knowledge and perspectives of foliar fertilization. *Critical Reviews in Plant Sciences* **2009**, *28*, 36-68.
46. Miwa, K.; Wakuta, S.; Takada, S.; Ide, K.; Takano, J.; Naito, S.; Omori, H.; Matsunaga, T.; Fujiwara, T. Roles of BOR2, a boron exporter, in cross linking of rhamnogalacturonan II and root elongation under boron limitation in Arabidopsis. *Plant physiology* **2013**, *163*, 1699-1709.
47. Hegazi, E.S.; El-Motaium, R.A.; Yehia, T.A.; Hashim, M.E. Effect of foliar boron application on boron, chlorophyll, phenol, sugars and hormones concentration of olive (*Olea europaea* L.) buds, leaves, and fruits. *Journal of Plant Nutrition* **2018**, *41*, 749-765.
48. Zhao, D.; Oosterhuis, D.M. Cotton carbon exchange, nonstructural carbohydrates, and boron distribution in tissues during development of boron deficiency. *Field Crops Research* **2002**, *78*, 75-87.
49. Feng, Y.N.; Cui, R.; Wang, S.L.; He, M.L.; Hua, Y.P.; Shi, L.; Ye, X.S.; Xu, F.S. Transcription factor BnaA9.WRKY47 contributes to the adaptation of Brassica napus to low boron stress by up-regulating the boric acid channel gene BnaA3.NIP5;1. *Plant Biotechnol. J.* **2019**, *14*.
50. Dinh, A.Q.; Naeem, A.; Sagervanshi, A.; Wimmer, M.A.; Mühlhling, K.H. Boron uptake and distribution by oilseed rape (*Brassica napus* L.) as affected by different nitrogen forms under low and high boron supply. *Plant Physiology and Biochemistry* **2021**, *161*, 156-165.
51. Wang, S.L.; Yoshinari, A.; Shimada, T.; Hara-Nishimura, I.; Mitani-Ueno, N.; Ma, J.F.; Naito, S.; Takano, J. Polar Localization of the NIP5;1 Boric Acid Channel Is Maintained by

Endocytosis and Facilitates Boron Transport in Arabidopsis Roots. *The Plant cell* **2017**, *29*, 824-842.

52. Wu, X.; Riaz, M.; Yan, L.; Jiang, C. Distribution and mobility of foliar-applied boron (10B) in citrange rootstock under different boron conditions. *Journal of Plant Growth Regulation* **2020**, *39*, 575-582.

53. Shah, S.; Karunarathna, N.L.; Jung, C.; Emrani, N. An APETALA1 ortholog affects plant architecture and seed yield component in oilseed rape (*Brassica napus* L.). *BMC plant biology* **2018**, *18*, 380.

54. Livak, K.J.; Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(T)(-Delta Delta C) method. *Methods* **2001**, *25*, 402-408.

**Figure Legends:**

**Fig. 1.** Leaves and roots of *B. napus* responses to different B treatments in nutrient solution grown for 42 days. +B, 25  $\mu\text{M}$  B in NS; -B, 0.25  $\mu\text{M}$  B in NS; -B+RR, 0.25  $\mu\text{M}$  B in NS for the first two weeks and then 25  $\mu\text{M}$  B for the latter two weeks; -B+LR<sub>30</sub>, 0.25  $\mu\text{M}$  B in NS and foliar-applied 30 mM B at two weeks; -B+LR<sub>60</sub>, 0.25  $\mu\text{M}$  B in NS and foliar-applied 60 mM B at two weeks; -B+LR<sub>150</sub>, 0.25  $\mu\text{M}$  B in NS and foliar-applied 150 mM B at two weeks.

**Fig. 2.** Root (A) and shoot (B) dry matters and their responses to different B treatments in nutrient solution grown *Brassica napus* for 42 days. +B, 25  $\mu\text{M}$  B in NS; -B, 0.25  $\mu\text{M}$  B in NS; -B+RR, 0.25  $\mu\text{M}$  B in NS for the first two weeks and then 25  $\mu\text{M}$  B for the latter two weeks; -B+LR<sub>30</sub>, 0.25  $\mu\text{M}$  B in NS and foliar-applied 30 mM B at two weeks; -B+LR<sub>60</sub>, 0.25  $\mu\text{M}$  B in NS and foliar-applied 60 mM B at two weeks; -B+LR<sub>150</sub>, 0.25  $\mu\text{M}$  B in NS and foliar-applied 150 mM B at two weeks. The data  $\pm$  SE are means of five independent pot replicates. Different letters on bars indicate significant differences between treatments (ANOVA with Duncan's multiple range test  $p \leq 0.05$ ).

**Fig. 3.** Boron concentration in young leaves (A), mature leaves (B) and roots (C) of *Brassica napus* grown in nutrient solution under different B treatments for 42 days. +B, 25  $\mu\text{M}$  B in NS; -B, 0.25  $\mu\text{M}$  B in NS; -B+RR, 0.25  $\mu\text{M}$  B in NS for the first two weeks and then 25  $\mu\text{M}$  B for the latter two weeks; -B+LR<sub>30</sub>, 0.25  $\mu\text{M}$  B in NS and foliar-applied 30 mM B at two weeks; -B+LR<sub>60</sub>, 0.25  $\mu\text{M}$  B in NS and foliar-applied 60 mM B at two weeks; -B+LR<sub>150</sub>, 0.25  $\mu\text{M}$  B in NS and foliar-applied 150 mM B at two weeks. The data  $\pm$  SE are means of five independent pot replicates. Different letters on bars indicate significant differences between treatments (ANOVA with Duncan's multiple range test  $p \leq 0.05$ ).

**Fig. 4.** Changes in net CO<sub>2</sub> assimilation rate (A;  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) and transpiration rate (B;  $\mu\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) of *Brassica napus* grown in nutrient solution under different B treatments for 42 days. +B, 25  $\mu\text{M}$  B in NS; -B, 0.25  $\mu\text{M}$  B in NS; -B+RR, 0.25  $\mu\text{M}$  B in NS for the first two weeks and then 25  $\mu\text{M}$  B for the latter two weeks; -B+LR<sub>30</sub>, 0.25  $\mu\text{M}$  B in NS and foliar-applied 30 mM B at two weeks; -B+LR<sub>60</sub>, 0.25  $\mu\text{M}$  B in NS and foliar-applied 60 mM B at two weeks; -B+LR<sub>150</sub>, 0.25  $\mu\text{M}$  B in NS and foliar-applied 150 mM B at two weeks. The data  $\pm$  SE are means of four independent pot replicates. Different letters on bars indicate significant differences between treatments (ANOVA with Duncan's multiple range test  $p \leq 0.05$ ).

**Fig. 5.** Glucose, fructose and sucrose concentrations in the roots (A), mature leaves (B) and young leaves (C) of *Brassica napus* grown in nutrient solution under different B treatments for 42 days. +B, 25  $\mu\text{M}$  B in NS; -B, 0.25  $\mu\text{M}$  B in NS; -B+RR, 0.25  $\mu\text{M}$  B in NS for the first two weeks and then 25  $\mu\text{M}$  B for the latter two weeks; -B+LR<sub>30</sub>, 0.25  $\mu\text{M}$  B in NS and foliar-applied

30 mM B at two weeks; -B+LR<sub>60</sub>, 0.25 µM B in NS and foliar-applied 60 mM B at two weeks; -B+LR<sub>150</sub>, 0.25 µM B in NS and foliar-applied 150 mM B at two weeks. The data ± SE are means of five independent pot replicates. Different letters on bars indicate significant differences between treatments (ANOVA with Duncan's multiple range test  $p \leq 0.05$ ).

**Fig. 6.** The fold change in the relative expression of *BnaBOR1;2* and *BnaNIP5;1* gene in roots (A, C) and leaves (B, D) of *Brassica napus* grown in nutrient solution under different B treatment for 42 days. +B, 25 µM B in NS; -B, 0.25 µM B in NS; -B+RR, 0.25 µM B in NS for the first two weeks and then 25 µM B for the latter two weeks; -B+LR<sub>30</sub>, 0.25 µM B in NS and foliar-applied 30 mM B at two weeks; -B+LR<sub>60</sub>, 0.25 µM B in NS and foliar-applied 60 mM B at two weeks; -B+LR<sub>150</sub>, 0.25 µM B in NS and foliar-applied 150 mM B at two weeks. Bars represent means of three biological replicates, technically replicated two times ± SE. Different letters on bars indicate significant differences between treatments (ANOVA with Duncan's multiple range test  $p \leq 0.05$ ).

Fig. 1.

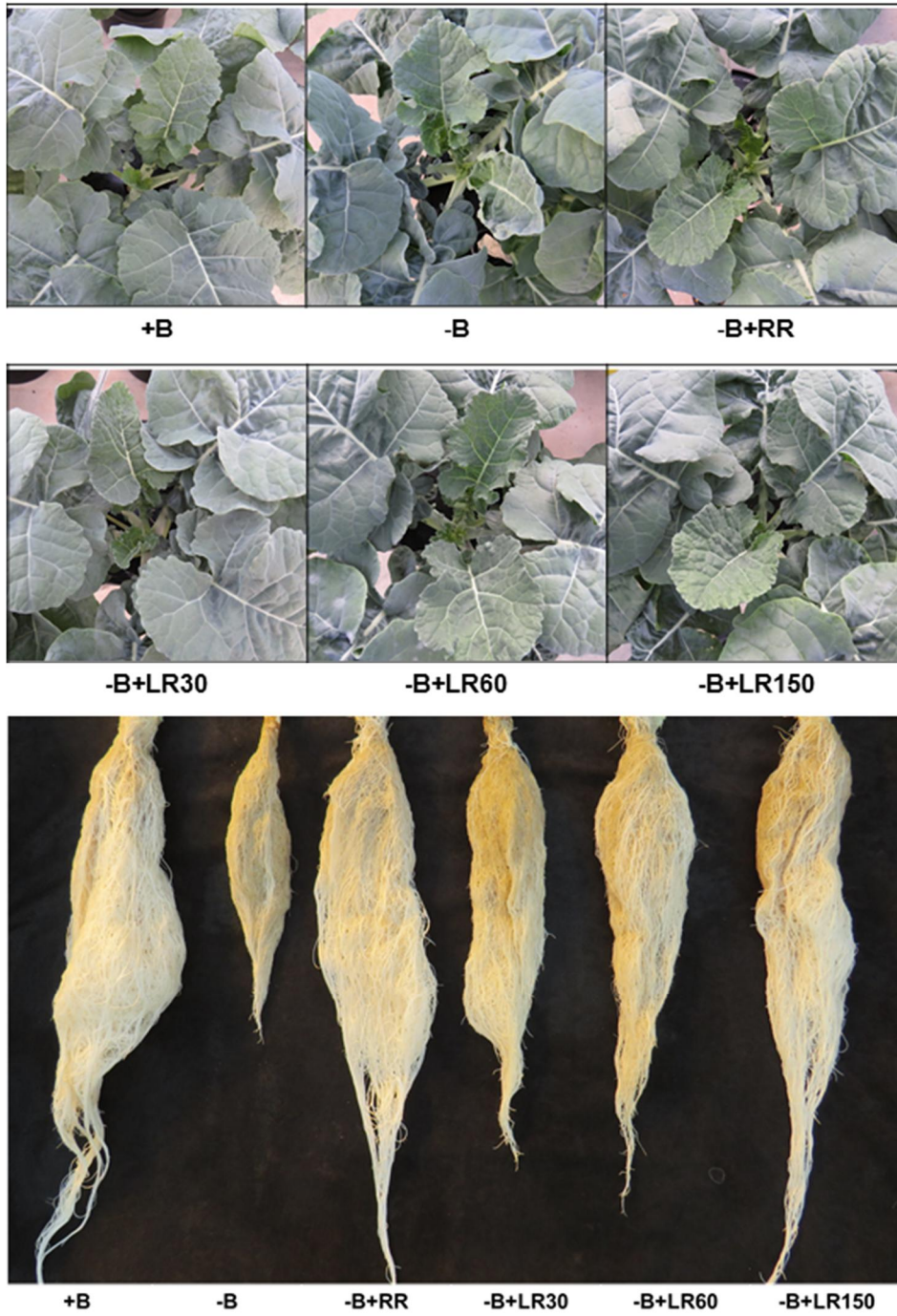


Fig. 2.

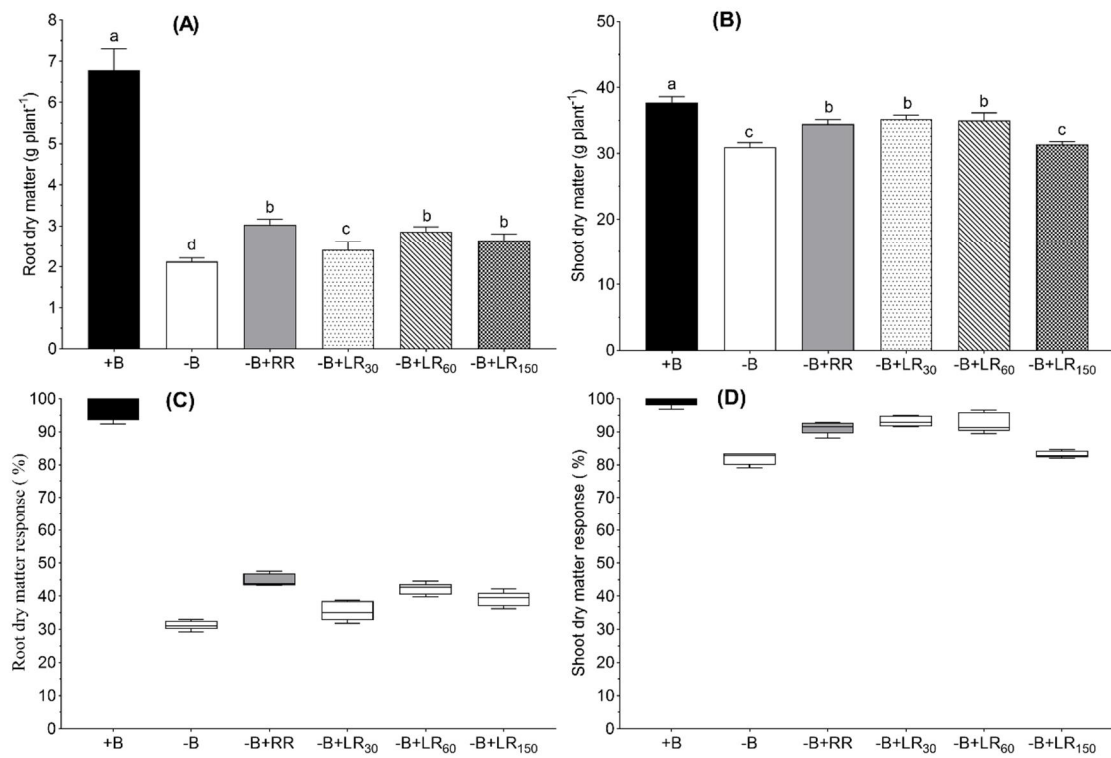


Fig. 3.

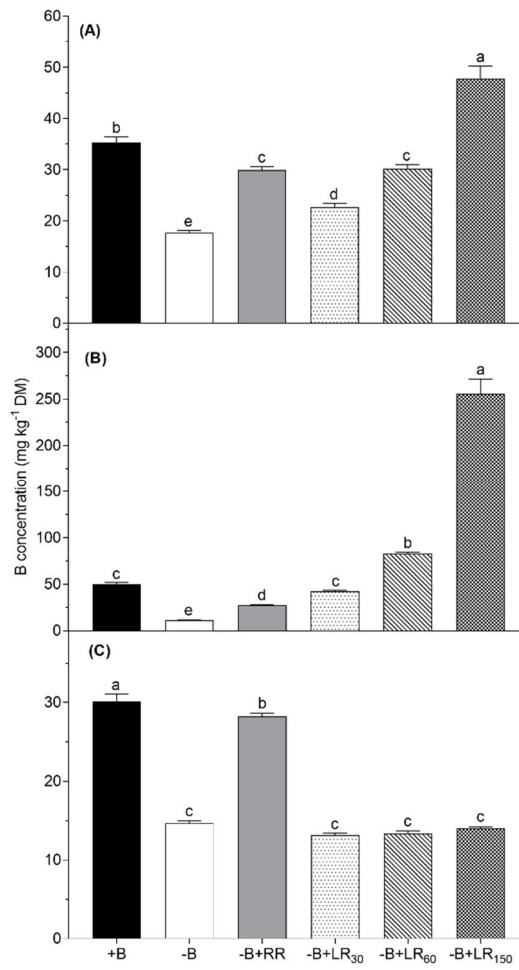


Fig. 4.

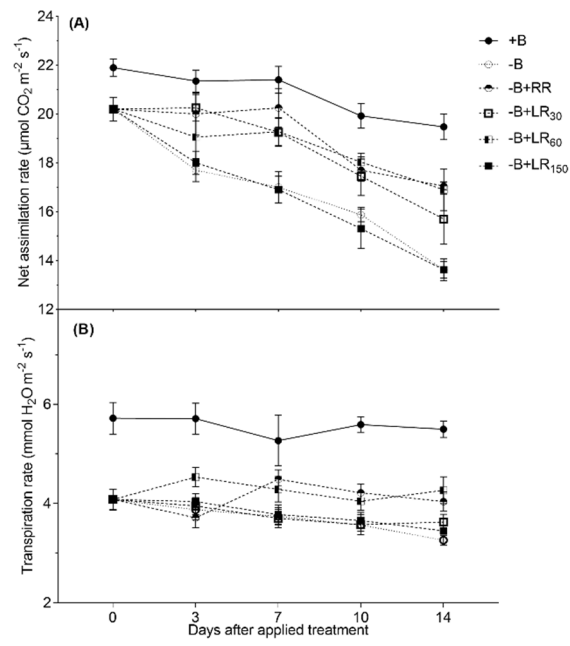




Fig. 5.

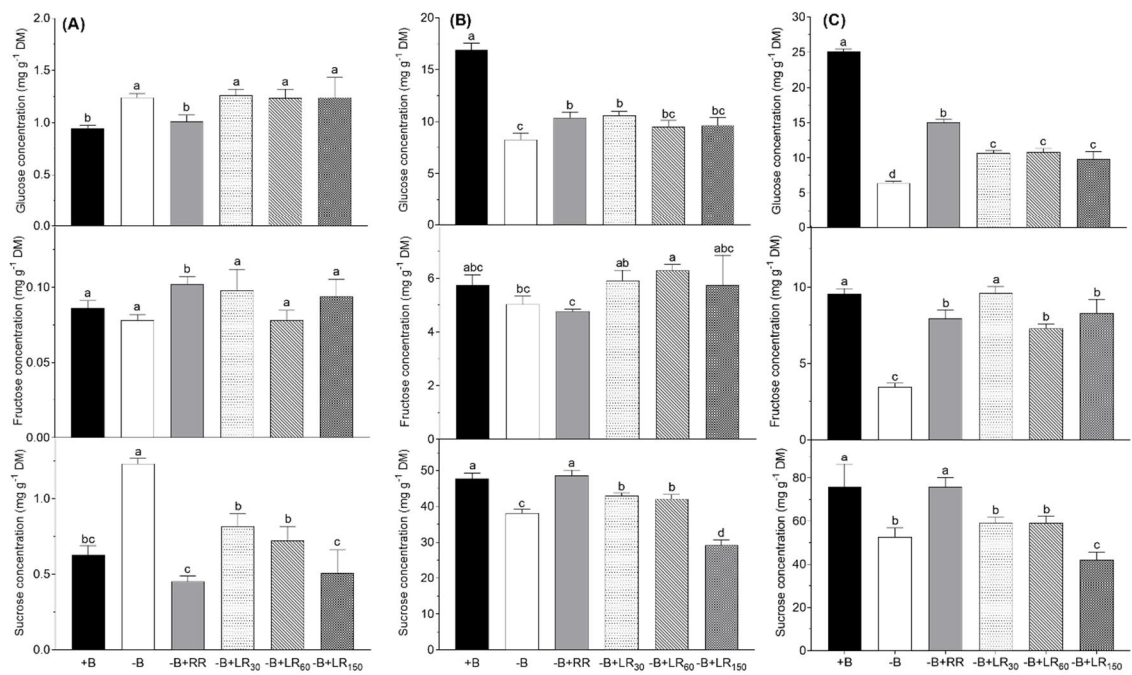
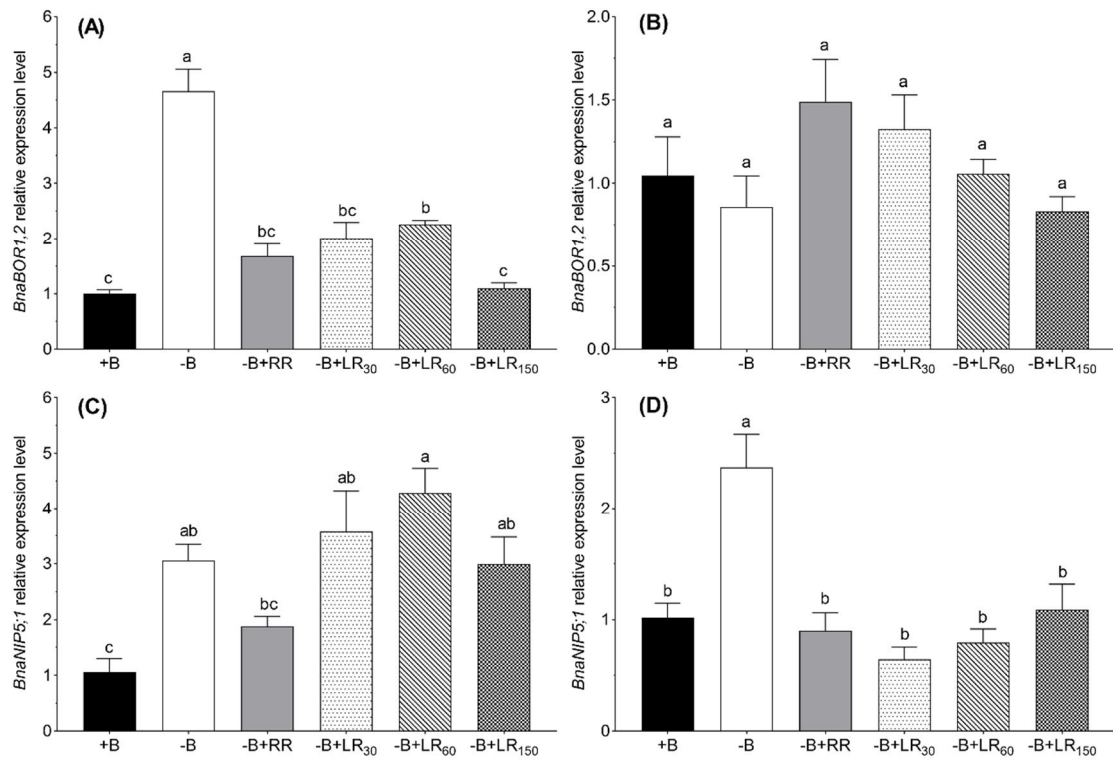


Fig. 6.



## Chapter 4

### **Boron Uptake and Distribution by Oilseed Rape (*Brassica napus* L.) as Affected by Different Nitrogen Forms under Low and High Boron Supply<sup>1</sup>**

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Research article

Boron uptake and distribution by oilseed rape (*Brassica napus* L.) as affected by different nitrogen forms under low and high boron supplyAnh Quang Dinh<sup>a,b</sup>, Asif Naeem<sup>a</sup>, Amit Sagervanshi<sup>a</sup>, Monika A. Wimmer<sup>a,1</sup>, Karl H. Mühling<sup>a,\*</sup><sup>a</sup> Institute for Plant Nutrition and Soil Science, Kiel University, Hermann Rodewald Strasse 2, D-24118, Kiel, Germany<sup>b</sup> Faculty of Agriculture and Forestry, Dalat University, Da Lat, Lam Dong Province, Viet Nam

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## ABSTRACT

Ammonium ( $\text{NH}_4^+$ ) and nitrate ( $\text{NO}_3^-$ ) conversely alter pH of the rooting medium, and thus differentially affect the equilibrium between boric acid and borate in soil solution. This can alter boron (B) uptake by plants, which is passive under high, but facilitated (boric acid) or active (borate) under low B supply. Therefore, the effect of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  forms was investigated on the growth,  $^{10}\text{B}$  uptake rate and accumulation, and expression of B transporters in *Brassica napus* grown with low (1  $\mu\text{M}$ ) or high (100  $\mu\text{M}$ )  $^{10}\text{B}$  for five days in the nutrient solution. At the low  $^{10}\text{B}$  level,  $\text{NO}_3^-$ -fed plants had the same specific  $^{10}\text{B}$  uptake rate,  $^{10}\text{B}$  accumulation and xylem  $^{10}\text{B}$  concentration as  $\text{NH}_4\text{NO}_3$ -fed plants but these attributes were reduced at the high  $^{10}\text{B}$  level. *BnaBOR1;2* and *BnaNIP5;1* were upregulated in roots of  $\text{NO}_3^-$ -fed plants at low  $^{10}\text{B}$  supply.  $\text{NH}_4^+$ -fed plants had substantially lower dry matters; due to nutrient solution acidification (2.0 units)-induced deficiency of nitrogen, potassium, magnesium, and iron in plant shoots. Reduced transpiration rates resulted in lower  $^{10}\text{B}$  uptake rate and accumulation in the roots and shoots of  $\text{NH}_4^+$ -fed plants. *BnaNIP5;1* in roots, while both *BnaBOR1;2* and *BnaNIP5;1* in shoots were upregulated in  $\text{NH}_4^+$ -fed plants at low  $^{10}\text{B}$  level. Collectively,  $\text{NH}_4^+$ -induced acidity and consequent lowering of  $^{10}\text{B}$  uptake induced the upregulation of B transport mechanisms, even at marginal  $^{10}\text{B}$  concentrations, while  $\text{NO}_3^-$ -induced alkalization resulted in altered B distribution between roots and shoots due to restricted B transport, especially at higher  $^{10}\text{B}$  supply.

## 1. Introduction

Boron (B) is a micronutrient for vascular plants, diatoms, and some green algae (Loomis and Durst, 1992). The primary established role of B in plants is to crosslink rhamnogalacturonan-II (RG-II) molecules of the cell wall, thus maintaining cell wall structural integrity and apical growth (Brown et al., 2002; Bolaños et al., 2004; Goldbach and Wimmer, 2007; Marschner, 2012). The B requirement of plants varies from species to species, and oilseed rape (*Brassica napus* L.) is considered one of the highest B requiring plants, which often suffers from yield and quality losses due to B deficiency, especially in Northern Europe, Canada and China (Gupta, 1980; Pommerrenig et al., 2018). In addition, soils low in B (<0.25 mg B  $\text{kg}^{-1}$  soil) are found in more than 80 countries of the world (Shorrocks, 1997), and are likely insufficient for canola

production, which requires more than 0.5 mg B  $\text{kg}^{-1}$  soil to achieve its actual yield potential (Pommerrenig et al., 2018). Rape is extremely sensitive to B deficiency, and deficiency symptoms include inhibition of root and shoot meristem activity, cell elongation, flower development, and ultimately reduced yield (Shorrocks, 1997; Eggert and von Wirén, 2016; Wang et al., 2007).

In soil solution, B is mainly present in the form of boric acid and borate (Marschner, 2012). Boric acid is a weak monobasic acid with a  $\text{pK}_a$  value of 9.25, which acts as Lewis acid by accepting a hydroxyl ion ( $\text{OH}^-$ ) to form the anionic borate  $\text{B}(\text{OH})_4^-$  in an aqueous solution. The  $\text{pK}_a$  value indicates that the borate anion  $\text{B}(\text{OH})_4^-$  is present in significant amounts only in soils with alkaline pH, while at soil pH values of less than 7, more than 99% of B exists as uncharged boric acid. The latter is readily available for passive plant uptake, especially under sufficient B

**Abbreviations:** B, boron; DM, dry matter; ICP-MS, inductively coupled plasma mass spectrometer;  $\text{NH}_4^+$ , ammonium;  $\text{NH}_4\text{NO}_3$ , ammonium nitrate;  $\text{NO}_3^-$ , nitrate; N, nitrogen; qRT-PCR, quantitative reverse transcriptase-polymerase chain reaction.

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supply (Marschner, 2012; Wimmer et al., 2015). At increasing soil pH, passive B uptake is thus reduced due to the progressive shift of B from boric acid to the borate anion (Lauchli and Grattan, 2017). Under limited B supply, B can also be taken up by plants via facilitated diffusion through channels such as nodulin 26-like intrinsic protein 5; 1 (NIP5; 1) located on the soil side of root cells (Takano et al., 2006), or via active uptake through transporters such as BOR1, which acts in loading B into the xylem sap (Brown et al., 2002; Takano et al., 2002; Miwa et al., 2007; Miwa and Fujiwara, 2010). Since NIP5; 1 allows facilitated diffusion of boric acid, while BOR1 transports the borate anion rather than the uncharged boric acid, a change in external pH is likely to affect B inflow into cells and B loading into the xylem to different extent under low and high B supply, respectively.

Boron uptake by plants may depend upon the forms of nitrogen (N) supplied since these can alter the soil solution pH: supply of ammonium ( $\text{NH}_4^+$ ) decreases the pH while nitrate ( $\text{NO}_3^-$ ) results in the opposite effect (Marschner, 2012). When plants take up  $\text{NH}_4^+$ ,  $\text{H}^+$  is exuded to regulate cytosolic pH and charge balance, and the pH of the rhizosphere decreases. In contrast, the uptake of  $\text{NO}_3^-$  can cause  $\text{H}^+$  influx or  $\text{OH}^-$  efflux with a concomitant increase in rhizosphere pH (Gerendas et al., 1998). In both cases, the boric acid – borate equilibrium will be shifted. The effect of different N forms on B uptake by plants has been addressed in several studies, but with quite different outcomes. McCrimmon et al. (1992) found higher B uptake and concentrations in root and verdure tissues of  $\text{NO}_3^-$ -fed as compared to  $\text{NH}_4^+$ -fed bentgrass plants. Similarly, lower B accumulation in  $\text{NH}_4^+$ -fed compared to  $\text{NO}_3^-$ -fed wheat plants has been reported by Wang and Below (1998). On the other hand,  $\text{NH}_4^+$  application to high B soils resulted in higher B accumulation in the roots of olive plants as compared to  $\text{NO}_3^-$  application (Chatzissavvidis et al., 2007). And finally, other studies did not indicate significant effects of N forms on B uptake by cucumber Alt and Schwarz (1973) or tobacco Tsadilas et al. (2005). The reason for such contrasting results is not clear, but may be partly explained by the above mentioned dependence of the pH response upon the level of B supply. Given that passive diffusion of boric acid is the dominant B uptake pathway under optimum B supply, while B requirements under low B supply can be met through facilitated and/or active uptake of boric acid or borate, this work is based on the hypothesis that the effects of N forms might be different depending on the B supply level. More specifically, we hypothesize that a nitrate-induced increase in pH would shift the equilibrium towards borate and thus reduce B uptake especially at optimum B supply, where passive diffusion dominates, while the opposite effect would be expected under ammonium supply. We additionally tested the question whether  $\text{NH}_4^+$  and  $\text{NO}_3^-$  have contrasting effects on B uptake at low B supply.

To this end, N was supplied in the form of  $\text{NH}_4^+$  alone,  $\text{NO}_3^-$  alone, or  $\text{NH}_4^+/\text{NO}_3^-$  mix (50% each) to the roots of oilseed rape grown with low and high  $^{10}\text{B}$  supply. To elucidate the genetic mechanisms governing the treatment response, expressions of the B transporter *BnaBOR1;2* and the channel *BnaNIP5;1* were also investigated. *B. napus* was selected for this study because of its known high B requirement, which was expected to result in clear differences in treatment response at high and low B levels.

## 2. Materials and methods

### 2.1. Growth conditions

The seeds of winter oilseed rape (*Brassica napus* L. cv. Alpage) were obtained from Norddeutsche Pflanzenzucht Lembke (NPZ), Hohenlieth, Germany. Seeds were soaked in aerated 1 mM  $\text{CaSO}_4$  solution for 24 h, germinated on a filter paper moistened with 1 mM  $\text{CaSO}_4$  at 22 °C for 4 days in darkness, and then exposed to light for 2 days. Thus, six-day-old uniform-sized seedlings were transferred to plastic pots (4 seedlings  $\text{pot}^{-1}$ ) containing 2 L of one quarter-strength nutrient solution. The strength of the nutrient solution was increased to one-half and to full-strength on the 4th and 8th day after transplantation, respectively. The composition of the full-strength nutrient solution was as follows:

2.0 mM  $\text{Ca}(\text{NO}_3)_2$ , 0.5 mM  $\text{K}_2\text{SO}_4$ , 0.25 mM  $\text{KH}_2\text{PO}_4$ , 0.325 mM  $\text{MgSO}_4$ , 50  $\mu\text{M}$   $\text{NaCl}$ , 1  $\mu\text{M}$   $\text{H}_3^{11}\text{BO}_3$  (99%  $^{11}\text{B}$  atom abundance, Sigma-Aldrich, USA), 2  $\mu\text{M}$   $\text{MnSO}_4$ , 0.4  $\mu\text{M}$   $\text{ZnSO}_4$ , 0.4  $\mu\text{M}$   $\text{CuSO}_4$ , 0.1  $\mu\text{M}$   $\text{Na}_2\text{MoO}_4$  and 40  $\mu\text{M}$   $\text{Fe-EDTA}$ . The inherent pH of the nutrient solution was 6.0. The nutrient solution was continuously aerated to provide oxygen to roots. To replenish the nutrients depleted by plant uptake, the nutrient solution was replaced every 2 days. The plants were grown under controlled conditions in a growth chamber adjusted to 14 h  $\text{d}^{-1}$  photoperiod with 350  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  light intensity (recorded by a light meter, Li-198, Lincoln, USA), 60% relative humidity and 22 °C (day)/18 °C (night) temperature.

### 2.2. Application of treatments

After a growth period of 14 days under the same conditions (pre-treatment), plants from four pots were harvested to determine the initial  $^{10}\text{B}$  content for the calculation of  $^{10}\text{B}$  uptake rates at the completion of the treatment period. Simultaneously, roots of the other plants were thoroughly rinsed with double deionized water and transferred to nutrient solution containing either 1  $\mu\text{M}$   $^{10}\text{B}$  (low B) or 100  $\mu\text{M}$   $^{10}\text{B}$  (high B) as  $^{10}\text{B}$ -boric acid (99%  $^{10}\text{B}$  atom abundance, Sigma-Aldrich, USA). We selected 1 and 100  $\mu\text{M}$  B levels, as low and high B treatments, for this study based upon a preliminary trial. The lower B level (1  $\mu\text{M}$ ) is at the edge of being critical and was “just-sufficient” to sustain plant growth for the initial 5 days after treatment application. We expected that a decrease in B uptake due to a change in B form induced by pH alteration might be reflected as B deficiency at this B level. The high B treatment refers to a B supply, which was in the higher optimum range, but not yet toxic. At each  $^{10}\text{B}$  level, plants were supplied with 4 mM of nitrogen (N) as either ammonium plus nitrate ( $\text{NH}_4^+ + \text{NO}_3^-$ ), ammonium ( $\text{NH}_4^+$ ) or nitrate ( $\text{NO}_3^-$ ). The N containing salts were  $\text{NH}_4\text{NO}_3$ ,  $(\text{NH}_4)_2\text{SO}_4$  and  $\text{Ca}(\text{NO}_3)_2$ , respectively. To balance calcium (Ca) in the treatments of  $\text{NH}_4^+$  and  $\text{NH}_4\text{NO}_3$ , 2 mM  $\text{CaSO}_4$  was added to the nutrient solution. The experiment followed a two-factor full factorial design. There were three identical sets of plant cultivations, each having four replicates. One set of plant cultivation was used for recording dry matter yield and determining B uptake by plants, the 2nd set for obtaining xylem sap and 3rd set for transporter studies. After the onset of the N/B treatments, the nutrient solution was replaced every 24 h. Double deionized water (18.2  $\text{M}\Omega$ ) was used for the preparation of nutrient solutions to avoid B contamination. To monitor the effect of different forms of N on the pH of the nutrient solution, the pH was measured every 4 h and 6 times a day during the cultivation period.

Plants were grown for 5 days following application of N and  $^{10}\text{B}$  treatments. Water loss was determined daily during this period by weighing each pot before replacing the nutrient solution. Mean evaporation was determined using four pots with nutrient solution but without plants. The volume of water transpired from each pot was calculated by subtracting the water loss from the pots without plants from those with plants every 24 h for five days after treatment application. These measurements were performed only on one set of the cultivation.

### 2.3. Harvesting and analytical methods

After 5 days of treatment application, roots and shoots were harvested separately, washed with deionized water, and dried at 65 °C for 72 h for the determination of dry matter. The growth rate of roots and shoots was calculated from the difference in dry matter between the first and second harvest (before and after the treatment period). The dried tissue samples were ground to fine powder using a ball-mill grinder. Approximately 200 mg of dried material was digested in 10 mL of 69%  $\text{HNO}_3$  (ROTIPURAN Supra for ICP, 69%) in a closed-vessel 1800 W microwave digestion system (MARS 6 Xpress, CEM Corporation, Matthews, USA) adjusted to the following conditions: 2 min at 100 °C, 1 min at 120 °C, 20 min at 180 °C and 20 min cooling time. Afterward, the digested samples were diluted with 2%  $\text{HNO}_3$  to 100 mL and stored at

4 °C until further analysis. The concentration of  $^{10}\text{B}$  was determined by inductively coupled plasma mass spectrometer (ICP-MS; Agilent 7700, Agilent Technologies Inc., USA) using Be as an internal standard. To avoid B contamination from glassware, only plasticware was used. Boron ( $^{10}\text{B}$ ) contents were calculated by multiplying  $^{10}\text{B}$  concentrations with the dry matter of that plant. Specific  $^{10}\text{B}$  uptake rate ( $I_M$ ) was calculated according to Bellaloui and Brown (1998) using the following formula:

$$I_M (\mu\text{g } ^{10}\text{B g}^{-1} \text{ root DM d}^{-1}) = [(\ln R_2 - \ln R_1) / (5)] \times [(M_2 - M_1) / (R_2 - R_1)]$$

Where,  $R_1$  and  $R_2$  are the root dry matter at the beginning and end of the treatment period,  $M_1$  and  $M_2$  are the  $^{10}\text{B}$  contents at the beginning and at the end of the treatment period, and 5 refers to the number of treatment days.

Nitrogen concentrations in the shoots were determined according to the Dumas combustion method using a CNS elemental analyzer (Flash EA 1112 NCS, Thermo Fisher Scientific, Waltham, MA, USA). Macronutrients including phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) and micronutrients including iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), and molybdenum (Mo) in the shoots were determined by ICP-MS (Agilent 7700, Agilent Technologies Inc., USA) following digestion in a mixture of concentrated nitric acid and perchloric acid.

Xylem sap was collected following the method described by Wu et al. (2015). Briefly, plant shoots were cut at 2 cm above the roots, soft rubber tubes were fixed over the decapitated stem and the xylem sap was collected by micropipette for 1 h. The first drop of the exudates was discarded. The volume of the xylem sap was recorded and 2 mL of it was used for  $^{10}\text{B}$  determination.

#### 2.4. RNA extraction, cDNA synthesis, and reverse transcription PCR

Root and shoot samples from the 3rd plant cultivation were harvested separately, washed in running deionized water, frozen in liquid nitrogen, and stored at  $-80$  °C. The frozen samples were ground to powder using a pestle and mortar. Total RNA was isolated from the powdered roots and shoots with TRIZOL reagent (Invitrogen, CA, USA). The RNA samples were shock-frozen in liquid nitrogen and stored at  $-80$  °C for further analysis. The amount of RNA was quantified using a NanoDrop spectrophotometer (ND1000, Thermo Fisher Scientific, USA).

The cDNA was synthesized following the manufacturer's instructions in the Verso™ cDNA kit from Thermo Fisher Scientific. The reaction was carried out in 20  $\mu\text{L}$  of total volume. The reverse transcription was conducted at 42 °C for 30 min followed by inactivation of reverse transcriptase at 95 °C for 2 min. The cDNA was aliquoted and stored at  $-20$  °C for further use. Amplification of cDNA was performed according to the manufacturer's instructions provided with Dream taq™ DNA polymerase (Fermentas). The PCR reaction components were mixed on ice and reaction tubes were put into a T Gradient Thermocycler (Biometra, Germany). Negative controls (NTC) were used to control the specificity and reliability of the PCR. The PCR was performed as follows: initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 10 s, primer annealing at 56 °C for 15 s, and extension 72 °C for 20 s. All of the primers were tested and validated by PCR amplification to ensure their specificity. PCR products obtained from the above reaction were run on agarose gels to ensure the correct length of the amplicon. Furthermore, gene validation was confirmed by the sequencing of the amplicon (Sanger sequencing, IKMB Kiel). Nucleotide sequences obtained from Sanger Sequencing were checked *in silico* by BLASTN against the nucleotide collection database and it revealed that the correct genes were amplified.

#### 2.5. Relative quantification of the real-time PCR data

The quality of synthesized cDNA (1/10 dilution) was checked by standard PCR. Quantitative RT-PCR was conducted by using PowerUp™ SYBR™ Green Master Mix (Applied Biosystems) with family-specific

conserved primers (Table S1) on a CFX96 Real-Time System (Bio-Rad Laboratories GmbH, München, Germany). For each reaction, the total volume was 20  $\mu\text{L}$  containing 100 nM of each primer and 2  $\mu\text{L}$  of diluted cDNA templates, and the sequence of cycling conditions was as follows: 95 °C for 3 min, 40 cycles of 95 °C for 10 s, 60 °C for 30 s, 72 °C for 30 s, and 95 °C for 10 min. Three biological replicates and two technical replicates were used for each treatment. Transcript levels of genes were normalized with endogenous control (*BnaActin*), and the fold expression changes of target mRNAs were determined using the  $2^{-\Delta\Delta\text{Ct}}$  method (Livak and Schmittgen, 2001).

#### 2.6. Statistical analysis

The data were statistically analyzed following two-way ANOVA, which was performed using SPSS software (version 22.0). Significant differences among the means were determined by Duncan Multiple Range (DMR) test using a confidence level of 95% ( $P \leq 0.05$ ). Data are presented as mean  $\pm$  standard error (SE), and graphs were prepared using GraphPad Prism 8.

### 3. Results

#### 3.1. Changes in pH of the nutrient solution and water transpiration

Since the changes in pH of the nutrient solution in response to each N form were similar for low and high  $^{10}\text{B}$ , only mean values of both  $^{10}\text{B}$  levels are reported. Moreover, as the pH changes were similar every day, the mean data of all five days are presented (Fig. 1). The initial pH of the nutrient solution was 6.0. The pH of the nutrient solution containing  $\text{NO}_3^-$  increased progressively and reached a value of 7.5 at the final measurement taken after 24 h (Fig. 1). On the other hand, a progressive decrease was recorded for the nutrient solution containing  $\text{NH}_4^+$ , with a final pH of 4.0. Nutrient solution containing both  $\text{NH}_4^+$  and  $\text{NO}_3^-$  did not show any significant change in pH, and the final value was approximately 6.3.

Water transpiration by plants was not affected by the  $^{10}\text{B}$  supply level, but there was a significant difference between the N-forms (Fig. 2). On average,  $\text{NH}_4^+$ -fed plants transpired 25% and 30% less water as compared to  $\text{NH}_4\text{NO}_3$  and  $\text{NO}_3^-$ -fed plants, respectively. There was no difference in water transpiration between  $\text{NH}_4\text{NO}_3$ - and  $\text{NO}_3^-$ -fed plants.

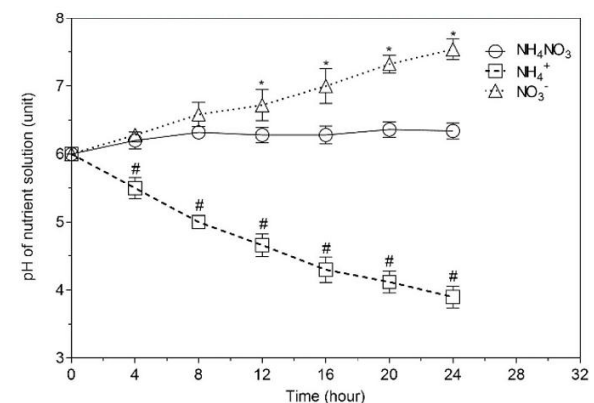
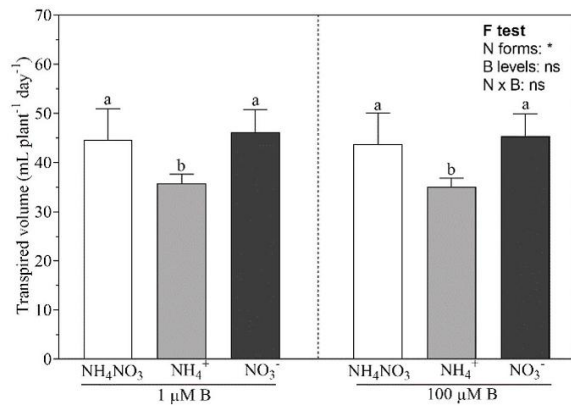


Fig. 1. Changes in pH of the nutrient solutions containing different forms of N ( $\text{NH}_4\text{NO}_3$ ,  $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) and grown with *Brassica napus* during a period of 24 h. Data points are means ( $\pm$ SE) of ten independent pot replicates. For each time point, significant differences ( $P = 0.05$ ) between  $\text{NH}_4\text{NO}_3$  and  $\text{NO}_3^-$  are indicated by asterisks (\*), and between  $\text{NH}_4\text{NO}_3$  and  $\text{NH}_4^+$  by diamonds (#).



**Fig. 2.** Volume of water transpired by *Brassica napus* grown with low (1  $\mu\text{M}$ ) and high (100  $\mu\text{M}$ ) levels of  $^{10}\text{B}$  and different forms of N ( $\text{NH}_4\text{NO}_3$ ,  $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) in nutrient solution for 5 days. Bars represent means ( $\pm\text{SE}$ ) of four independent pot replicates. Different letters indicate significant differences at  $P = 0.05$ . Level of significance: ns, non-significant; \* $P < 0.05$ .

### 3.2. Growth rate and dry matter yield

Although growth rates of both roots and shoots tended to be higher at high  $^{10}\text{B}$  as compared to low  $^{10}\text{B}$  supply irrespective of the N forms, the differences were statistically nonsignificant (Table 1). Regardless of the  $^{10}\text{B}$  level, root and shoot growth rates of  $\text{NH}_4^+$ -fed plants were 45% and 47%, respectively, slower as compared to  $\text{NH}_4\text{NO}_3$ -fed plants. However,  $\text{NO}_3^-$ -fed plants had the same root and shoot growth rates as  $\text{NH}_4\text{NO}_3$ -fed plants at both  $^{10}\text{B}$  levels. Similar results were also recorded for root and shoot dry matter:  $\text{NH}_4^+$ -fed plants produced 31% less root dry matter and 27% less shoot dry matter as compared to  $\text{NH}_4\text{NO}_3$ -fed plants (Fig. 3).

### 3.3. $^{10}\text{B}$ concentration and accumulation in roots and shoots

Compared to plants supplied with low  $^{10}\text{B}$  level, plants supplied with high  $^{10}\text{B}$  level had 2-fold higher  $^{10}\text{B}$  concentrations in roots, regardless of the N form supplied (Fig. 4A), and 6- ( $\text{NH}_4\text{NO}_3$  and  $\text{NO}_3^-$ ) to 15-fold ( $\text{NH}_4^+$ ) higher concentrations in shoots (Fig. 4B). The effect of N forms was independent of the B supply level in roots, where  $^{10}\text{B}$  concentrations of  $\text{NO}_3^-$ -fed plants were 13% higher, and those of  $\text{NH}_4^+$ -fed plants were 21–25% lower under both  $^{10}\text{B}$  levels as compared to  $\text{NH}_4\text{NO}_3$ -fed plants. In shoots, however, the effect of N forms depended upon the  $^{10}\text{B}$  level. Compared to  $\text{NH}_4\text{NO}_3$ -fed plants, shoot  $^{10}\text{B}$  concentration of  $\text{NH}_4^+$ -fed plants was decreased by 63% at low  $^{10}\text{B}$  level, and by only 9% at high  $^{10}\text{B}$  level, while those of  $\text{NO}_3^-$ -fed plants were not changed at low  $^{10}\text{B}$  level, and reduced by 12% at high  $^{10}\text{B}$  level. There was no difference in shoot  $^{10}\text{B}$  concentrations between  $\text{NH}_4^+$ - and  $\text{NO}_3^-$ -fed plants.

**Table 1**

Growth rates of roots and shoots of *Brassica napus* L. grown with low (1  $\mu\text{M}$ ) and high (100  $\mu\text{M}$ ) levels of  $^{10}\text{B}$  and different forms of N ( $\text{NH}_4\text{NO}_3$ ,  $\text{NH}_4^+$ , and  $\text{NO}_3^-$ ) in nutrient solution for 5 days.

B level	Root growth rate (mg DM day <sup>-1</sup> )			Shoot growth rate (mg DM day <sup>-1</sup> )		
	$\text{NH}_4\text{NO}_3$	$\text{NH}_4^+$	$\text{NO}_3^-$	$\text{NH}_4\text{NO}_3$	$\text{NH}_4^+$	$\text{NO}_3^-$
Low	36.3 $\pm$ 0.7	17.5 $\pm$ 1.6	39.5 $\pm$ 2.2	231 $\pm$ 2.7	149 $\pm$ 8.5	204 $\pm$ 10.8
	41.0 $\pm$ 0.5	24.7 $\pm$ 3.1	44.8 $\pm$ 2.6	251 $\pm$ 7.5	160 $\pm$ 15.6	218 $\pm$ 0.0
	Mean	38.6A	20.7B	42.1A	241.1A	154.4C

Values ( $\pm\text{SE}$ ) are the mean of four independent pot replicates. For each parameter, different letters indicate significant differences between treatments at  $P = 0.05$ .

Regardless of the N form, plants supplied with a high level of  $^{10}\text{B}$  had 1.9–2.5-fold higher  $^{10}\text{B}$  accumulation in roots and 4.3–9.2-fold higher accumulation in shoots as compared to plants supplied with a low  $^{10}\text{B}$  level (Fig. 4C and D). The effect of N forms on  $^{10}\text{B}$  accumulation depended on the level of  $^{10}\text{B}$  supplied.  $\text{NH}_4^+$  supply always reduced  $^{10}\text{B}$  accumulation, but the effect was more pronounced at low  $^{10}\text{B}$  supply with a reduction of 54% for roots and 73% for shoots, while at high  $^{10}\text{B}$  supply, reductions amounted to 39% for roots and 34% for shoots (Fig. 4C).  $\text{NO}_3^-$ -fed plants accumulated statistically similar quantities of  $^{10}\text{B}$  both in roots and shoots as compared to  $\text{NH}_4\text{NO}_3$ -fed plants at low  $^{10}\text{B}$  supply. However, at high  $^{10}\text{B}$  supply,  $\text{NO}_3^-$ -fed plants accumulated 32% more  $^{10}\text{B}$  in roots, and 21% less  $^{10}\text{B}$  in shoots as compared to  $\text{NH}_4\text{NO}_3$ -fed plants (Fig. 4D).

### 3.4. Relative $^{10}\text{B}$ distribution, $^{10}\text{B}$ concentration in xylem sap and specific $^{10}\text{B}$ uptake rate

Compared to  $\text{NH}_4\text{NO}_3$ -fed plants,  $\text{NO}_3^-$ -fed plants accumulated relatively more  $^{10}\text{B}$  in roots, and less in shoots, at both  $^{10}\text{B}$  supply levels (Fig. 5A). On the other hand, the effect of  $\text{NH}_4^+$  depended on the  $^{10}\text{B}$  supply; while roots retained more  $^{10}\text{B}$  at low  $^{10}\text{B}$  supply, no significant change in distribution was observed at high  $^{10}\text{B}$  supply, compared to  $\text{NH}_4\text{NO}_3$ -fed plants. Concentrations of  $^{10}\text{B}$  in the xylem sap of plants supplied with high  $^{10}\text{B}$  level was 5.6–8.5-fold higher compared to plants supplied with low  $^{10}\text{B}$  level (Fig. 5B). Nitrogen forms did not affect the  $^{10}\text{B}$  concentration in the xylem sap at low  $^{10}\text{B}$  supply. However, at high  $^{10}\text{B}$  supply,  $\text{NH}_4^+$  nutrition increased the xylem  $^{10}\text{B}$  concentration by 17%, whereas  $\text{NO}_3^-$  nutrition reduced it by 16% as compared to  $\text{NH}_4\text{NO}_3$  nutrition. Specific  $^{10}\text{B}$  uptake rates of plants at high  $^{10}\text{B}$  supply were 5.1–12.3-fold higher than those of plants at low  $^{10}\text{B}$  supply (Fig. 5C). At low  $^{10}\text{B}$  level, the  $^{10}\text{B}$  uptake rate of  $\text{NH}_4^+$ -fed plants was 61% lower than that of  $\text{NH}_4\text{NO}_3$ -fed plants, while  $\text{NO}_3^-$ -fed plants had no effect. At high  $^{10}\text{B}$  level, both  $\text{NH}_4^+$  and  $\text{NO}_3^-$  nutrition resulted in a 24% reduction of the  $^{10}\text{B}$  uptake rate. A highly positive linear correlation ( $R^2 = 0.97$ ) was found between specific  $^{10}\text{B}$  uptake rate and  $^{10}\text{B}$  content in *B. napus* (Fig. 5D).

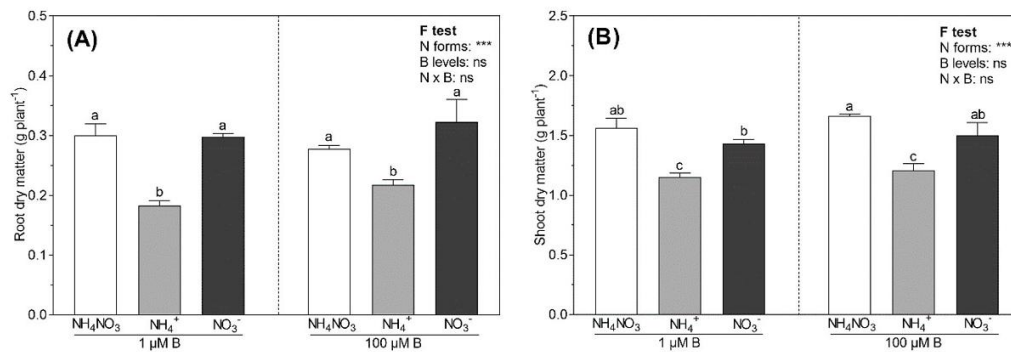
### 3.5. Concentration of macronutrients and micronutrients in the shoots

The concentrations of macronutrients (N, P, K, Ca, Mg) in shoots did not differ between the  $^{10}\text{B}$  levels, however, there were significant effects of the N forms (Fig. 6A–E). The concentrations of N and P in shoots of  $\text{NO}_3^-$ -fed plants were similar, but those of K, Ca and Mg were 7%, 7% and 8% higher, respectively, as compared to  $\text{NH}_4\text{NO}_3$ -fed plants. In  $\text{NH}_4^+$ -fed plants, shoot concentrations of N, P, K, Ca and Mg were 52%, 59%, 35%, 41% and 44% lower as compared to  $\text{NH}_4\text{NO}_3$ -fed plants.

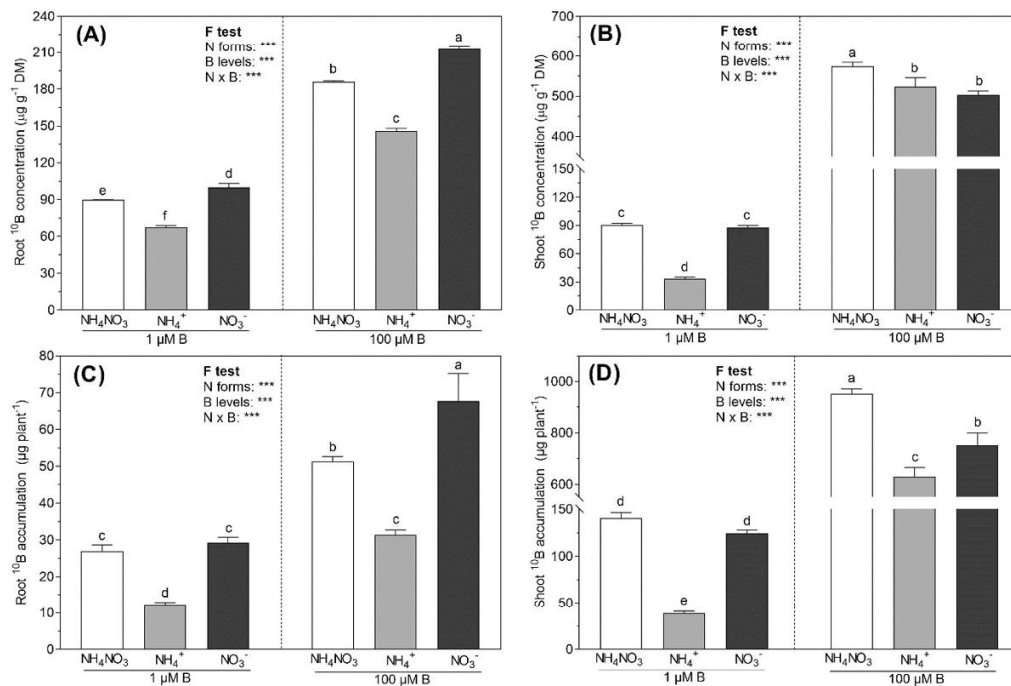
With the exceptions of Cu, Fe and Mo concentrations in shoots, which were 24%, 9% and 18% higher at the high compared to the low  $^{10}\text{B}$  level in  $\text{NH}_4\text{NO}_3$ -fed plants, the concentrations of other micronutrients did not differ between the  $^{10}\text{B}$  levels (Fig. 6F–J). The effect of  $\text{NO}_3^-$  nutrition on micronutrient concentrations was nutrient specific: concentrations of Zn and Mo were statistically similar to those of  $\text{NH}_4\text{NO}_3$ -fed plants at either  $^{10}\text{B}$  level, but that of Mn was 18% higher. Cu concentrations were higher in  $\text{NO}_3^-$ -fed plants only at the low  $^{10}\text{B}$  level, while Fe concentrations were reduced at high B supply (Fig. 6I–F). However,  $\text{NH}_4^+$ -fed plants had 43%, 45%, 33% and 21% lower concentrations of Fe, Mn, Mo and Zn in shoots, respectively, as compared to  $\text{NH}_4\text{NO}_3$ -fed plants.

### 3.6. Relative expression of *BnaBOR1; 2* and *BnaNIP5; 1* in roots and shoots

The relative expression of both *BnaBOR1;2* and *BnaNIP5;1* was differentially affected by N forms and B level (Fig. 7). At the low level of  $^{10}\text{B}$  supply, relative expression of *BnaBOR1;2* was not significantly affected in roots of  $\text{NH}_4^+$ -fed as compared to  $\text{NH}_4\text{NO}_3$ -fed plants, but it increased more than 2-fold in roots of  $\text{NO}_3^-$ -fed plants. At the high  $^{10}\text{B}$



**Fig. 3.** Root (A) and shoot (B) dry matter yields of *Brassica napus* grown with low (1 μM) and high (100 μM) levels of B and different forms of N (NH<sub>4</sub>NO<sub>3</sub>, NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>) in nutrient solution for 5 days. Bars represent means (±SE) of four independent pot replicates. Different letters indicate significant differences at  $P = 0.05$ . Level of significance: ns, non-significant; \*\*\* $P < 0.001$ .



**Fig. 4.** <sup>10</sup>Boron concentration in roots (A) and shoots (B) and <sup>10</sup>B accumulation in roots (C) and shoots (D) of *Brassica napus* grown with low (1 μM) and high (100 μM) levels of <sup>10</sup>B and different forms of N (NH<sub>4</sub>NO<sub>3</sub>, NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>) in nutrient solution for 5 days. Bars represent means (±SE) of four independent pot replicates. Different letters indicate significant differences at  $P = 0.05$ . Level of significance: ns, non-significant; \*\*\* $P < 0.001$ .

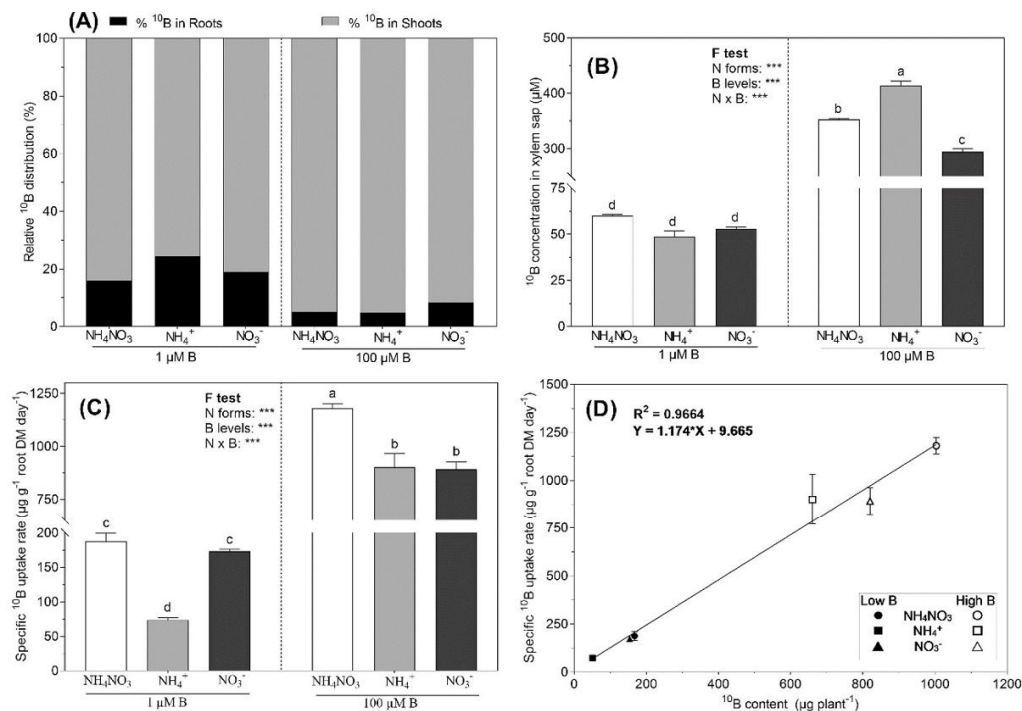
level, the relative expression of *BnaBOR1;2* in roots of plants was statistically similar between all the N forms. In shoots, the relative expression of *BnaBOR1;2* was 23 and 13-fold higher in NH<sub>4</sub><sup>+</sup>-fed plants at low and high <sup>10</sup>B level, respectively, as compared to NH<sub>4</sub>NO<sub>3</sub>-fed plants (Fig. 7B). However, no effect was observed in NO<sub>3</sub><sup>-</sup>-fed plants.

At low <sup>10</sup>B level, the relative expression of *BnaNIP5;1* was increased 6-fold in roots and 72-fold in shoots by NH<sub>4</sub><sup>+</sup> nutrition as compared to NH<sub>4</sub>NO<sub>3</sub> supply, whereas it was not significantly changed by NO<sub>3</sub><sup>-</sup> nutrition (Fig. 7C and D). At high <sup>10</sup>B level, there was no significant effect of the different N forms.

#### 4. Discussion

At the 100 μM <sup>10</sup>B level, <sup>10</sup>B concentrations and accumulation by *B. napus* roots and shoots were substantially higher than those recorded at the 1 μM <sup>10</sup>B level (Fig. 4), confirming the common observation that increasing the external supply of B increases its concentration in plant tissues (Eggert and von Wiren, 2016; Koohkan and Maftoun, 2016). This is also well in line with higher specific <sup>10</sup>B uptake rates in *B. napus* plants at higher external <sup>10</sup>B level (Fig. 5C), which strongly correlated with the <sup>10</sup>B content in plant tissues (Fig. 5D). Several fold higher <sup>10</sup>B concentrations and contents in shoots than in roots (Fig. 4) are in line with the accumulation of B at the end of the transpiration stream (Bellaloui and Brown, 1998). Overall, low and high <sup>10</sup>B levels resulted in similar root





**Fig. 5.** Relative distribution of  $^{10}\text{B}$  between roots and shoots (A),  $^{10}\text{B}$  concentrations in xylem sap (B), specific  $^{10}\text{B}$  uptake rates (C), and the correlation between specific  $^{10}\text{B}$  uptake rate and  $^{10}\text{B}$  content (D) in *Brassica napus* grown with low (1  $\mu\text{M}$ ) and high (100  $\mu\text{M}$ ) levels of  $^{10}\text{B}$  and different forms of N ( $\text{NH}_4\text{NO}_3$ ,  $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) in nutrient solution for 5 days. Data points represent means ( $\pm$ SE) of four independent replicates. Different letters indicate significant differences at  $P = 0.05$ . Level of significance: ns, non-significant; \*\*\* $P < 0.001$ .

and shoot dry matter (Fig. 3), indicating that a concentration of 1  $\mu\text{M}$   $^{10}\text{B}$ , even though clearly causing B deficiency in rape grown for longer time (45 days) (Asad et al., 2002), did not inhibit plant growth when supplied for only 5 days. Accordingly, the concentrations of  $^{10}\text{B}$  at 1  $\mu\text{M}$   $^{10}\text{B}$  supply were well above the reported critical level (18 mg B  $\text{kg}^{-1}$  DM) for *B. napus* (Asad et al., 2002) in all treatments. Nevertheless, we already observed an upregulation of two genes related to B uptake and transport under low  $^{10}\text{B}$  supply (Fig. 7), both in roots ( $\text{NH}_4^+$  and  $\text{NO}_3^-$  nutrition) and in shoots ( $\text{NH}_4^+$  nutrition). Under limited B supply, B uptake and translocation by plants can be enhanced by induction of facilitated and active transport mechanisms through channel proteins belonging to the NIP and the BOR family of efflux transporters (Brown et al., 2002; Takano et al., 2002; Miwa et al., 2010). While BOR1 regulates B loading into the xylem under limited B supply, and is tightly controlled by external B availability (Takano et al., 2005), the channel NIP5;1 facilitates the transport of  $\text{H}_3\text{BO}_3$  into the cell following a concentration gradient (Takano et al., 2006). Although it is well known that the *BnaNIP5;1* and *BnaBOR1;2* genes are ubiquitously expressed and have a significant role in B absorption and translocation in *B. napus* under all B supply levels (Yang et al., 2013; Diehn et al., 2019; Feng et al., 2019), especially *BnaNIP5;1* was strongly expressed under B limiting conditions in *B. napus* (Diehn et al., 2019). In the present study, expression of both genes in roots was not affected at high B supply, suggesting that *BnaBOR1;2* and *BnaNIP5;1* had low level of involvement in B uptake under high B supply, which is fully in line with observations by Yang et al. (2013) and Diehn et al. (2019). However, the observed upregulation of both genes under low B supply might indicate that, even though total B concentrations in roots were still higher than the critical level for canola, roots either perceived the drop in B concentrations ( $\text{NH}_4^+$  nutrition), or the decline in free (non-bound) B ( $\text{NO}_3^-$  nutrition),

and responded by increasing the uptake capacity before an absolute deficiency occurred.

Nitrogen forms differed in their effect on  $^{10}\text{B}$  concentrations and  $^{10}\text{B}$  accumulation in canola plants, depending on the supplied  $^{10}\text{B}$  level. Compared to  $\text{NH}_4\text{NO}_3$ -fed plants,  $\text{NO}_3^-$ -fed plants accumulated relatively more  $^{10}\text{B}$  in roots, and less in shoots, at both  $^{10}\text{B}$  supply levels (Fig. 5A), which is similar to results obtained for bentgrass (McCrimmon and Karnok, 1992). The higher B proportion in roots of  $\text{NO}_3^-$ -fed plants indicates that  $\text{NO}_3^-$  supply tended to restrict B translocation to shoots, which resulted in a higher  $^{10}\text{B}$  accumulation in roots. This is also supported by the lower B concentrations in the xylem sap of  $\text{NO}_3^-$ -fed plants at all  $^{10}\text{B}$  levels (even though significant only at high B supply, Fig. 5B). Since transpiration rates were similar between  $\text{NO}_3^-$ -fed and  $\text{NH}_4\text{NO}_3$ -fed plants (Fig. 2), this higher  $^{10}\text{B}$  retention in roots suggests that the mechanism for translocation of B depended on the pH of the nutrient solution, and not on reduced transpiration rates. Indeed, the pH of the nutrient solution containing  $\text{NO}_3^-$  substantially increased (by 1.5 units per 24 h), while that of  $\text{NH}_4^+$  containing nutrient solution decreased (by 2.0 units per 24 h) as compared to nutrient solution containing  $\text{NH}_4\text{NO}_3$  during 5 days of growth (Fig. 1). One could thus speculate that at pH 7.5, a larger proportion of B was present as the anionic borate ion, which might have been bound more strongly in the cell walls (bearing positive charges) of roots (Matoh, 1997), thus reducing free B in roots and B transport into the shoots. This possibility is further supported by previous studies indicating a stabilization of B-complexes as well as acceleration of the B-complex formation by divalent cations (Chormova and Fry, 2015; Funakawa and Miwa, 2015; O'Neill et al., 1996; Ishii et al., 1999). Such a local reduction of free boric acid would also explain, at least in part, why *BnaBOR1;2* (and to some extent also *BnaNIP5;1*) expression was upregulated in roots, but not in shoots of  $\text{NO}_3^-$ -fed plants

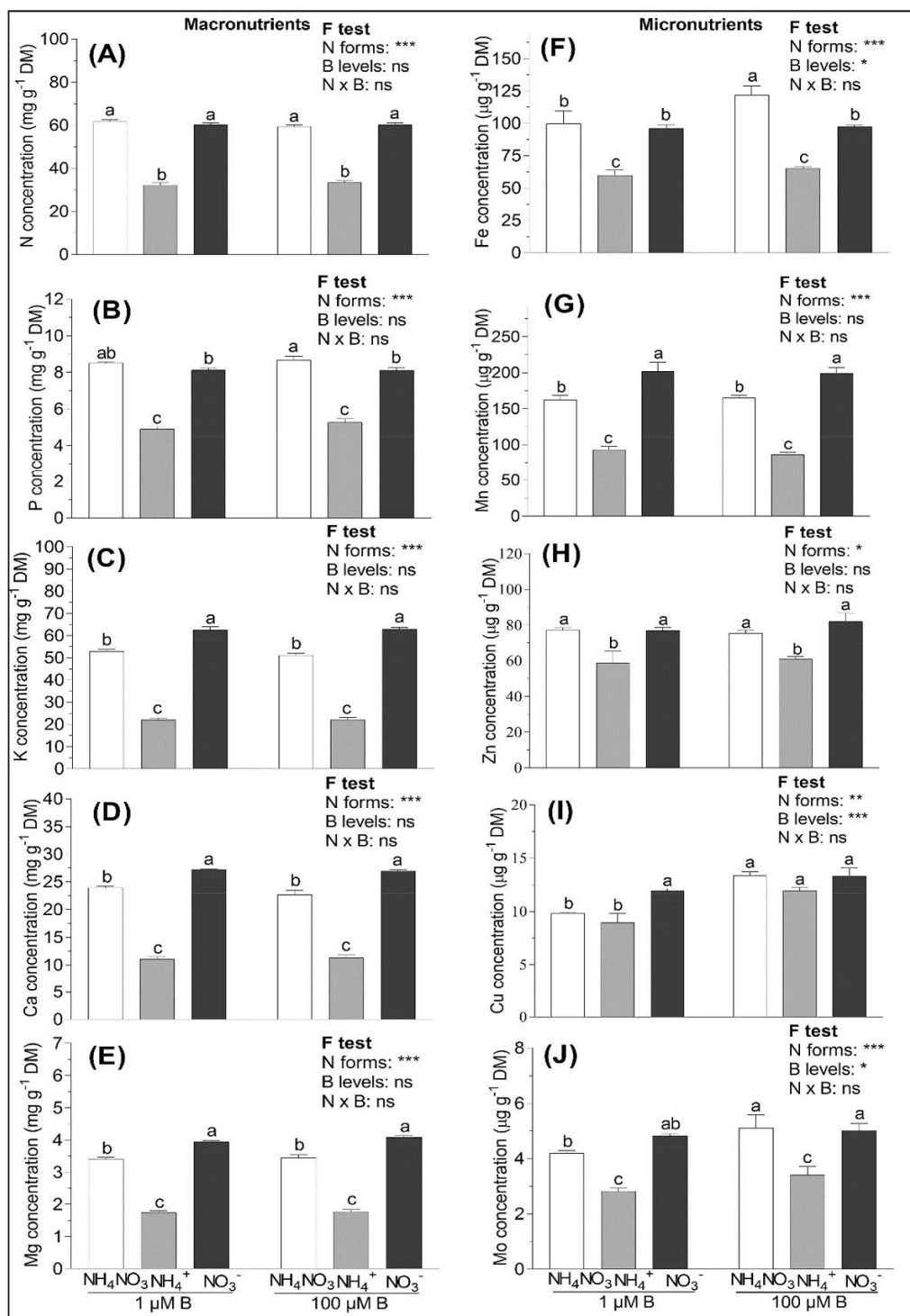


Fig. 6. Concentrations of macronutrients (A–E) and micronutrients (F–J) in shoots of *Brassica napus* grown with low (1 μM) and high (100 μM) levels of <sup>10</sup>B and different forms of N (NH<sub>4</sub>NO<sub>3</sub>, NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>) in nutrient solution for 5 days. Bars represent means (±SE) of four independent pot replicates. Different letters indicate significant differences at *P* = 0.05. Level of significance: ns, non-significant. \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001.

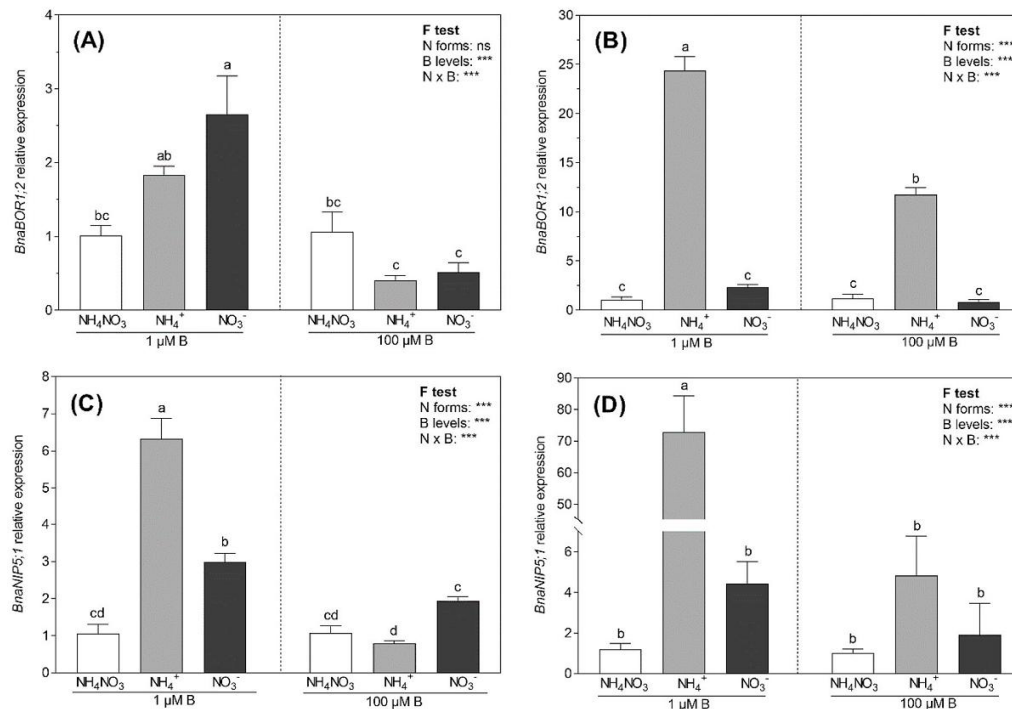


Fig. 7. Relative expression of *BnaBOR1;2* and *BnaNIP5;1* gene in roots (A, C) and shoots (B, D) of *Brassica napus* grown with low (1  $\mu\text{M}$ ) and high (100  $\mu\text{M}$ ) level of  $^{10}\text{B}$  and different nitrogen forms ( $\text{NH}_4\text{NO}_3$ ,  $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) in nutrient solution for 5 days. Bars represent means ( $\pm$ SE) of three independent pot replicates. Different letters indicate significant differences at  $P = 0.05$ . Level of significance: ns, non-significant, \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

as compared to  $\text{NH}_4\text{NO}_3$ -fed plants under low B supply, even though total B concentrations in roots were almost identical between both the treatments. To clarify this question, it will be interesting to investigate the ratio between free and bound B fractions in roots under different N forms in future experiments.

The situation was different under  $\text{NH}_4^+$  supply, where root and shoot dry matter, transpiration rates,  $^{10}\text{B}$  concentrations and  $^{10}\text{B}$  contents were always significantly lower than under  $\text{NH}_4\text{NO}_3$  nutrition, regardless of the external  $^{10}\text{B}$  level (Figs. 2–4). The reduced dry matter production cannot be attributed to the  $\text{NH}_4^+$ -induced decrease in  $^{10}\text{B}$  concentration, since on the one hand  $^{10}\text{B}$  concentration in  $\text{NH}_4^+$ -fed plants at 100  $\mu\text{M}$  was still much higher than that of plants grown with  $\text{NH}_4\text{NO}_3$  and/or  $\text{NO}_3^-$  at 1  $\mu\text{M}$   $^{10}\text{B}$ , and on the other hand tissue  $^{10}\text{B}$  concentrations were always higher than the reported critical level of 18  $\text{mg B kg}^{-1}$  DM for *B. napus* (Asad et al., 2002). The reduction in  $^{10}\text{B}$  content can be explained largely by the reduced dry matter, which was likely an effect of acidity toxicity. At high  $^{10}\text{B}$  supply, where B is mainly taken up by passive diffusion (Wimmer and Eichert, 2013), reduced  $^{10}\text{B}$  accumulation might be assumed to be associated with the lower transpiration rates, which result in an overall lower water flow and thus B supply into the plants. However,  $^{10}\text{B}$  accumulation was equally reduced under low  $^{10}\text{B}$  supply, which indicates that not the decline in transpiration, but rather the decline in pH to 4.0 was toxic to the plants by restricting the uptake of other nutrients and leading to nutrient deficiencies. In fact, the pH of the  $\text{NH}_4^+$ -containing nutrient solution recorded after every 24 h of plant growth was much lower than the critical soil pH values (4.9–5.7) reported for canola growth in two ultisols (Abdulaha-Al Baqay et al., 2017).

Acidity is known to inhibit plant growth by a combination of several factors, including among others proton toxicity as well as deficiencies of Mg, Ca, P and Mo (Marschner, 2012). Accordingly, in the present study,

concentrations of all measured macro- and micronutrients (with the exception of Cu) were significantly reduced in  $\text{NH}_4^+$ -fed plants (Fig. 6), which could have been a consequence of inhibited root growth in combination with acid-induced inhibition of cation uptake (Marschner, 2012). The mean N concentration in the shoots of  $\text{NH}_4^+$ -fed plants (33  $\text{mg g}^{-1}$  DM) was less than the concentration (34  $\text{mg g}^{-1}$  DM) at which Brennan et al. (2019) found 5–15% yield loss in canola plants in Australia. Similarly, the mean K concentration (22  $\text{mg g}^{-1}$  DM) was lower than 30  $\text{mg g}^{-1}$  DM, at which canola plants suffer from 25% or more yield loss (Brennan et al., 2019). Brennan and Bolland (2006) reported that canola plants responded to K fertilization at a tissue concentration of less than 45  $\text{mg kg}^{-1}$  DM. The shoot Ca concentration of  $\text{NH}_4^+$ -fed plants was also less than the value required for the normal growth of canola plants, i.e. 16  $\text{mg g}^{-1}$  DM (Campbell, 2000). Among micronutrients, only the shoot concentration of Fe decreased to a level which is deficient for brassica plant growth, i.e. below 100  $\mu\text{g g}^{-1}$  DM (Brennan et al., 2019). It can thus be concluded that  $\text{NH}_4^+$ -induced acidity was the main factor leading to growth inhibition and nutrient deficiencies, irrespective of the  $^{10}\text{B}$  supply level.

Despite this overall effect,  $^{10}\text{B}$  xylem concentration and  $^{10}\text{B}$  distribution between roots and shoots was differently affected by  $\text{NH}_4^+$  nutrition depending on the  $^{10}\text{B}$  supply level: compared to  $\text{NH}_4\text{NO}_3$  nutrition, xylem B concentration was not changed but relatively more  $^{10}\text{B}$  accumulated in roots under low  $^{10}\text{B}$  supply, while xylem  $^{10}\text{B}$  concentration was increased but no change in distribution was observed under high  $^{10}\text{B}$  supply (Fig. 5B). At this point we can only speculate that this difference might be related to the upregulation of B transporters/channels under low B supply, while at the same time translocation into the shoot was inhibited by the reduced transpiration rates, leading to accumulation of B in the roots. At the high  $^{10}\text{B}$  supply, reduced transpiration rates in combination with a high influx rate by diffusion

through the membrane at an external pH of 4.0, where nearly 100% of the external B is present in the form of the uncharged boric acid, may have caused a concentration effect, which could explain the higher  $^{10}\text{B}$  concentration in the xylem sap of  $\text{NH}_4^+$ -fed compared to  $\text{NH}_4\text{NO}_3$ -fed plants.

Overall, upregulation of *BnaNIP5;1* and *BnaBORI;2* under low  $^{10}\text{B}$  supply was significantly more pronounced under  $\text{NH}_4^+$  compared to  $\text{NO}_3^-$  nutrition (with the exception of *BnaBORI;2* in roots; Fig. 7). This is in accordance with the significantly lower  $^{10}\text{B}$  concentrations in roots, shoots and xylem of  $\text{NH}_4^+$ -fed plants. The strong upregulation of both genes in the shoots of  $\text{NH}_4^+$ -fed plants might indicate that shoots had a higher requirement for B compared to roots, and in addition suggests an important role of shoots to ensure B fluxes throughout the plant especially under low B supply.

In a previous study, Tsadilas et al. (2005) reported that  $\text{NH}_4^+$  fertilization did not affect the pH of an artificially limed acidic soil and hence could not counteract the liming-induced decline in hot water extractable B as well as the reduced B accumulation in tobacco plants. This indicates the fundamental differences between soil and hydroponic studies. In soil, the applied lime was obviously capable of buffering the acidic effect produced by  $\text{NH}_4^+$  uptake by the plants, before it could affect B uptake. Hence, our finding that  $\text{NH}_4^+$  nutrition suppresses B uptake by lowering the pH of the nutrient solution with an original pH value of 6.5 cannot be extrapolated to an alkaline soil where  $\text{NH}_4^+$  supply is not expected to induce such a sharp decline in pH to negatively affect plant growth. Rather, in alkaline soils,  $\text{NH}_4^+$  supply is expected to induce a moderate decline in rhizosphere pH that would reduce B binding and thus have a beneficial effect on B uptake by the plants, especially at lower B supply.

Overall, the results of the present study suggest that: 1) N forms in nutrient solution have an impact on B uptake and transport into the shoot, which depends on the supplied B level, 2) ammonium-induced acidity can induce upregulation of B transport mechanisms, even though B concentrations do not indicate B deficiency, 3) nitrate-induced alkalization results in altered B distribution between roots and shoots due to a restriction of B transport.

#### Author contributions

K. H. M. and M. W. conceptualization of the hypothesis, designed the experiments and critically reviewed the manuscript. A. Q. D. conducted the experiments. A. Q. D. and A.N. analyzed the data and wrote the manuscript. A. Q. D. and A. S. performed qRT-PCR analysis. All authors read and approved the final manuscript.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.plaphy.2021.02.009>.

#### References

- Abdulah-Al Baquy, M., Li, J.Y., Xu, C.Y., Mehmood, K., Xu, R.K., 2017. Determination of critical pH and Al concentration of acidic Ultisols for wheat and canola crops. *Solid Earth* 8, 149–159.
- Alt, D., Schwarz, W., 1973. Boron toxicity, boron uptake and boron distribution in young cucumber plants under influence of N-form. *Plant Soil* 39, 277–283.
- Asad, A., Blamey, F.P.C., Edwards, D.G., 2002. Dry matter production and boron concentrations of vegetative and reproductive tissues of canola and sunflower plants grown in nutrient solution. *Plant Soil* 243, 243–252.
- Bellaloui, N., Brown, P.H., 1998. Cultivar differences in boron uptake and distribution in celery (*Aptium graveolens*), tomato (*Lycopersicon esculentum*) and wheat (*Triticum aestivum*). *Plant Soil* 198, 153–158.
- Bolaños, L., Lukaszewski, K., Bonilla, I., Blevins, D., 2004. Why boron? *Plant Physiol. Biochem.* 42, 907–912.
- Brennan, R., Blake, A., Sharma, D., Bucat, J., Seymour, M., Harries, M., 2019. Critical nutrient levels for canola in Western Australia. <https://www.wagrigwagovau/soil-nutrients/critical-nutrient-levels-canola-western-australia>.
- Brennan, R.F., Bolland, M.D.A., 2006. Soil and tissue tests to predict the potassium requirements of canola in south-western Australia. *Aust. J. Exp. Agric.* 46, 675–679.
- Brown, P.H., Bellaloui, N., Wimmer, M.A., Bassil, E.S., Ruiz, J., Hu, H., Pfeffer, H., Dannel, F., Romheld, V., 2002. Boron in plant biology. *Plant Biol.* 4, 205–223.
- Campbell, C., 2000. Reference sufficiency ranges for plant analysis in the southern region of the United States. <https://agris.fao.org/agris-search/search.do?recordID=US201300090515>. Retrieved on November, 2020.
- Chatzissavvidis, C.A., Therios, I.N., Antonopoulou, C., 2007. Effect of nitrogen source on olives growing in soils with high boron content. *Aust. J. Exp. Agric.* 47, 1491–1497.
- Chormova, D., Fry, S.C., 2015. Boron bridging of rhamnogalacturonan-II is promoted in vitro by cationic chaperones, including polyhistidine and wall glycoproteins. *New Phytol.* 209, 241–251.
- Diehn, T.A., Bienert, M.D., Pommerrenig, B., Liu, Z.J., Spitzer, C., Bernhardt, N., Fuge, J., Bieber, A., Riche, N., Chaumont, F., Bienert, G.P., 2019. Boron demanding tissues of *Brassica napus* express specific sets of functional Nodulin26-like Intrinsic Proteins and BOR1 transporters. *Plant J.* 100, 68–82.
- Eggert, K., von Wiren, N., 2016. The role of boron nutrition in seed vigour of oilseed rape (*Brassica napus* L.). *Plant Soil* 402, 63–76.
- Feng, Y.N., Cui, R., Wang, S.L., He, M.L., Hua, Y.P., Shi, L., Ye, X.S., Xu, F.S., 2019. Transcription factor BnaA9.WRKY47 contributes to the adaptation of *Brassica napus* to low boron stress by up-regulating the boric acid channel gene BnaA3.NIP5;1. *Plant Biotechnol. J.* 18, 1241–1254.
- Funakawa, H., Miwa, K., 2015. Synthesis of borate cross-linked rhamnogalacturonan II. *Front. Plant Sci.* 6, 223.
- Gerendas, J., Heeschen, V., Kahl, S., Ratcliffe, R.G., Rudolph, H., 1998. An investigation of N metabolism and pH regulation in Sphagnum using in vivo nuclear magnetic resonance and stable isotope mass spectrometry. *Isot. Environ. Health Stud.* 34, 21–29.
- Goldbach, H.E., Wimmer, M.A., 2007. Boron in plants and animals: is there a role beyond cell-wall structure? *J. Plant Nutr. Soil Sci.* 170, 39–48.
- Gupta, U.C., 1980. Boron nutrition of crops. *Adv. Agron.* 31, 273–307.
- Ishii, T., Matsunaga, T., Pellerin, P., O'Neill, M.A., Darvill, A., Albersheim, P., 1999. The plant cell wall polysaccharide rhamnogalacturonan-II self-assembles into a covalently cross-linked dimer. *J. Biol. Chem.* 274, 13098–13104.
- Koohkan, H., Maftoun, M., 2016. Effect of nitrogen-boron interaction on plant growth and tissue nutrient concentration of canola (*Brassica napus* L.). *J. Plant Nutr.* 39, 922–931.
- Lauchli, A., Grattan, S.R., 2017. Plant stress under non-optimal soil pH. In: Shabala, S. (Ed.), *Plant Stress Physiology*. CABI Publishing, Wallingford, UK, pp. 201–216.
- Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(T)(-Delta Delta C) method. *Methods* 25, 402–408.
- Loomis, W.D., Durst, R.W., 1992. Chemistry and biology of boron. *Biofactors* 3, 229–239.
- Marschner, P., 2012. *Marschner's Mineral Nutrition of Higher Plants*. Elsevier Academic Press Inc., San Diego, USA.
- Matoh, T., 1997. Boron in plant cell walls. *Plant Soil* 193, 59–70.
- McCrimmon, J.N., Karnok, K.J., 1992. Nitrogen form and the seasonal root and shoot response of creeping bentgrass. *Commun. Soil Sci. Plant Anal.* 23, 1071–1088.
- McCrimmon, J.N., Mills, H.A., Karnok, K.J., 1992. Effect of N-form on macronutrient and micronutrient concentration and uptake of creeping bentgrass. *J. Plant Nutr.* 15, 1269–1289.
- Miwa, K., Fujiwara, T., 2010. Boron transport in plants: co-ordinated regulation of transporters. *Ann. Bot.* 105, 1103–1108.
- Miwa, K., Tanaka, M., Kamiya, T., Fujiwara, T., 2010. Molecular mechanisms of boron transport in plants: involvement of arabidopsis NIP5;1 and NIP6;1. In: Jahn, T.P., Bienert, G.P. (Eds.), *Mips and Their Role in the Exchange of Metalloids*. Springer-Verlag Berlin, Berlin, pp. 83–96.
- Miwa, K., Takano, J., Omori, H., Seki, M., Shinozaki, K., Fujiwara, T., 2007. Plants tolerant of high boron levels. *Science* 318, 1417, 1417.
- O'Neill, M.A., Warrenfeltz, D., Kates, K., Pellerin, P., Doco, T., Darvill, A.G., Albersheim, P., 1996. Rhamnogalacturonan-II, a pectic polysaccharide in the walls of growing plant cell, forms a dimer that is covalently cross-linked by a borate ester. *J. Biol. Chem.* 271, 22923–22930.
- Pommerrenig, B., Junker, A., Abreu, I., Bieber, A., Fuge, J., Willner, E., Bienert, M.D., Altmann, T., Bienert, G.P., 2018. Identification of rapeseed (*Brassica napus*) cultivars with a high tolerance to boron-deficient conditions. *Front. Plant Sci.* 9, 1142.
- Shorrocks, V.M., 1997. The occurrence and correction of boron deficiency. *Plant Soil* 193, 121–148.

- Takano, J., Miwa, K., Yuan, L.X., von Wiren, N., Fujiwara, T., 2005. Endocytosis and degradation of BOR1, a boron transporter of *Arabidopsis thaliana*, regulated by boron availability. *Proc. Natl. Acad. Sci. U.S.A.* 102, 12276–12281.
- Takano, J., Wada, M., Ludewig, U., Schaaf, G., von Wiren, N., Fujiwara, T., 2006. The *Arabidopsis* major intrinsic protein NIP5;1 is essential for efficient boron uptake and plant development under boron limitation. *Plant Cell* 18, 1498–1509.
- Takano, J., Noguchi, K., Yasumori, M., Kobayashi, M., Gajdos, Z., Miwa, K., Hayashi, H., Yoneyama, T., Fujiwara, T., 2002. *Arabidopsis* boron transporter for xylem loading. *Nature* 420, 337–340.
- Tsadilas, C.D., Kassioti, T., Mitsios, I.K., 2005. Influence of liming and nitrogen forms on boron uptake by tobacco. *Commun. Soil Sci. Plant Anal.* 36, 701–708.
- Wang, X.T., Below, F.E., 1998. Accumulation and partitioning of mineral nutrients in wheat as influenced by nitrogen form. *J. Plant Nutr.* 21, 49–61.
- Wang, L.H., Xu, F.S., Wang, Y.H., 2007. Effects of B, Mo, Zn interaction on the seed yield and quality of *Brassica napus*. *Plant Nutr. Fert. Sci.* 19, 53–59.
- Wimmer, M.A., Eichert, T., 2013. Review: mechanisms for boron deficiency-mediated changes in plant water relations. *Plant Sci.* 203, 25–32.
- Wimmer, M.A., Goldberg, S., Gupta, U.C., 2015. Boron. In: Barker, A., Pilbeam, D. (Eds.), *Handbook of Plant Nutrition*, second ed. CRC Press, Florida, USA, pp. 305–346.
- Wu, Z.C., Zhao, X.H., Sun, X.C., Tan, Q.L., Tang, Y.F., Nie, Z.J., Hu, C.X., 2015. Xylem transport and gene expression play decisive roles in cadmium accumulation in shoots of two oilseed rape cultivars (*Brassica napus* L.). *Chemosphere* 119, 1217–1223.
- Yang, L., Zhang, Q., Dou, J.N., Li, L., Guo, L.F., Shi, L., Xu, F.S., 2013. Characteristics of root boron nutrition confer high boron efficiency in *Brassica napus* cultivars. *Plant Soil* 371, 95–104.

## Supplementary data

**Table S1.** Primers of *BnaBOR1;2* and *BnaNIP5;1* designed for real-time PCR.

Genes	Primers	Genbank accession number
<i>BnaBOR1;2</i>	F-5' - CCGTTGTACAGGAGATGCTTG -3'	GU827643
	R-5' - AACCCAAGAAGAAGCAAGTCG -3'	
<i>BnaNIP5;1</i>	F-5' - GGGGCTCACTTAAATCCATCAC -3'	KT899999
	R-5' - CCAACACTAACAGACGGAACAG -3'	
<i>Actin</i>	F-5' - TCTGGTGATGGTGTGTCTCA -3'	AF111812
	R-5' - GGTC AACATGTACCCTCTCTCG -3'	

Primer design was done by using Primer3plus and primer-blast online software.

# **Chapter 5**

## **General Discussion**

## Chapter 5. General Discussion

Boron (B) is an essential element for the growth of vascular plants (Brown et al., 2002). The important roles of B in the plants have been comprehensively reviewed by Cakmak and Römheld (1997) and Brown et al. (2002). The uptake and translocation of B by plant roots are affected by several factors, i.e., plant species, B level in the rooting medium, soil nutrient status, forms of nutrients applied, soil pH etc. (Wimmer et al., 2015). Many of these factors have been studied previously; however, there is still a lack of knowledge in evaluating different B concentrations, foliar resupply of B and effects of N forms on B uptake and translocation, especially in oilseed rape (*Brassica napus* L.). Consequently, following three studies were conducted to explore these aspects of B nutrition. The second chapter describes about the critical concentration of B required for optimum growth of oilseed rape under hydroponic condition. In the third chapter, the physiological and growth responses of B-deficient oilseed rape plants to foliar and roots resupply of B were investigated. In the fourth chapter, the effect of different nitrogen forms on B uptake and distribution, and the relative expression of specific genes involved in B uptake was studied.

### 5.1. Effect of B supply on biomass and B concentration in oilseed rape

In this first experiment, hydroponic-based plant cultivation was conducted to establish the critical concentration of B required for optimum growth of oilseed rape. Based on the results of this experiment, two B levels, critical and optimum, were identified for the other experiments. With an optimistic expectation, covering a wide range of B supply from lowest to highest, the growth response of plants was found to follow the optimum curve (Chapter 2, Fig. 2). The present work depicted a similar normal distribution curve as expatiated by Huang et al. (1996) and Asad et al. (1997). Nonetheless, slight symptoms of B toxicity on roots and leaves of oilseed rape plants were observed at the highest B rate (1000  $\mu\text{M}$  B) even before significant growth retardation. It was found that at the highest rate of B supply (1000  $\mu\text{M}$ ), the roots and shoots dry matters of oilseed rape were reduced as compared to the sufficient B rate (25  $\mu\text{M}$  B) (Chapter 2, Fig. 2). This finding is in accordance with what was reported by Reid et al. (2004). Obvious symptoms of deficiency such as reduced leaf elongation, downward cupping, brittleness of tissues and darker appearance of roots and shoots were observed in oilseed rape grown at the lowest rate of B supply (0.25  $\mu\text{M}$  B) (Chapter 2, Fig.1; Chapter 3, Fig. 1). This observation agrees with the findings of Asad et al. (1997) and Savic et al. (2012) who reported the deformation and brittleness of leaves accompanied with the growth retardation at the apical meristems of roots and shoots as possible symptoms of B deficiency in plants. The maximum vegetative growth and biomass performance of oilseed rape plants were obtained at 25  $\mu\text{M}$



external B concentration. Based on the definition of critical value according to Goldbach et al. (2000) and Reid et al. (2004) (the minimum tissue or external concentration of a nutrient required to produce 90% of the maximum yield or biomass), the critical B concentration in the nutrient solution for oilseed rape in the present work was established to be 1  $\mu\text{M}$  B. In this study, B deficiency symptoms occurred on roots and leaves with B concentration less than 14  $\text{mg B kg}^{-1}$  DM. This concentration lies within the range of the critical B concentration (10-14  $\text{mg B kg}^{-1}$  DM) for the full expanded leaves of *B. napus* (cv. Eureka.) reported by Huang et al. (1996), while lower than that of reported by Asad et al. (2002) (18  $\text{mg B kg}^{-1}$  DM) 75 days old oilseed rape grown in the nutrient solution.

The B concentrations in different parts of oilseed rape plants in the current study also showed that B concentrations in the shoot are higher than in the corresponding roots. The results in this study support the notion that the transpiration stream significantly induces B distribution in oilseed rape, which is denoted in the accumulation of B (Wimmer & Eichert, 2013). It could also be explained in general that increasing external B concentration in the nutrient solution remarkably increases the internal B concentration of oilseed rape (Asad et al., 2002; Reid et al., 2004; Eggert & von Wiren, 2016). This clearly indicates that B concentrations in plant parts are directly proportional to the external B concentration (Chapter 2, Fig. 2; Chapter 3, Fig. 2), which is in agreement with observation by Koohkan and Maftoun (2016).

Increasing B supply in the nutrient solution and resupply B via the roots and leaves to B-deficient plants significantly increased B concentration in the mature leaves and young leaves of oilseed rape plants as compared to the root tissues (Chapter 2, Fig. 3; Chapter 3, Fig. 3). Similarly, in broccoli under sufficient B supply (Shelp et al., 1996), sunflower (Asad et al., 2003; Mator & Ochiai, 2005) and peanut (Konsaeng et al., 2010), foliar-applied B has been shown to absorb by the leaves. In addition, Eichert and Goldbach (2010) and Stangoulis et al. (2010) have mentioned that B can be translocated from the mature leaves to the young leaves when deficiency is prolonging to several days. It is well known that B is transported to the new leaves via transpiration stream (Brown & Shelp, 1997; Shelp et al., 1998), and B may be transferred from xylem to phloem by a B transporter to the young tissues (Shelp et al., 1998; Takano et al., 2002). Some other studies in the literature have demonstrated that the concentration of B in roots was less affected during the short-term B deficiency than that in shoots (Dannel et al., 1998). This study showed that B concentration in the roots did not increase by leaf application of B even at the high B supply level (Chapter 3, Fig. 3). This was in agreement with Asad et al. (2003), who found that foliar-applied B did not affect B concentration in roots of sunflower. The possible reasons might be restricted transport of B

from treated leaves to roots and its recycling to shoots via xylem loading (Eichert & Goldbach, 2010). The other reason could be related to the dilution effect since B content in the roots of foliar B treated plants was not significantly different compared with the B-deficient plants (Fernández et al., 2013)

The increase of B supply in the nutrient solution significantly affected the distribution of B in the roots and shoots of oilseed rape plants. As expected, the relative distribution of B showed that B accumulated more in the roots under low B supply compared to the treatments with high B supply (Chapter 2, Fig. 3; Chapter 4, Fig. 4). These results correspond with those of Asad et al. (2001); Huang et al. (1996); Matoh (1997); Goldbach and Wimmer (2007) who found that B in the roots is bound to the cell wall of the roots and lacks translocation from root to shoot under low B supply.

## **5.2. Photosynthesis as influences by foliar B application**

Foliar application and roots resupply of B to B-deficient oilseed rape plants led to increased photosynthesis and transpiration rate as compared with the B-deficient plants (Chapter 3, Fig. 3), which were well in line with the increased roots and shoot biomass under these treatments. It is known that B deficiency or toxicity significantly reduces photosynthesis and transpiration rate. These effects seem to be related to several consequences such as reduced biomass and decreased protein and chlorophyll in the leaves (Reid et al., 2004; Landi et al., 2013; Chen et al., 2014). In this study, one-time foliar application of 60 mM B and resupply B via the roots significantly increased photosynthesis rate; however, it did not reach the level of positive control. These effects can be explained by the increased roots and shoot biomass, which might be due to the reason that plant have more capability to expand the leaves and accumulate more chlorophyll in the leaves (Wimmer & Eichert, 2013). The higher foliar application of B (150 mM) to B-deficient plants showed slight B toxicity which resulted in lower photosynthesis and reduced shoots biomass. The possible reason for this may be the disruption of stomatal function and reduced leaves water status (Wimmer & Eichert, 2013).

## **5.3. Impact of foliage and roots resupply B on the expression of B transporter genes**

With regard to the uptake and distribution of B with the exogenous B resupply via the roots or via the leaves to B-deficient plants, it was found that under B deficiency, B resupply via the leaves to B-deficient plants reversed the B-deficiency induced upregulation of *BnaNIP5;1* and *BnaBORI;2* in the roots but not to the level of B sufficient +B control plants (Chapter 3; Fig. 6). Since B concentration in the roots and leaves were close to the critical level for B deficiency, *BnaNIP5;1* channel and *BnaBORI;2* transporters were highly upregulated to enhance the absorption and transport of boric acid into the root cell and xylem to meet the B demands of

plants. Comparatively, Diehn et al. (2019) indicated that *BnaNIP5;1*, and *BnaBOR1;2* and its isoforms showed significant upregulation in the roots and leaves of oilseed rape under B-deficiency, suggesting that these genes are particularly important to deliver B to younger untreated leaves under B limiting period. Similarly, Yang et al. (2013) showed that upregulation of *BnaBOR1;2* and *BnaNIP5;1* might have played a fundamental role in B translocation in oilseed rape. Interestingly, foliar application of B to B-deficient oilseed rape plants induced upregulation in the relative expression of *BnaNIP5;1* and *BnaBOR1;2* in the roots but not in the leaves (Chapter 3; Fig. 6). This was supported by an increase in B concentration in the leaves of the foliage resupply treatments, while roots B concentration did not significantly differ from that of B-deficient oilseed rape plants. This might be explained by the moderate mobility of B in oilseed rape (Stangoulis et al., 2010), the free B can be translocation into the new leaves through the phloem, but not to the roots. Together with the increase in roots dry matter, low B concentration in the roots and upregulation of *BnaNIP5;1* in the foliar B treatments, B deficient conditions stimulated gene expression for the absorption of B into roots.

#### **5.4. Specific B uptake rates and B distribution in oilseed rape as affected by nitrogen forms**

The specific B-uptake rates is determined during the vegetative stage of growth from the total B content per plant and the root dry weights during the uptake period, when shoot and root growth are expected to be approximately exponential (Bellaloui & Brown, 1998; Wimmer et al., 2005). In this study, the effect of N forms on specific B uptake rate under low and high B supply in the nutrient solution was investigated. It was found that oilseed rape roots treated with  $\text{NH}_4\text{NO}_3$  had the highest specific B uptake rates at both low and high B supply levels (Chapter 3; Fig. 6B). At the low B supply, the specific B uptake rates were found to be higher in  $\text{NO}_3^-$ -fed plants compared with  $\text{NH}_4^+$ -fed plants in the present study. Since  $\text{NH}_4\text{NO}_3$  and  $\text{NO}_3^-$  treated plants had almost identical growth rates and dry matter of both roots and shoots, the lower B concentration in  $\text{NH}_4^+$  fed plant in xylem sap resulted in lower B uptake rate as well as B accumulation in the shoots compared with  $\text{NH}_4\text{NO}_3$  and  $\text{NO}_3^-$  fed plants under low B supply. In addition,  $\text{NH}_4\text{NO}_3$  and  $\text{NO}_3^-$  treated plants had higher water transpiration, which could be the reason for higher B uptake because plants absorb B via transpiration pull (Hu & Brown, 1997; Wimmer & Eichert, 2013). Nevertheless, the specific B uptake rates in  $\text{NH}_4^+$  and  $\text{NO}_3^-$  treated plants were not significantly different under high B supply. At an external pH of 4.0, nearly 100% of the external B is present in the form of the uncharged boric acid (Lauchli & Grattan, 2017). Consequently, a high B influx rate due to its diffusion through the membrane in

combination with reduced transpiration rates could explain the higher B concentration in the xylem sap of  $\text{NH}_4^+$ -fed plants compared to  $\text{NH}_4\text{NO}_3$ -fed plants.

Since B is taken up by plant via passive diffusion of  $\text{B}(\text{OH})_3$  in the root cell due to the high permeability of boric acid to plant cell membrane under high B supply (Hu & Brown, 1997), it is reasoned that low and high pH induced by  $\text{NH}_4^+$  and  $\text{NO}_3^-$  supply, respectively, is the primary factor controlling B uptake in oilseed rape plants. Thus, B uptake under adequate B supply could be associated with passive process (Brown et al., 2002) and facilitated uptake via MIPs channels, which can transport uncharged molecules (Dannel et al., 2002) and might be enhanced by  $\text{NH}_4^+$  as hypothesized. Brown et al. (2002) and Smith et al. (2013) reported that under slightly basic pH, only 10% of the B activity is represented in the form of  $\text{B}(\text{OH})_4^-$  and it is not likely reason to explain reduced plant B uptake. An increase in pH induced by  $\text{NO}_3^-$  nutrition in this study did not affect B uptake under high B supply.

The availability of B in the plants and its absorption is affected by the pH of the soil solution (Smith et al., 2013). Under low B supply, the relative expression of *BnaBOR1;2* and *BnaNIP5;1* were strongly upregulated in the shoots of  $\text{NH}_4^+$ -treated plants, this result indicated that shoots of oilseed rape plants have a higher requirement for B compare to roots. In addition, the results in this study confirm the important role of these genes to ensure the influx of B into the roots. In a previous study, Tsadilas et al. (2005) reported that  $\text{NH}_4^+$  fertilization did not affect the pH of an artificially limed acidic soil and hence could not counteract the liming-induced decline in hot water extractable B as well as the reduced B accumulation in tobacco plants. This shows the fundamental differences between soil and hydroponic studies. In soil, the applied lime was probably capable of buffering the acidic effect produced by  $\text{NH}_4^+$  uptake by the plants, before it could affect B uptake. Hence, our results that  $\text{NH}_4^+$  nutrition decreases B uptake by lowering the pH of the nutrient solution having initial pH value of 6.5 cannot be extrapolated to an alkaline soil where  $\text{NH}_4^+$  supply is not expected to induce such a sharp decline in pH to negatively affect plant growth. Rather, in alkaline soils,  $\text{NH}_4^+$  supply is likely to persuade a moderate decline in rhizosphere pH that would reduce B binding and thus would have a beneficial effect on B uptake by the plants, especially at lower B supply.

### **5.5. Ammonium nutrition enhances the expression of *BnaBOR1;2* and *BnaNIP5;1* in the roots and shoots of oilseed rape under marginal B supply**

The responses of specific genes related to the absorption and distribution of B in oilseed rape, *i.e.* *BnaBOR1;2* and *BnaNIP5;1* (Yang et al., 2013; Hua et al., 2017; Diehn et al., 2019) were investigated under different N forms at low and high B supply. Diehn et al. (2019), Feng et al. (2019) and (Yang et al., 2013) reported that the *BnaNIP5;1* and *BnaBOR1;2* genes are

ubiquitously expressed and have a significant role in B absorption and translocation in oilseed rape under all B supply levels, especially *BnaNIP5;1* was strongly expressed under B limiting conditions in oilseed rape. In this study, the expression level of both genes in roots was not affected at high B supply, suggesting that *BnaBORI;2* and *BnaNIP5;1* had low level of involvement in B uptake under high B supply, which is fully in line with observations by Yang et al. (2013) and Diehn et al. (2019). The relative expressions of these genes were upregulated under low B supply, even the given B concentration in the nutrient solution was still higher than the critical concentration for oilseed rape. This may be explained possibly by the fact that the roots perceived the drop in B concentrations under  $\text{NH}_4^+$  nutrition or the decline in free B in  $\text{NO}_3^-$  nutrition and responded in advance by increasing the uptake capacity before an absolute deficiency occurred. A local reduction in free boric acid under  $\text{NO}_3^-$  nutrition would also explain, at least in part, why *BnaBORI;2* (and to some extent also *BnNIP5;1*) expression was upregulated in roots, but not in shoots of  $\text{NO}_3^-$ -fed plants as compared to  $\text{NH}_4\text{NO}_3$ -fed plants under low B supply (Chapter 4, Fig. 7), even though total B concentrations in roots were almost identical between both the treatments. Hence, under acidic soil conditions,  $\text{NH}_4^+$  nutrition can induce upregulation of B transport mechanisms, even though B concentrations do not indicate B deficiency, while  $\text{NO}_3^-$  nutrition results in altered B distribution between roots and shoots due to a restriction of B transport.

## 5.6. References

- Asad, A., Bell, R. W., & Dell, B. (2001). A critical comparison of the external and internal boron requirements for contrasting species in boron-buffered solution culture *Plant and Soil*, 233(1), 31-45.
- Asad, A., Bell, R. W., Dell, B., & Huang, L. (1997). External boron requirements for Canola (*Brassica napus* L) in boron buffered solution culture *Annals of Botany*, 80(1), 65-73.
- Asad, A., Blamey, F. P. C., & Edwards, D. G. (2002). Dry matter production and boron concentrations of vegetative and reproductive tissues of canola and sunflower plants grown in nutrient solution *Plant and Soil*, 243(2), 243-252.
- Asad, A., Blamey, F. P. C., & Edwards, D. G. (2003). Effects of boron foliar applications on vegetative and reproductive growth of sunflower *Annals of Botany*, 92(4), 565-570.
- 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(2), 153-158.
- Brown, P. H., Bellaloui, N., Wimmer, M. A., Bassil, E. S., Ruiz, J., Hu, H., Pfeffer, H., Dannel, F., & Romheld, V. (2002). Boron in plant biology *Plant Biology*, 4(2), 205-223.
- Brown, P. H., & Shelp, B. J. (1997). Boron mobility in plants *Plant and soil*, 193(1), 85-101.
- Cakmak, I., & Römheld, V. (1997). Boron deficiency-induced impairments of cellular functions in plants *Plant and Soil*, 193(1), 71-83.
- Chen, M., Mishra, S., Heckathorn, S. A., Frantz, J. M., & Krause, C. (2014). Proteomic analysis of *Arabidopsis thaliana* leaves in response to acute boron deficiency and toxicity reveals effects on photosynthesis, carbohydrate metabolism, and protein synthesis *Journal of plant physiology*, 171(3-4), 235-242.
- Dannel, F., Pfeffer, H., & Romheld, V. (2002). Update on boron in higher plants - Uptake, primary translocation and compartmentation *Plant Biology*, 4(2), 193-204.
- 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*, 153(5), 615-622.
- Diehn, T. A., Bienert, M. D., Pommerrenig, B., Liu, Z. J., Spitzer, C., Bernhardt, N., Fuge, J., Bieber, A., Richet, N., Chaumont, F., & Bienert, G. P. (2019). Boron demanding tissues of *Brassica napus* express specific sets of functional Nodulin26-like Intrinsic Proteins and BOR1 transporters *Plant Journal*, 100(1), 68-82.
- Eggert, K., & von Wiren, N. (2016). The role of boron nutrition in seed vigour of oilseed rape (*Brassica napus* L.) *Plant and Soil*, 402(1-2), 63-76.
- Eichert, T., & Goldbach, H. E. (2010). Transpiration rate affects the mobility of foliar-applied boron in *Ricinus communis* L. cv. Impala *Plant and soil*, 328(1), 165-174.
- Feng, Y. N., Cui, R., Wang, S. L., He, M. L., Hua, Y. P., Shi, L., Ye, X. S., & Xu, F. S. (2019). Transcription factor BnaA9.WRKY47 contributes to the adaptation of *Brassica napus*

- to low boron stress by up-regulating the boric acid channel gene BnaA3.NIP5;1 *Plant Biotechnology Journal*, 14.
- Fernández, V., Sotiropoulos, T., & Brown, P. (2013). Foliar Fertilization - Scientific Principles and Field Practices. International Fertilizer Industry Association (IFA), Paris, France.
- Goldbach, H. E., & Wimmer, M. A. (2007). Boron in plants and animals: Is there a role beyond cell-wall structure? *Journal of Plant Nutrition and Soil Science*, 170(1), 39-48.
- Goldbach, H. E., Wimmer, M. A., & Findelee, P. (2000). Discussion paper: Boron - How can the critical level be defined? *Journal of Plant Nutrition and Soil Science*, 163(1), 115-121.
- Hu, H. N., & Brown, P. H. (1997). Absorption of boron by plant roots *Plant and Soil*, 193(1-2), 49-58.
- Hua, Y. P., Feng, Y. N., Zhou, T., & Xu, F. S. (2017). Genome-scale mRNA transcriptomic insights into the responses of oilseed rape (*Brassica napus* L.) to varying boron availabilities *Plant and Soil*, 416(1-2), 205-225.
- Huang, L. B., Ye, Z. Q., & Bell, R. W. (1996). The importance of sampling immature leaves for the diagnosis of boron deficiency in oilseed rape (*Brassica napus* cv Eureka) *Plant and Soil*, 183(2), 187-198.
- Konsaeng, S., Dell, B., & Rerkasem, B. (2010). Boron mobility in peanut (*Arachis hypogaea* L.) *Plant and Soil*, 330(1), 281-289.
- Koohkan, H., & Maftoun, M. (2016). Effect of nitrogen- boron interaction on plant growth and tissue nutrient concentration of canola (*Brassica napus* L.) *Journal of Plant Nutrition*, 39(7), 922-931.
- Landi, M., Pardossi, A., Remorini, D., & Guidi, L. (2013). Antioxidant and photosynthetic response of a purple-leaved and a green-leaved cultivar of sweet basil (*Ocimum basilicum*) to boron excess *Environmental and Experimental Botany*, 85, 64-75.
- Lauchli, A., & Grattan, S. R. (2017). *Plant Stress under Non-optimal Soil pH*. Cabi Publishing-C a B Int.
- Matoh, T. (1997). Boron in plant cell walls *Plant and Soil*, 193(1-2), 59-70.
- Matoh, T., & Ochiai, K. (2005). Distribution and partitioning of newly taken-up boron in sunflower *Plant and Soil*, 278(1-2), 351-360.
- Reid, R. J., Hayes, J. E., Post, A., Stangoulis, J. C. R., & Graham, R. D. (2004). A critical analysis of the causes of boron toxicity in plants *Plant, Cell & Environment*, 27(11), 1405-1414.
- Savic, J., Romheld, V., & Nikolic, M. (2012). Oilseed rape (*Brassica napus* L.) genotypic variation in response to boron deficiency *Turkish Journal of Agriculture and Forestry*, 36(4), 408-414.

- Shelp, B. J., Kitheka, A. M., Vanderpool, R. A., Van Cauwenberghe, O. R., & Spiers, G. A. (1998). Xylem-to-phloem transfer of boron in broccoli and lupin during early reproductive growth *Physiologia Plantarum*, 104(4), 533-540.
- Shelp, B. J., Vivekanandan, P., Vanderpool, R. A., & Kitheka, A. M. (1996). Translocation and effectiveness of foliar-fertilized boron in broccoli plants of varying boron status *Plant and Soil*, 183(2), 309-313.
- Smith, T. E., Grattan, S. R., Grieve, C. M., Poss, J. A., Lauchli, A. E., & Suarez, D. L. (2013). pH dependent salinity-boron interactions impact yield, biomass, evapotranspiration and boron uptake in broccoli (*Brassica oleracea* L.) *Plant and Soil*, 370(1-2), 541-554.
- Stangoulis, J., Tate, M., Graham, R., Bucknall, M., Palmer, L., Boughton, B., & Reid, R. (2010). The Mechanism of Boron Mobility in Wheat and Canola Phloem *Plant Physiology*, 153(2), 876-881.
- Takano, J., Noguchi, K., Yasumori, M., Kobayashi, M., Gajdos, Z., Miwa, K., Hayashi, H., Yoneyama, T., & Fujiwara, T. (2002). Arabidopsis boron transporter for xylem loading *Nature*, 420(6913), 337-340.
- Tsadilas, C. D., Kassioti, T., & Mitsios, I. K. (2005). Influence of liming and nitrogen forms on boron uptake by tobacco *Communications in Soil Science and Plant Analysis*, 36(4-6), 701-708.
- Wimmer, M. A., Bassil, E. S., Brown, P. H., & Lauchli, A. (2005). Boron response in wheat is genotype-dependent and related to boron uptake, translocation, allocation, plant phenological development and growth rate *Functional Plant Biology*, 32(6), 507-515.
- Wimmer, M. A., & Eichert, T. (2013). Review: mechanisms for boron deficiency-mediated changes in plant water relations *Plant Sci*, 203-204, 25-32.
- Wimmer, M. A., Goldberg, S., & Gupta, U. C. (2015). Boron. In *Barker A (Ed), Pilbeam D (Ed) Handbook of Plant Nutrition* (2<sup>nd</sup> ed., pp. 305-346). CRC Press.
- Yang, L., Zhang, Q., Dou, J. N., Li, L., Guo, L. F., Shi, L., & Xu, F. S. (2013). Characteristics of root boron nutrition confer high boron efficiency in *Brassica napus* cultivars *Plant and Soil*, 371(1-2), 95-104.



## Chapter 6: Summary

Boron (B) is an essential micronutrient for vascular plants and its plant availability is reduced by soil liming, high soil pH, and drought periods during growth stages. This work, consisting of three independent hydroponics experiments, aimed at investigating the growth and physiological response of oilseed rape (*Brassica napus* L.) plants to B supply. In the first experiment, the influence of B supply on the growth and distribution of B in oilseed rape was investigated. The results showed that oilseed rape plants supplied with 0.25  $\mu\text{M}$  B in the nutrient solution showed severe symptoms of B deficiency, which were not seen in the plants supplied with 1.0  $\mu\text{M}$  B. The increasing B supply in the nutrient solution significantly increased the translocation factor, and hence increased B shoot to root ratio in oilseed rape plants.

In the second experiment, the effects of continuous B resupply via roots and one-time foliar application leaves on growth and physiological response, the relative expression of specific genes involved in B absorption and transport in B-deficient oilseed rape were evaluated. It was found that continuous root resupply and one-time foliar resupply B to B-deficient plants partially retrieved B concentration in the leaves and dry matter production, with a corresponding increase in the net carbon assimilation, transpiration rate and sugar accumulation. The finding from this work provided evidence that the relative expression of *BnaNIP5;1* and *BnaBOR1;2* strongly upregulated in the roots and leaves of B-deficient oilseed rape plants. However, gene expression of *BnaNIP5;1* and *BnaBOR1;2* was upregulated only in roots, which could be attributed to the low B concentration in roots in the foliar-applied B treatments. The effects of foliar B resupply on the growth of oilseed rape suggest that dual resupply via roots and foliage is needed to achieve a complete growth recovery of the B deficiency in oilseed rape.

In the third experiment, the mechanisms underlying the uptake and distribution of B in oilseed rape under different nitrogen forms were elucidated. The concentration of B in the oilseed rape was not affected by the N forms, whereas it depended on the levels of B supplied. Marginal B supply significantly decreased specific B uptake rates as well as B concentrations and accumulation in roots and shoots as compared to the high B supply. The  $\text{NO}_3^-$ -fed plants showed reduced xylem B concentrations and accumulated relatively more B in roots than that recorded with other N forms. The relative expression of *BnaNIP5;1* was upregulated in roots, while both *BnaBOR1;2* and *BnaNIP5;1* were upregulated in shoots in the  $\text{NH}_4^+$ -fed plants under marginal B supply. Nitrate nutrition led to up-regulation of *BnaBOR1;2* and *BnaNIP5;1* in roots only. Nitrate-induced alkalization results in altered B distribution between roots and shoots due to

a restriction of B transport, while  $\text{NH}_4^+$ -induced acidity can induce upregulation of B transport mechanisms, even though B concentrations do not indicate B deficiency.

## Zusammenfassung

Bor (B) ist ein essentieller Mikronährstoff für Gefäßpflanzen. Die Pflanzenverfügbarkeit von B wird durch Bodenkalkung, hohen Boden-pH-Wert und Trockenperioden reduziert. Die vorgelegte Arbeit, bestehend aus drei unabhängigen Hydrokulturversuchen, hatte zum Ziel, das Wachstum und die physiologische Reaktion von Rapspflanzen (*Brassica napus* L.) auf die B-Versorgung unter verschiedenen Wachstumsbedingungen zu untersuchen.

Im ersten Experiment wurde der Einfluss der B-Versorgung auf das Wachstum und die Verteilung von B in Raps untersucht. Die Ergebnisse zeigten, dass Rapspflanzen, die mit 0,25  $\mu\text{M}$  B in der Nährlösung versorgt wurden, schwere B-Mangelsymptome aufwiesen, die bei den Pflanzen, die mit 1,0  $\mu\text{M}$  B versorgt wurden, nicht zu sehen waren. Die zunehmende B-Versorgung in der Nährlösung erhöhte signifikant den Translokationsfaktor und steigerte somit das Spross-Wurzel-B-Verhältnis in Rapspflanzen.

Im zweiten Experiment wurden die Auswirkungen einer kontinuierlichen B-Versorgung über die Wurzeln und einer einmaligen Blattapplikation auf das Wachstum untersucht. Außerdem wurde die physiologische Reaktion sowie die relative Expression spezifischer Gene, die an der B-Absorption und dem B-Transport in B-defizientem Raps beteiligt sind, gemessen. Es konnte festgestellt werden, dass die kontinuierliche Wurzelzufuhr und die einmalige Blattapplikation die B-Konzentration in den Blättern und die Trockenmasseproduktion von Pflanzen mit Bormangel teilweise wiederherstellte. Dies hatte einen Anstieg der Netto-Kohlenstoffassimilation, der Transpirationsrate und der Zuckerakkumulation zur Folge. Die Ergebnisse dieser Arbeit lieferten den Beweis, dass die relative Expression von *BnaNIP5;1* und *BnaBOR1;2* in den Wurzeln und Blättern von B-defizienten Rapspflanzen stark hochreguliert waren. Nach einer B-Blattapplikation war die Genexpression von *BnaNIP5;1* und *BnaBOR1;2* jedoch nur in den Wurzeln hochreguliert, was auf die niedrigen B-Konzentration in den Wurzeln zurückzuführen werden konnte. Die Auswirkungen einer B-Blattapplikation auf das Wachstum von Raps deuteten darauf hin, dass eine Blattapplikation zusätzlich zur Wurzelapplikation notwendig ist, um eine vollständige Erholung des Wachstums bei B-Mangel in Raps zu erreichen.

Im dritten Versuch wurden die Mechanismen, die der Aufnahme und Verteilung von B in Raps unter verschiedenen Stickstoffformen zugrunde liegen, aufgeklärt. Die B-Konzentration in Rapspflanzen wurde durch die N-Formen nicht beeinflusst. Jedoch hatte die Höhe der B-Konzentration in der Nährlösung einen Einfluss auf die Aufnahmerate von B. Eine geringe B-

Versorgung verringerte signifikant die spezifischen B-Aufnahmeraten sowie die B-Konzentrationen und die Akkumulation in Wurzeln und Sprossen im Vergleich zu einer hohen B-Versorgung. Die mit  $\text{NO}_3^-$  versorgten Pflanzen zeigten eine reduzierte B-Konzentration im Xylem. Andererseits akkumulierten sie relativ mehr B in den Wurzeln, im Vergleich zu Pflanzen mit einer  $\text{NH}_4^+$ -Ernährung. Unter marginaler B-Versorgung war in Rapspflanzen mit einer  $\text{NH}_4^+$ -Ernährung die relative Expression nur von *BnaNIP5;1* in den Wurzeln hochreguliert, während sowohl *BnaBOR1;2* als auch *BnaNIP5;1* im Spross eine erhöhte Expression aufzeigten. Die Düngung mit  $\text{NO}_3^-$  führte nur in den Wurzeln zu einer Hochregulierung von *BnaBOR1;2* und *BnaNIP5;1*. Außerdem kam es zu einer veränderten B-Verteilung zwischen Wurzeln und Spross aufgrund einer Einschränkung des B-Transports. Dies kann auf eine  $\text{NO}_3^-$ -induzierte Alkalisierung zurückgeführt werden. Dahingegen kam es unter  $\text{NH}_4^+$ -Ernährung zu einer Hochregulierung von B-Transportmechanismen, obwohl die B-Konzentrationen über dem kritischen Wert eines B-Mangels lagen. Dies wird durch die versauernde Wirkung einer  $\text{NH}_4^+$ -Ernährung auf den pH-Wert der Nährlösung zurückgeführt.

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## Publications

1. Dinh, A. Q., Naeem, A., Sagervanshi, A., Wimmer, M. A., & Mühling, K. H. (2021). Boron uptake and distribution by oilseed rape (*Brassica napus* L.) as affected by different nitrogen forms under low and high boron supply. *Plant Physiology and Biochemistry*, 161, 156-165.
2. Dinh, A.Q., Naeem, A., Sagervanshi, A., Mühling, K.H (2021). One-Time Foliar Application and Continuous Resupply via Roots Equally Improved the Growth and Physiological Response of B-Deficient Oilseed Rape. *Plants*, 10, 866. <https://doi.org/10.3390/plants10050866>