

Effect of low boron supply in turnip plants under drought stress

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Abstract

Turnip (*Brassica rapa* L.) plants were grown in *Perlite* with low ($< 2.5 \mu\text{M}$) or adequate ($25 \mu\text{M}$) boron supply under well-watered and drought conditions for 12 weeks. Dry mass of leaves and roots was reduced under drought by about 61 and 56 % in plants supplied adequately with B, while up to 84 and 74 % under B starvation. Drought reduced B content by about 70 and 82 % for B-sufficient and B-deficient plants, respectively. According to the chlorophyll fluorescence parameters, the photosynthesis processes conserved their normal activities under low B supply in well-watered plants, while a serious damage to photosystem 2 occurred under drought stress. Stomatal limitation was the most important cause for a 17 % lower net photosynthetic rate (P_N) of drought stressed B-sufficient plants. In B-deficient plants, however, both stomatal and non-stomatal limitations were involved in 53 % reduction of P_N . Low B supply reduced strongly leaf water potential.

Additional key words: boron deficiency, carotenoids, chlorophyll, fluorescence, gas exchange, water potential.

Boron is a micronutrient required for normal growth of higher plants. However, its function in plants has not yet been fully understood (Bolaños *et al.* 2004, Matas *et al.* 2009). It was reported that B deficiency decreases plant photosynthetic capacity due to reduction of chlorophyll (Chl) content, Hill reaction activity (El-Shintinavy 1999), photosynthetic electron transport and activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and fructose-1,6-bisphosphatase (Han *et al.* 2009).

Soil water is one of the important factors affecting B availability for plant roots (Marschner 1995). Roots absorb B mainly as non-dissociated boric acid which is transported to the leaves *via* transpiration stream. Therefore, water stress may accentuate B deficiency. However, very little information is available on this relationship and evidences on the effect of water stress on B nutrition are controversial. Water stress has been shown to depress B content in barley and black gram plants (Gupta 1979, Noppakoonwong 1991) and

depresses B uptake and transport into young leaves in wheat (Huang *et al.* 1997). In contrast, another report on wheat showed that B concentration of flag leaf and ear was not affected by irrigation (Pant *et al.* 1998).

Turnip is an important vegetable crop that is known to be susceptible to B deficiency (Shorrocks 1997). However, detailed researches on physiological consequences of low B supply in this plant subjected to drought stress are rare. This work was aimed to study the combination effect of water stress and low B supply on growth, water relations, B content and photosynthesis in turnip plants.

Seeds of turnip (*Brassica rapa* L.) plants were surface-sterilized and germinated in the dark. Seven-day-old young seedlings were transferred to the pots filled with *Perlite* (1 - 2 mm particles) irrigated for two weeks with water or 50 % nutrient solution with low B ($< 2.5 \mu\text{M}$ as contamination of nutrient solution) at field capacity. Thereafter, plants were irrigated with water and

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Abbreviations: Chl - chlorophyll; E - transpiration rate; ETR - electron transport rate; F_0 - initial fluorescence of dark adapted leaves; F'_0 - initial fluorescence of light adapted leaves; F_m - maximum fluorescence of dark adapted leaves; F'_m - maximum fluorescence of light adapted leaves; F_s - steady-state fluorescence of light adapted leaves; F_v - variable fluorescence of dark adapted leaves; g_s - stomatal conductance; PFD - photon flux density; P_N - net photosynthetic rate; qN - non-photochemical quenching; qP - photochemical quenching; RCs - reaction centers; Φ_{PS2} - effective quantum yield of PS 2; ψ_s - osmotic potential; ψ_w - water potential.

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full nutrient solution, pH 6.5 (Johnson *et al.* 1957), containing either low or adequate (25 μM) B supply either at 100 % (control) or 30 % field capacity (drought treatment) by daily weighing. The volume of nutrient solution used for irrigation was started with 200 cm^3 per plant per week in the earlier growth phase and increased gradually and reached up to 500 cm^3 at the final phase of growth. After daily irrigation with the nutrient solution, water was used in order to keep the pots at the two watering regimes. Plants were grown under controlled environmental conditions with a 14-h photoperiod at a photon flux density of about 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$, day/night temperature of 25/18 $^{\circ}\text{C}$ and relative humidity of 70/80 %.

After growing for 12 weeks (13 weeks after sowing), plants were harvested. After drying at 70 $^{\circ}\text{C}$ for 2 d and determination of dry mass, samples were dry-ashed and B was determined following the azomethine-H method as described by Lohse (1982). Before harvest, Chl fluorescence and gas exchange parameters were determined.

Chlorophyll fluorescence parameters were recorded using a portable fluorometer (*OSF1, ADC Bioscientific, Hoddesdon, UK*) for both dark adapted and light adapted leaves. Measurements were carried out on the second youngest, fully expanded and attached leaf. Dark-adapted leaves were used for determination of initial (F_0), maximum (F_m), variable ($F_v = F_m - F_0$) fluorescence as well as maximum quantum yield of photosystem 2 (PS 2) (F_v/F_m). Light adapted leaves were used for measurement of steady-state (F_s) and maximum (F'_m) fluorescence. Following calculations were made:

$$F'_0 = F_0 / [(F_v/F_m) + (F_0/F'_m)],$$

$$F'_v/F'_m = [F'_m - F'_0] / F'_m],$$

$$qP = [(F'_m - F_s) / (F'_m - F'_0)],$$

$$qN = (1 - [(F'_m - F'_0) / (F_m - F_0)]),$$

$$\Phi_{PS2} = [(F'_m - F_s) / F'_m],$$

$$ETR = \Phi_{PS2} \times PFD \times 0.84 \times 0.5$$

(Krall and Edwards 1992).

Net photosynthetic rate (P_N), transpiration rate (E) and stomatal conductance (g_s) were measured in the same leaf with a calibrated portable gas exchange system (*LCA-4, ADC Bioscientific, Hoddesdon, UK*) between 10:00 and 13:00. The measurements were conducted under photon flux density (PFD) at the leaf surface of 350 $\mu\text{mol m}^{-2} \text{s}^{-1}$ measured by a quantum sensor attached to the leaf chamber of the gas exchange unit.

Leaf contents of chlorophyll (Chl) *a*, Chl *b* and carotenoids (Car) were determined according to Lichtenthaler and Wellburn (1985). Leaf osmotic and water potential were measured by an osmometer (*Micro-Osmometer, Heman Roebling Messtechnik, Berlin, Germany*) and a pressure chamber (*DTK-7000, Kyoto, Japan*), respectively, in the second youngest leaf harvested at 1 h after light switch on in the growth chamber. Experiments were undertaken in complete randomized block design with 4 replications. Statistical analyses were carried out by Tukey test ($P < 0.05$) using *Sigma Stat (3.02)*.

Under B deficiency, shoot and root dry mass was reduced by about 27 and 48 %, respectively, compared with control plants (Table 1). Effect of B deficiency on cell expansion in growing regions that leads to rapid cessation of root elongation and reduction of leaf expansion has been attributed to the role of B in cell wall structure and function (Marschner 1995). Other effects of B deficiency including loss of membrane integrity, accumulation of phenolics and induction of imbalance between production and scavenging of reactive oxygen species could be also involved in the impairment of plants growth as was demonstrated for turnip plants (Hajiboland and Farhanghi 2010). Dry mass of leaves and roots was reduced under drought conditions by about 61 and 56 %, respectively, when plants were supplied adequately with B, while up to 84 and 74 % under B starvation (Table 1). Data implied that, impairment of growth in B-deficient plants was markedly higher when they were subjected to drought stress compared with well watered conditions. In turn, effect of drought stress on the inhibition of plants dry matter production was greater for B-deficient compared with B-sufficient plants.

Boron content of leaves, roots and plant total B content declined dramatically not only at low B supply but also under drought conditions. Reduction of B content due to low B supply was higher for drought stressed (90 %) compared with well-watered (83 %) plants. In turn, effect of drought conditions on the reduction of B content was more pronounced in B-deficient (82 %) than B-sufficient (70 %) plants (Table 1). Similar trend was observed for B concentration (B content per dry mass unit), however, due to greater growth reduction of drought stressed plants compared with well-watered ones under low B supply, differences between these two treatments were not significant. Roots absorb B mainly as non-dissociated H_3BO_3 which is transported to the leaves *via* the xylem (Hu and Brown 1997) by transpiration stream (Kozlowski and Pallardy 1997). There has been an active debate concerning a relationship between the ion and water transport in plants (Clarkson *et al.* 2000). Since nutrient uptake is linked to water availability, an inhibition of root hydraulic conductivity and stomatal conductance in drought stressed plants could lead to reduced B uptake irrespective to the B supply level. In our experiment, transpiration was strongly lowered by drought stress as the result of lower stomatal conductance (see below). It was reported that, transpiration is among the key factors affecting the uptake and tissue concentration of B and other essential mineral nutrients (Hu and Brown 1997). Therefore, reduction of water transport could result in nutrient deficiencies as observed for B in this work and for Zn in red cabbage plants (Hajiboland and Amirazad 2010).

Leaf Chl *a+b* content was reduced by low B supply, but the effect of drought was not significant. In contrast to Chl, carotenoid content expressed on a fresh mass

Table 1. Growth parameters, B content, photosynthetic pigments, parameters of chlorophyll fluorescence and of gas exchange in turnip plants grown for 3 months with adequate (+B) and low (-B) boron supply under sufficient water supply (control) or under drought stress. The means refer to 4 repetitions \pm SD. Data of each row followed by the same letter are not significantly different at $P < 0.05$.

Parameters	Control	Drought		
	+B	-B	+B	
Shoot DM [mg plant ⁻¹]	5051 \pm 416 ^a	3684 \pm 180 ^b	1975 \pm 205 ^c	957 \pm 175 ^d
Root DM [mg plant ⁻¹]	284 \pm 35 ^a	149 \pm 28 ^{ab}	127 \pm 33 ^c	39 \pm 12 ^d
Tuber DM [mg plant ⁻¹]	986 \pm 28 ^a	294 \pm 80 ^b	91 \pm 18 ^c	37 \pm 9 ^d
Shoot B content [μ g plant ⁻¹]	1313 \pm 219 ^a	214 \pm 73 ^b	421 \pm 174 ^b	41 \pm 10 ^c
Root B content [μ g plant ⁻¹]	58 \pm 13 ^a	15 \pm 2 ^b	22 \pm 1 ^b	2 \pm 1 ^c
Tuber B content [μ g plant ⁻¹]	148 \pm 12 ^a	24 \pm 2 ^b	8 \pm 2 ^b	2 \pm 1 ^c
Total B content [μ g plant ⁻¹]	1520 \pm 222 ^a	253 \pm 71 ^b	451 \pm 174 ^b	45 \pm 11 ^c
Shoot B concentration [μ g g ⁻¹ (d.m.)]	256 \pm 81 ^a	59 \pm 20 ^b	211 \pm 74 ^a	45 \pm 15 ^b
Root B concentration [μ g g ⁻¹ (d.m.)]	227 \pm 6 ^a	125 \pm 35 ^{ab}	161 \pm 44 ^a	64 \pm 29 ^b
Tuber B concentration [μ g g ⁻¹ (d.m.)]	160 \pm 49 ^a	87 \pm 27 ^{ab}	106 \pm 35 ^{ab}	50 \pm 25 ^b
Chl <i>a+b</i> [mg g ⁻¹ (f.m.)]	3.02 \pm 0.11 ^a	2.34 \pm 0.21 ^{bc}	2.65 \pm 0.97 ^{ab}	1.97 \pm 0.34 ^c
Carotenoids [μ mol g ⁻¹ (f.m.)]	934 \pm 29 ^a	669 \pm 32 ^c	715 \pm 39 ^b	589 \pm 33 ^d
Chl <i>a+b</i> [mg g ⁻¹ (d.m.)]	10.4 \pm 0.38 ^{ab}	6.57 \pm 0.59 ^b	12.5 \pm 4.57 ^a	6.05 \pm 1.04 ^b
Carotenoids [mmol g ⁻¹ (d.m.)]	3.21 \pm 0.1 ^a	1.88 \pm 0.09 ^b	3.37 \pm 0.18 ^a	1.81 \pm 0.1 ^b
F_v/F_m	0.85 \pm 0.02 ^a	0.83 \pm 0.01 ^a	0.85 \pm 0.02 ^a	0.81 \pm 0.00 ^b
F'_v/F'_m	0.81 \pm 0.01 ^b	0.82 \pm 0.00 ^{ab}	0.84 \pm 0.02 ^a	0.84 \pm 0.01 ^a
q _P	0.99 \pm 0.01 ^a	0.95 \pm 0.01 ^b	0.93 \pm 0.02 ^{ab}	0.92 \pm 0.02 ^b
q _N	0.19 \pm 0.06 ^a	0.17 \pm 0.06 ^a	0.04 \pm 0.01 ^b	0.07 \pm 0.01 ^b
Φ_{PS2}	0.80 \pm 0.01 ^a	0.79 \pm 0.01 ^{ab}	0.78 \pm 0.01 ^{bc}	0.77 \pm 0.01 ^c
ETR	134 \pm 1.2 ^a	132 \pm 1.5 ^a	132 \pm 1.2 ^a	129 \pm 1.4 ^b
P_N [μ mol m ⁻² s ⁻¹]	10.2 \pm 0.3 ^a	5.9 \pm 0.2 ^c	8.5 \pm 0.4 ^b	2.8 \pm 0.5 ^d
E [mmol m ⁻² s ⁻¹]	4.6 \pm 0.1 ^a	3.1 \pm 0.6 ^b	4.4 \pm 0.4 ^a	2.1 \pm 1.1 ^b
g_s [mol m ⁻² s ⁻¹]	0.97 \pm 0.1 ^a	0.35 \pm 0.1 ^b	0.76 \pm 0.2 ^a	0.19 \pm 0.1 ^b
Osmotic potential [MPa]	-0.89 \pm 0.03 ^b	-0.99 \pm 0.05 ^a	-0.93 \pm 0.04 ^{ab}	-1.31 \pm 0.03 ^c
Water potential [MPa]	-0.06 \pm 0.01 ^a	-0.18 \pm 0.01 ^b	-0.15 \pm 0.02 ^b	-0.27 \pm 0.02 ^c

basis decreased by both low B supply and drought significantly, effect of B deficiency was more prominent in control (97 %) compared with drought stressed plants (18 %). Similarly, carotenoid content in B-sufficient plants decreased by drought stress up to 23 %, while this reduction in B-deficient plants was only 12 % (Table 1).

In contrast to other reports (Han *et al.* 2009), low B supply did not influence maximum quantum yield of PS 2 (F_v/F_m), excitation energy capture by open PS 2 reaction centres (F'_v/F'_m), non-photochemical quenching (qN), effective quantum yield of PS 2 (Φ_{PS2}) and electron transport rate (ETR) in well watered plants (Table 1). These data indicated that the photosynthetic processes conserved their normal activities under B starvation when the plants are adequately watered. However, the photochemical quenching (qP), that reflects the capacity to utilize absorbed energy through metabolism and growth, decreased significantly in plants subjected to B deficiency. Reduction of photochemical quenching could be related to photoinhibition rather than to a direct damage to PS 2 (Baker and Bowyer 1994). One of the causes of photoinhibition was likely lower Chl content of B-deficient leaves (Table 1). Leaf Chl content had a

crucial role for the susceptibility to photoinhibition and leaves with less Chl were found more susceptible to photoinhibition (Han *et al.* 2009).

In contrast to well watered plants, low B supply significantly diminished F_v/F_m , Φ_{PS2} and ETR in drought-stressed turnip (Table 1). In turn, effect of drought stress on reduction of these parameters was significant only in low B leaves. The non-photochemical quenching (qN), that reflects the capacity to dissipate excess absorbed energy as heat, was surprisingly depressed under drought conditions irrespective to the B nutrition. This reduction was accompanied by considerable decline in leaf content of carotenoids (Table 1). Our data demonstrated that, drought intensified inhibitory effects of B deficiency on the photochemistry of leaves. Significantly lower F_v/F_m , Φ_{PS2} and ETR in plants under the combination of low B and drought stress indicated severe damage to PS 2. Reduction of qN can be linked to photoinhibition and damage of PS 2 under combination of B deficiency and drought conditions.

Reduction of P_N due to low B supply was greater under drought (67 %) than under sufficient water supply (42 %; Table 1). In turn, P_N in B-sufficient plants was

impaired due to water stress by about 17 %, while this impairment was greater for B-deficient plants (52 %). Boron deficiency lowered transpiration rate (E) and stomatal conductance (g_s) significantly, reduction was greater in stressed than in well watered plants (Table 1). Under B deficiency conditions, depressed P_N was mostly the result of decreased g_s . Thus stomatal limitation prevailed as was reported for other plant species (Han *et al.* 2009). Under combination of B deficiency and drought, changes in g_s were also important, however, changes in photochemical parameters of leaves under combined effect of low B and drought stress suggested that non-stomatal limitations was also involved (Table 1).

Low B supply caused significant reduction of leaf osmotic potential in both well-watered and drought stressed plants. Significant effect of drought stress on

reduction of leaf osmotic potential was observed only in B-deficient plants. Drought conditions lowered leaf water potential in both B-deficient and B-sufficient plants (Table 1). Significantly lower water potential in B-deficient plants together with lower transpiration rate suggested lower water uptake or water transport. In fact, water transport through the roots is very sensitive to various environmental factors including low temperature (Wan *et al.* 1999), salinity (Carvajal *et al.* 2000) and nutrient stress (Clarkson *et al.* 2000). Boron deficiency likely reduces root hydraulic conductivity (Apostol and Zwiazek 2004) due to disturbance in the formation of functional xylem vessels as well as reduction of new root growth. More investigations are needed for the mechanisms involve in reduction of B uptake under drought stress and water uptake under B deficiency.

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