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BRIEF COMMUNICATION

Effects of boron on growth, and chlorophyll and mineral contents of shoots of the apple rootstock MM 106 cultured *in vitro*

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Abstract

The *in vitro* cultures of apple rootstock MM 106 produced the highest fresh mass (FM) when 0.1 mM B was included in the culture medium. By increasing B concentration of the culture medium from 0.1 to 6.0 mM, FM and contents of B, P, Ca, and Mg in explants increased, whereas K, Fe, Mn, and Zn contents decreased. SPAD units of leaves characterizing chlorophyll contents declined as B concentration of the culture medium increased from 0.1 to 6.0 mM.

Additional key words: cell proliferation, micropropagation, mineral nutrition.

Boron (B) is required for normal growth and development of all higher plants (Brown *et al*. 2002). B is involved in cell wall intactness and synthesis (Hu *et al*. 1996) and in plasma membrane integrity (Marschner 1995, O'Neil *et al*. 2001). Renukdas *et al*. (2003) reported that B influences somatic embryogenesis in papaya (*Carica papaya* L.). There is no evidence for a direct role for B in photosynthesis (Shelp 1993). B deficiency reduces chlorophyll (Chl) and soluble protein contents of leaves, which results in an inhibition of Hill reaction and net photosynthetic rate (Sharma and Ramchandra 1990). Furthermore, B deficiency induced changes in translocation of $^{14}CO_2$ photosynthates into primary and secondary metabolites (Dixit *et al*. 2002). In studies of kiwifruit leaf anatomy, B toxicity induced a decrease of the volume of mesophyll cells, and an increase of the volume of intercellular spaces and cell damage (Sotiropoulos *et al*. 2002). However, the effect of B toxicity on Chl content of leaves of *in vitro* cultures has not been studied yet. The aim of this study was to find the effects of B on growth, and nutrient and Chl contents of the apple rootstock shoots cultured *in vitro*.

The explants employed were shoots of the apple (*Malus domestica* Borkh.) rootstock MM 106 of about 25 mm in length preserved from previous *in vitro* cultures and maintained in the growth room. Each explant was transferred and grown in a 15 × 100 mm glass test tube containing 3 cm^3 of the MS culture medium (Murashige and Skoog 1962). The culture medium was supplemented with 30 kg m^{-3} sucrose, 1.5 g m^{-3} benzyladenine, and 1 g m^{-3} gibberellic acid. Five B treatments were included in the experiment: 0.1 (control), 0.5, 1.0, 3.0 and 6.0 mM. The pH of the media was adjusted to 5.8 before autoclaving at 121 °C for 15 min. The tubes were closed with aluminum foil and maintained in the growth room at temperature of 22 ± 1 °C and 16-h photoperiod with irradiance of 45 μmol m^{-2} s^{-1} (400-700 nm) (cool white fluorescent light, supplied by *TLD 36W/84* lamps). After eight weeks in culture, fresh mass (FM) of explants was measured. Leaf Chl content was estimated non-destructively in leaves by using a SPAD meter (*Minolta model 502,* Osaka, Japan). For determination of the mineral composition, leaves and stems of each plantlet were harvested and rinsed twice with distilled water. These organs were then dried at 68 °C for 48 h, ground to pass a 30-mesh screen, and dry ashed at 530 °C for 16 h. Ca, K, Mg, Zn, Fe, and Mn were determined by atomic absorption spectrometry (*Perkin-Elmer model 2380,* Wellesley, MA, USA). P was determined colorimetrically by the ammonium phosphovanadomolybdate method and B by the azomethine-H method (Wolf 1974). Each treatment included fifteen replicates (tubes). The

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Abbreviations: Chl - chlorophyll; FM - fresh mass.
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experiment was conducted twice, and the reported values are means of the two experiments. The statistical design employed was the randomized complete block one. Differences between the means were evaluated by the Duncan's multiple range test at $P \leq 0.05$.

The *in vitro* cultures of MM 106 shoots produced the highest FM when 0.1 mM B was included in the medium (Table 1). By increasing B concentration of the culture medium, FM of explants significantly decreased as was shown also for maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L.) by Ismail (2003). The highest shoot length at 0.1 mM B may be ascribed to increases in cell elongation and/or cell division rates. By increasing B concentration in the culture medium, the B content of tomato cell suspensions were in near equilibrium with B concentration in the culture medium (Seresinhe and Oertli 1991). Therefore, if the supply of B to the culture medium is high, then B is accumulated in leaf cell walls and finally may intrude into cytoplasm and thus disturb cytoplasmic metabolism. This results in B toxicity and reduced growth of the explants (Matoh 1997).

Regarding ion content of explants, B, P, Ca, and Mg contents of the explants increased, whereas K, Fe, Mn, and Zn contents decreased with increasing B concentration of the culture medium from 0.1 to 6.0 mM (Table 1). B might influence either uptake or transport of mineral elements within the plants. Reduced uptake rate for phosphate as one of the early events of B deficiency was found by Goldbach (1985). Schon *et al*. (1990) have shown that 48-h B treatment of sunflower roots induced hyperpolarization of cell membranes and increased K^+ accumulation. In addition to this, the previous researchers postulated that H^+-ATPase may be regulated either directly by B or indirectly through the stimulation caused by the B-induced K^+ accumulation. In contrast, in our experiments higher B concentrations in the culture medium (> 0.1 mM) exerted a negative effect on the plasma membrane properties of explants and thus decreased K^+ uptake. In addition, B might influence plant reactions to environmental stimuli by modifying their response to auxins (Goldbach *et al*. 1990).

Chl content (SPAD units) of leaves declined as B concentration of the culture medium increased from 0.1 to 6.0 mM (Table 1). This may be ascribed to the decrease of Fe content of explants at the higher B concentrations of the culture medium as Fe is essential for Chl synthesis (Montvedt *et al*. 1991). El-Shintinawy (1999) reported that B deficiency decreased CO_2 assimilation in sunflower and spinach leaves and reduced energy transfer from photosystem 2 to photosystem 1 (Kastori *et al*. 1995).

Table 1. Effect of B concentration of the culture medium on fresh mass, chlorophyll (Chl) content (SPAD units), and the contents of P, K, Ca, Mg, Fe, Mn, Zn, and B of the explants. Means followed by the same letter in the same row are not significantly different (Duncan's multiple range test P=0.05).

Parameters	B concentration [mM]				
	0.1	0.5	1.0	3.0	6.0
Fresh mass [g]	0.2 a	0.16 ab	0.13 bc	0.12 bc	0.11 c
Chl content (SPAD units)	33.0 a	32.0 a	31.0 ab	29.0 bc	26.0 c
P [g kg^{-1}(DM)]	3.3 c	3.3 c	3.8 bc	3.9 b	5.3 a
K [g kg^{-1}(DM)]	14.0 a	12.7 b	12.2 b	12.4 b	5.1 c
Ca [g kg^{-1}(DM)]	4.2 cd	4.0 d	4.4 c	5.2 b	5.7 a
Mg [g kg^{-1}(DM)]	1.0 b	0.9 b	0.9 b	1.4 a	1.4 a
Fe [mg kg^{-1}(DM)]	105.0 a	68.0 b	55.0 c	49.0 cd	57.0 c
Mn [mg kg^{-1}(DM)]	79.0 a	68.0 b	70.0 b	65.0 c	18.0 d
Zn [mg kg^{-1}(DM)]	65.0 a	41.0 bc	43.0 b	39.0 c	17.0 d
B [mg kg^{-1}(DM)]	85.0 e	113.0 d	185.0 c	327.0 b	508.0 a

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