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BRIEF COMMUNICATION

Response of the pear rootstock to boron and salinity *in vitro*

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Abstract

The effects of boron and NaCl induced salinity on growth and mineral composition of the pear (*Pyrus communis* L.) rootstock OH × F 333 shoots cultured *in vitro* were investigated. Shoots were grown *in vitro* for seven weeks on a Murashige and Skoog medium containing two B concentrations (0.1 and 2 mM) combined with five NaCl concentrations (0, 10, 20, 40, and 80 mM). The longest shoots were produced at 0.1 mM B and 80 mM NaCl, but highest number of shoots were produced at 0.1 mM B and 0 - 20 mM NaCl. Inclusion of 20 and 40 mM NaCl in the culture medium significantly increased fresh mass of cultures compared to 0 mM NaCl for all B concentrations tested. The concentrations of P, K, Ca, Mg, Na, Fe, Mn and Zn of plants were affected by B and NaCl concentration of the medium.

Additional key words: cell proliferation, mineral nutrition, osmotic stress, *Pyrus communis*.

Pear rootstocks affect the nutritional status of the scion and proper choice of rootstocks can ameliorate the detrimental effects of salinity (Woodbridge 1973). Reduction in growth of crops by salinity and B toxicity has been well documented (Seresinhe and Oertli 1991, Sotiropoulos *et al*. 2002, Ismail 2003/4). Salinity and B can also affect organogenesis in *in vitro* cultures (Dimassi-Theriou 1998). Salinity significantly affected the growth of *in vitro* cultures of *Prunus cerasifera* (Dimassi-Theriou 1998), *Actinidia deliciosa* (Sotiropoulos *et al*. 2004), *Prunus persica* and *Prunus persica* × *Prunus amygdalus* rootstocks (Sotiropoulos *et al*. 2005). Dimassi-Theriou (1998), reported that NaCl at low concentrations exerts a significant effect on shoot proliferation *in vitro*. NaCl at low concentrations *in vitro* exert a positive effect on plant growth due to the increased osmolarity (Flowers and Läuchli 1983). Boron is required for normal growth and development of higher plants (Brown and Hu 1996). Boron is essential for cell wall integrity and synthesis and plasma membrane integrity (Hu *et al*. 1996). Renukdas *et al*. (2003) reported that B influences somatic embryogenesis in papaya (*Carica papaya* L.). The objective of this study was to investigate the effect of various B and NaCl concentrations on growth and nutritional status of the pear rootstock shoots cultured *in vitro*.

The explants were shoots of the pear (*Pyrus communis* L.) rootstock Old Home × Farmingdale (OH × F 333) from previous *in vitro* cultures maintained in the growth room. The shoots (about 25 mm in length) were transferred and grown in a 15 × 100 mm glass test tube containing 5 cm^3 of the Murashige and Skoog (1962; MS) culture medium supplemented with 1 g m^{-3} benzyl-adenine (BA). Two B concentrations (0.1 and 2 mM) were combined with five NaCl concentrations (0, 10, 20, 40, and 80 mM). The pH of the media was adjusted to 5.8 before autoclaving at 121 °C for 15 min. The tubes were maintained in the growth room at 22 ± 1 °C and 16-h photoperiod (cool white fluorescent tubes, irradiance of 45 μmol m^{-2} s^{-1}, 400 - 700 nm). After seven weeks in culture, the number of shoots, length of shoots, and fresh mass (f.m.) of plantlets were measured. For determination of the mineral composition, leaves and stems from 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. After ashing, the residue was dissolved in 10 cm^3 of 6 M HCl and brought to a volume of 50 cm^3. These extracts were measured for K, Ca, Mg, Na, Mn, Fe, and Zn by atomic absorption spectrometry (*Perkin-Elmer* model *2380*, Wellesley, USA).

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Phosphorus was determined colorimetrically by the ammonium phosphovanadomolybdate method and B by the azomethine-H method (Wolf 1974). Each treatment included fifteen replicates (tubes). The experiment was conducted and repeated twice, and the reported data are the means of the two experiments. The statistical design adopted was the randomized complete block. Differences between means were evaluated using Duncan's multiple range test at $P \leq 0.05$.

The growth of pear plantlets was a function of B and NaCl concentrations of the culture medium. The highest shoot length was recorded when 0.1 mM B plus 80 mM NaCl were added in the culture medium (Table 1). More shoots were produced with 0.1 mM B compared to 2 mM when NaCl concentration was 0 - 20 mM (Table 1). The lowest number of shoots was recorded when 2 mM B and 80 mM NaCl were added in the culture medium. Inclusion of 20 and 40 mM NaCl in the culture medium significantly increased fresh mass of cultures compared to 0 mM NaCl when B concentration in the medium was 0.1 mM (Table 1). Growth enhancement due to the presence of NaCl at low concentrations has been reported for various *in vitro* cultures such as *Suaeda aegyptiaca* (Eshel 1985), *Prunus cerasifera* (Dimassi-Theriou 1998) and *Oryza sativa* (Lutts *et al.* 1999). Fresh mass of cultures with 0.1 mM B was significantly higher than that of 2 mM for all NaCl concentrations tested. Mouhtaridou *et al*. (2004) reported that the *in vitro* cultures of apple shoots produced the highest fresh mass when 0.1 mM B was included in the medium in comparison to higher B concentrations. When B supply to the culture medium is high, then B is accumulated in leaf cell walls and finally may intrude into cytoplasm and disturb cytoplasmic metabolism. This results in B toxicity and reduced growth of cultures (Matoh 1997). The data indicate a general stunting of plant growth due to NaCl osmotic stress or ion toxicity. Plants are stressed in two ways under salinity: by the increase in osmotic potential as a result of high solute content, and by the toxic effects of high concentrations of ions. Plants develop various mechanisms in order to alleviate the effects of salinity (Greenway and Munns 1980). Whichever mechanisms account for salt tolerance, there must be an energy cost. The energy required to make the organic compounds used for osmotic regulation, and the carbon skeletons required, may limit the growth of plants (Stavarek and Rains 1984).

Table 1. Effect of B and NaCl concentrations of the culture medium on the number of shoots per explant, shoot length, and fresh mass of the pear rootstock OH × F 333 *in vitro*. Significantly different means within columns according to Duncan's multiple range test ($P \leq 0.05$) are marked with different letters, $n = 15$.

B [mM]	NaCl [mM]	Number of shoots [explant^{-1}]	Shoot length [mm]	Fresh mass [g]
0.1	0	2.6 a	12.3 d	0.103 d
0.1	10	2.6 a	13.7 d	0.122 c
0.1	20	2.7 a	18.2 b	0.148 a
0.1	40	1.9 b	19.4 ab	0.142 ab
0.1	80	1.8 b	20.2 a	0.135 b
2.0	0	1.9 b	17.6 bc	0.120 c
2.0	10	1.7 b	16.6 c	0.103 d
2.0	20	1.7 b	17.4 bc	0.120 c
2.0	40	1.7 b	17.0 bc	0.116 c
2.0	80	1.0 c	18.6 b	0.100 d

The mineral composition of cultures was affected by B and NaCl concentration of the medium. By increasing B concentration in the culture medium from 0.1 to 2 mM, B content in the plantlets increased for the various NaCl treatments (Table 2). Seresinhe and Oertli (1991) have pointed out that by increasing B concentration in the culture medium, the B content of tomato cell suspensions were close to equilibrium with the

Table 2. Effect of B and NaCl concentrations of the culture medium on P, K, Ca, Mg, Na, Fe, Mn, Zn, and B concentrations of the pear rootstock OH × F 333 *in vitro*. Significantly different means within columns according to Duncan's multiple range test ($P \leq 0.05$) are marked with different letters, $n = 15$.

B [mM]	NaCl [mM]	P [g kg^{-1}(d.m.)]	K	Ca	Mg	Na	Fe [mg kg^{-1}(d.m.)]	Mn	Zn	B
0.1	0	0.13 d	0.65 b	0.24 a	0.06 a	0.59 h	144 c	154 c	106 a	56 f
0.1	10	0.25 bc	0.66 b	0.19 ab	0.06 a	1.10 f	139 cd	182 a	102 ab	53 f
0.1	20	0.19 c	0.63 b	0.16 b	0.05 a	1.47 e	92 g	176 ab	72 c	52 f
0.1	40	0.22 bc	0.67 b	0.17 b	0.05 a	2.47 b	102 f	143 d	63 cd	50 f
0.1	80	0.27 b	0.54 c	0.13 c	0.05 a	3.44 a	91 g	108 e	55 de	53 f
2.0	0	0.22 bc	0.74 a	0.20 ab	0.04 a	0.46 i	168 a	168 b	80 bc	285 e
2.0	10	0.19 c	0.66 b	0.22 ab	0.05 a	0.90 g	154 b	170 b	91 b	294 d
2.0	20	0.32 a	0.71 ab	0.17 b	0.05 a	1.48 e	131 d	178 ab	85 bc	311 c
2.0	40	0.32 a	0.62 b	0.12 c	0.05 a	1.99 d	111 e	152 c	66 cd	326 b
2.0	80	0.25 bc	0.55c	0.13 c	0.06 a	2.25 c	98 fg	73 f	39 e	349 a

B concentration of the culture medium. In the present study, when B concentration of the culture medium was 2 mM, the B content in the plantlets for the various NaCl treatments varied from 285 to 349 μg g^{-1}(d.m.). Inclusion of 0 - 80 mM NaCl in the culture medium containing 0.1 mM B did not significantly affect B content in the plantlets.

By raising the NaCl concentration from 10 to 80 mM, Na content in the plantlets increased for all the B treatments whereas K and Ca contents decreased (Table 2). These results are in agreement with *in vitro* studies for callus cultures of *Brassica campestris* (Paek *et al*. 1988). Salinity may increase energy consumption, required for osmotic regulation and competition of transported ions. This may subsequently lead to a reduction of contents of metabolically important ions such as K^+ and Ca^{2+} (Kwon *et al*. 1995).

Phosphorus contents in plantlets were significantly increased by increased NaCl in the culture medium from 0 to 40 mM for both B concentrations in the medium (Table 2). However, Mg content was not significantly affected by B and NaCl concentration in medium. By increasing NaCl concentration in the medium, Fe, Mn, and Zn contents in plantlets decreased. Nutrient imbalances may result from the effect of salinity on nutrient availability, competitive uptake, transport or partitioning within the plant or may be caused by physiological inactivation of a given nutrient resulting in an increase in the plant internal requirement for that essential element (Grattan and Grieve 1999).

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