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BRIEF COMMUNICATION

Effect of boron and methionine on growth and ion content in kiwifruit shoots cultured *in vitro*

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Abstract

The growth of kiwifruit explants was affected by boron (B) and methionine (Meth) in the culture medium. The longest shoots, the greatest number of shoots and the highest amount of fresh mass per explant were produced in Murashige and Skoog medium with 2 mM B and 2 μM Meth. Furthermore, by increasing B concentration in the culture medium from 0 to 2 mM, an increased rate of shoot proliferation was observed for the various Meth concentrations employed.

Additional key words: *Actinidia deliciosa*, cell proliferation, micropropagation.

Boron (B) is required for normal growth and development of all higher plants. B nutrition might affect polysaccharide synthesis, cell wall metabolism, plasmalemma permeability, and cell division (Matoh 1997). B can also affect organogenesis of *in vitro* kiwifruit shoot cultures (Sotiropoulos *et al*. 1998). Renukdas *et al*. (2003) reported that B influences somatic embryogenesis in papaya (*Carica papaya* L.). Chlorophyll content in the apple rootstock MM 106 leaves declined as B concentration of the medium increased from 0.1 to 6.0 mM (Mouhtaridou *et al*. 2004).

Methionine is a precursor of ethylene biosynthesis in plant tissues (Yang 1985). Other amino acids are also involved in evolution of ethylene during *in vitro* culture (Sudarsana Rao *et al*. 2001). Furthermore, it has been reported that tissues and organs of various plant species produce ethylene when cultured *in vitro* (Gonzalez *et al*. 1997). The endogenously produced ethylene accumulates in the vessel atmosphere and can exert an influence on explant growth and morphogenesis (Sauerbrey *et al*. 1988). In addition, exogenously supply of ethylene promoted regeneration of petunia plants *in vitro* (Dimassi-Theriou *et al*. 1993). The objective of this study was to investigate the effect of various B concentrations combined with different Meth concentrations on shoot proliferation, shoot length, fresh mass and ion content of *in vitro* produced shoots of *Actinidia deliciosa*.

The explants employed were kiwifruit [*Actinidia deliciosa* (A. Chev.) C.F. Liang et A.R. Ferguson var. *deliciosa* cv. Hayward] shoots 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 25 × 100 mm glass test tube containing 10 cm^3 of the Murashige and Skoog (1962; MS) culture medium. The culture medium was supplemented with 30 g dm^{-3} sucrose, 0.5 mg dm^{-3} benzyladenine, and 1 mg dm^{-3} gibberellic acid. Five B concentrations were included in the experiment: 0, 0.02, 0.1, 0.5 and 2 mM combined with four Meth concentrations (0, 2, 20 and 60 μM). 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 22 ± 1 °C and 16-h photoperiod with irradiance of 45 μmol m^{-2} s^{-1} (400 - 700 nm; cool white fluorescent lamps, supplied by *TLD 36W/84* lamps). After ten weeks, the number and length of shoots and the fresh mass per explant (callus excluded) were measured. 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, Mg and Mn were determined by atomic absorption

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Abbreviations: Meth - methionine; MS medium - Murashige and Skoog medium.
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spectrometry (*Perkin-Elmer 2380*, Wellesley, MA, USA). Phosphorus was determined colorimetrically by the ammonium phospho-vanadomolybdate method and N by the Kjeldahl procedure. Boron was determined by the azomethine-H method (Wolf 1974). Each treatment included fifteen replicates (tubes). The 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 growth of kiwifruit explants was affected by B and Meth concentrations of the culture medium. The longest shoots, and greatest number of shoots and fresh mass per explant were observed in medium with 2 mM B and 2 μM Meth, in comparison with the other treatments (Table 1). By increasing B concentration in the culture medium from 0 to 2 mM, an increased rate of shoot proliferation was observed for the various Meth concentrations employed. With regard to shoot length, by increasing B concentration of the medium from 0 to 2 mM, an increase of shoot length was measured in the presence of 0 and 2 μM Meth. However, the same trend was not observed in the presence of the two highest Meth concentrations (Table 1). A significant interaction between B and Meth was calculated concerning shoot proliferation ($P < 0.001$), FM per explant ($P < 0.001$) and shoot length ($P < 0.1$). The results obtained are in agreement with previous ones (Robertson and Loughman 1974, Hu and Brown 1994). 1 mM B in the medium doubled shoot proliferation of *A. deliciosa* in comparison to 0.2 mM B (Sotiropoulos *et al.* 1998). The increase in shoot proliferation at high B concentration indicated that

Table 1. Effect of B and Meth concentrations in the culture medium on the number of shoots per explant, mean shoot length and fresh mass per explant. Means followed by the same letter in the same column are not significantly different (Duncan's multiple range test $P \leq 0.05$).

B [mM]	Meth [μM]	Number of shoots [explant^{-1}]	Shoot length [mm]	Fresh mass [g explant^{-1}]
0.00	0	2.57 k*	12 d	0.68 i
0.02	0	2.86 ij	14 bcd	0.82 gh
0.10	0	3.00 hi	13 cd	0.89 fg
0.50	0	2.95 hij	20 ab	0.82 gh
2.00	0	3.11 gh	19 abc	0.94 ef
0.00	2	3.33 def	15 bcd	0.89 fg
0.02	2	3.50 bcd	17 bcd	0.94 ef
0.10	2	3.67 ab	19 abc	0.90 fg
0.50	2	3.59 abc	17 bcd	0.93 ef
2.00	2	3.78 a	24 a	1.22 a
0.00	20	2.78 j	16 bcd	0.97 ef
0.02	20	2.81 ij	14 bcd	1.08 cd
0.10	20	2.78 j	15 bcd	1.02 de
0.50	20	3.25 efg	13 cd	1.11 bcd
2.00	20	3.13 fgh	17 bcd	1.19 ab
0.00	60	2.65 kj	15 bcd	0.74 hi
0.02	60	3.13 fgh	14 bcd	0.90 fg
0.10	60	3.10 gh	16 bcd	0.77 hi
0.50	60	3.10 gh	17 bcd	0.82 gh
2.00	60	3.30 defg	17 bcd	0.82 gh

Table 2. Effect of B and Meth concentrations in the culture medium on B, N, P, Ca, Mg, and Mn contents of kiwifruit shoots *in vitro*. Means followed by the same letter in the same column are not significantly different (Duncan's multiple range test $P \leq 0.05$).

B [mM]	Meth [μM]	B [mg kg^{-1}(d.m.)]	N [g kg^{-1}(d.m.)]	P [g kg^{-1}(d.m.)]	Ca [g kg^{-1}(d.m.)]	Mg [g kg^{-1}(d.m.)]	Mn [mg kg^{-1}(d.m.)]
0.00	0	9 h	40.21 e	0.9 fg	2.2 fg	1.5 bcdef	152 f
0.02	0	30 g	40.22 e	0.6 g	2.0 g	1.5 bcdef	160 ef
0.10	0	45 g	40.25 e	0.9 fg	2.7 f	1.3 def	197 abcd
0.50	0	118 e	40.21 e	1.7 de	2.6 fg	1.1 ef	205 abc
2.00	0	320 bc	40.35 de	1.7 de	2.1 fg	1.0 f	220 ab
0.00	2	8 h	40.78 bc	2.0 cde	3.5 e	1.7 abcd	193 bcd
0.02	2	33 g	40.90 bc	2.0 cde	3.8 e	1.9 abc	185 cd
0.10	2	45 g	40.78 bc	2.3 cd	3.6 e	1.4 cdef	190 bcd
0.50	2	125 e	40.90 bc	2.0 cde	4.0 de	1.6 abcde	212 ab
2.00	2	330 ab	50.01 bc	2.3 cd	3.7 e	1.6 abcde	212 ab
0.00	20	7 h	40.70 cd	2.0 cde	3.4 e	2.1 a	222 a
0.02	20	45 g	40.65 cd	2.6 c	3.7 e	2.0 ab	198 abcd
0.10	20	50 g	40.70 cd	2.0 cde	4.6 d	1.7 abcd	190 bcd
0.50	20	120 e	40.68 cd	1.9 cde	4.6 d	1.6 abcde	220 ab
2.00	20	305 c	40.78 bc	2.3 cd	4.6 d	1.7 abcd	178 de
0.00	60	8 h	40.64 cd	3.3 b	8.3 b	1.4 cdef	188 bcd
0.02	60	48 g	40.65 cd	3.6 b	6.6 c	1.4 cdef	200 abcd
0.10	60	75 f	40.96 bc	3.8 ab	9.6 a	1.9 abc	185 cd
0.50	60	171 d	50.12 b	4.4 a	6.4 c	1.5 bcdef	178 de
2.00	60	349 a	50.87 a	3.8 ab	8.5 b	1.6 abcde	185 cd

high B concentrations promoted formation of axillary buds or induced bud burst and growth.

An enhanced ethylene production in the vessel atmosphere in response to the presence of Meth in the substrate was reported *in vitro* (Kepczynska *et al*. 2003). Inclusion of 10 μM Meth in the medium increased the endogenously produced ethylene by 25 times in comparison to the control and shoot proliferation and shoot length of the peach rootstock cultured *in vitro* (Dimassi-Theriou 1995). Furthermore, supplementing petunia cultures with ethylene (0.01-10 mg dm^{-3}) caused a marked increase of the number of *in vitro* produced shoots (Dimassi-Theriou *et al*. 1993).

By increasing B concentration in the medium from 0 to 2 mM, its content in kiwifruit plants increased too (Table 2). Maximum B concentration 349 mg kg^{-1}(d.m.) was recorded when 2 mM B and 60 μM Meth were included in the medium. The increase of B concentration of the medium did not alter significantly N content of cultures when Meth concentration was 0, 2 or 20 μM. The highest N content of plants was recorded when 2 mM B and 60 μM Meth were included in the medium (Table 2). Inclusion of 60 μM Meth in the medium resulted in an increase of P and Ca contents of plants (Table 2). Maximum Mg and Mn contents of plants were recorded in the presence of 0 mM B and 20 μM Meth in the medium (Table 2).

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