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BRIEF COMMUNICATION

Contents of macroelements and growth of sweet cherry rootstock *in vitro*

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Abstract

Rootstocks for sweet cherry (*Prunus canescens* Bois) Camil GM 79 were grown *in vitro* on Murashige and Skoog (MS) medium, and on MS medium with double- and half-strength macroelements. All the media contained 4.4 μM 6-benzyladenine, 0.5 μM α-naphthylacetic acid, 0.3 μM gibberellic acid, 20 g dm^{-3} sucrose and 7 g dm^{-3} agar. The chemical analyses were monitored on day 0 and 40 of culturing in callus, stem and leaves. Fresh and dry mass of shoots increased linearly up to the end of culture. The highest fresh and dry masses and also the content of Ca and Mg were registered in shoots grown on half-strength MS medium.

Additional key words: fresh and dry mass of explants, media, mineral composition, *Prunus canescens*.

A number of factors affect *in vitro* growth, mineral nutrition being among them. Studies carried out up to now indicate that mineral composition of many media is deficient in or in excess of some macroelements, which may induce abnormal growth, vitrification and many other undesirable effects on plants grown *in vitro*. Many media compositions have been developed via empirical methods. The second approach is analytical and includes determination of the mineral elements taken up by the plants and their concentration in the plant tissue and medium. Unfortunately such studies are rare.

Considering the significance of the mineral nutrition *in vitro*, the aim of the paper was to study the growth and macroelements content in sweet cherry rootstock as affected by different concentration of macroelements in the media.

Currently interesting sweet cherry (*Prunus canescens* Bois) rootstock Camil GM 79 was used. Murashige and Skoog (1962) (MS) medium, MS medium with double (MS 2×), and half (MS ½) strength macroelements were used. All the media contained agar in the concentration of 7 g dm^{-3} and sucrose, 20 g dm^{-3}, and following hormones: 4.4 μM 6-benzyladenine (BA), 0.5 μM α-naphthylacetic acid (NAA) and 0.3 μM gibberellic acid (GA_3). The pH of the medium was adjusted to 5.7 with 0.1 M KOH and sterilization was conducted at 120 °C for 20 min. Plants are cultivated in the growth room under 16-h photoperiod with irradiance on culture surface of 8.83 W m^{-2} (40 W white fluorescent lamps) and temperature of 25 ± 1 °C.

Fresh mass, dry mass and all chemical analyses were monitored on day 0 and 40 of culturing in callus, stem and leaves. Dry mass was determined after drying at 65 - 70 °C for 48 h. Plant material for determination of macroelements N, P, K, Ca and Mg was dried at 105 °C for 8 h, crumbled and 0.15 g stirred in ovens at 550 °C for 48 h. After that ash was diluted with 5 cm^3 3 M HCl up to boiling. Quantitatively filtrated solution was used as a basic solution from which the following was determined: potassium, calcium and magnesium with atomic absorption spectrophotometer (AAS) SP-191 *Pye Unicam* (England), phosphorous with UV/VIS spectrophotometer (*PU 8740, Pye Unicam*) by colorimetric vanadomolybdate method. Nitrogen was determined from an individual sample (0.20 g) according to Kjeldahl procedure on *Tecator-Kjeltec System 1003* (Hoganas, Sweden).

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Abbreviations: BA - 6-benzyladenine; DM - dry mass; FM - fresh mass; GA_3 - gibberellic acid; MS medium - Murashige and Skoog medium; NAA - α-naphthylacetic acid.
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The shoots of Camil had the highest FM and DM on MS ½ medium at the end of subculture. In terms of shoot parts, the highest FM and DM of leaves was registered also on MS ½ medium (Table 1). Mezzetti et al. (1991) also proved that FM and DM of *Actinidia deliciosa* explants grown *in vitro* were increasing during the first 30 d of culture, in particular FM and DM of leaves, whereas stem FM and DM rose insignificantly. FM and DM of Camil explants on all the media constantly increased up to the end of culture. The stated fact was also proved by Singha *et al.* (1987), who indicated that DM of pear cv. Seckel and crab apple cv. Almey explants had a linear increase on MS medium up to the end of culture. In addition, the increase in culture duration induced the rise in callus FM and DM in three wheat cultivars (Ćalović 1997).

The previous study (Ružić 1998) proved that Camil had the highest multiplication index on MS ½, which correlates to the highest FM and DM of the explants (Table 1). However, Singha *et al.* (1987) concluded via comparison of multiplication index in pear cv. Seckel and crab apple cv. Almey with DM, that the stated two parameters are not obligatory dependent. It is known that reduction in mineral content of MS medium favourably affects shoot rooting (Orlikowska 1992). However, even in the multiplication phase Camil reacted more effectively on the media with reduced content of macroelements, which proves the species specificity of mineral nutrition *in vitro*.

The correlation among FM, DM and multiplication in Camil was observed, but not in terms of the macroelements content in shoots. Only the content of Ca and Mg was highest on MS ½ medium (Table 2). The highest N and K amount was recorded with the shoots grown on MS 2×, and P on MS medium. In the callused cultures of sugar beet, Mezei *et al.* (1995) also did not find the correlation between DM and the element concentration. Ćalović (1997) only found correlation between DM and total N content in callus of three wheat cultivars.

According to the observed relations in this paper, the FM and DM may undoubtedly be a proper indicator of culture growth, since a linear dependence with the multiplication parameters was recorded. The complete correlation between FM and DM with macroelements content in shoots was not observed.

Table 1. Effect of content of macroelements in the medium on fresh (FM) and dry mass (DM) [g] of the explants during culturing. Means ± SD of three replications

Media	Explant	0 d FM	40 d FM	0 d DM	40 d DM
MS 2×	Callus	-	0.03 ± 0.01	-	0.010 ± 0.001
MS 2×	Stem	0.04 ± 0.01	0.13 ± 0.05	0.009 ± 0.001	0.020 ± 0.005
MS 2×	Leaf	0.04 ± 0.01	0.33 ± 0.08	0.009 ± 0.001	0.040 ± 0.006
MS	Callus	-	0.11 ± 0.02	-	0.020 ± 0.005
MS	Stem	0.05 ± 0.01	0.21 ± 0.01	0.005 ± 0.001	0.020 ± 0.001
MS	Leaf	0.04 ± 0.01	0.42 ± 0.06	0.006 ± 0.001	0.050 ± 0.001
MS ½	Callus	-	0.10 ± 0.01	-	0.010 ± 0.001
MS ½	Stem	0.03 ± 0.01	0.10 ± 0.01	0.003 ± 0.001	0.010 ± 0.003
MS ½	Leaf	0.05 ± 0.01	0.50 ± 0.01	0.006 ± 0.001	0.060 ± 0.003

Table 2. Contents of N, P, K, Ca and Mg [mg g^{-1}(DM)] in the different parts of the Camil explants during culturing on media with different content of macroelements.

Media	Explant	0 d N	40 d N	0 d P	40 d P	0 d K	40 d K	0 d Ca	40 d Ca	0 d Mg	40 d Mg
MS 2×	Callus	-	0.20	-	0.01	-	0.05	-	0.01	-	0
MS 2×	Stem	0.51	1.07	0.02	0.06	0.08	0.25	0.01	0.03	0.01	0.02
MS 2×	Leaf	0.41	1.85	0.01	0.08	0.13	0.67	0.02	0.06	0.01	0.03
MS	Callus	-	0.58	-	0.03	-	0.15	-	0.02	-	0.02
MS	Stem	0.18	0.68	0.01	0.03	0.07	0.36	0.03	0.03	0	0.02
MS	Leaf	0.17	1.50	0.01	0.09	0.09	0.65	0.02	0.08	0.01	0.06
MS ½	Callus	-	0.13	-	0.01	-	0	-	0.02	-	0.01
MS ½	Stem	0.05	0.17	0.01	0.01	0.04	0.15	0	0.02	0	0.01
MS ½	Leaf	0.13	1.10	0.01	0.07	0.08	0.59	0.01	0.12	0.01	0.06

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