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Influence of Various Types of Culture Vessels upon the *In Vitro* Multiplication of Highbush Blueberry (*Vaccinium corymbosum*)

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Abstract. Our paper presents aspects regarding the influence of culture vessels upon multiplication rate in the highbush blueberry (*Vaccinium corymbosum*) cultivar `Blue Crop`. Modified Woody Plant Medium (WPM) with 5 mg/l 2-Isopentenyladenine (2-Ip) and 20 mg/l ascorbic acid was used as culture medium. The following types of culture vessels were tested: Magenta GA7, 720 ml jars with metal caps vented with sponge filters, 720 ml jars with polycarbonate caps without venting, 720 ml jars with vented polycarbonate caps equipped with filters made of water-resistant bandaids, 320 ml jam jars with polycarbonate caps without venting and Eco 2 Box vessels with vented caps. The best growth and highest multiplication rates were obtained in the 720 ml jars with metal caps vented with sponge filters and the shoots were vigorous, with intense green color.

Keywords: micropropagation, Woody Plant Medium, Magenta GA7, Eco 2 box

Introduction. For blueberry micropropagation several basal media have been used: Anderson, Woody Plant Medium, Zimmermann & Broome medium, Economou & Read. Among the growth regulators used, Zeatin and 2-Isopentenyladenine ensured the highest proliferation rates (Chandler and Draper, 1986; Clapa *et al*., 2008; Eccher *et al*.,1986; Eccher and Noe, 1989; Orlikowska, 1986; Zimmerman and Broome, 1980).

Aims and Objectives. The aim of this research was to find out whether the various types of culture vessels influence the *in vitro* proliferation of the highbush blueberry (*Vaccinium corymbosum*) by their shape, volume (330 ml, 720 ml), material (glass, polycarbonate, polypropylene) as well as by the type of their lids (vented or not vented). The influence of culture vessels upon proliferation rate as well as shoot quality was investigated.

Materials and Methods. *Plant material used*: 2 cm long shoot fragments, containing 5-6 nodes, from *in vitro* cultured blueberry cultivar 'Blue Crop'.

Nutritive medium: Woody Plant Medium (Lloyd & McCown) prepared from stock solutions of macro- and micronutrients and stock solutions of vitamins B_1, $B_{6,}$ nicotinic acid, 5 mg/l 2-Isopentenyladenine and 20 mg/l vitamin C and pH was adjusted to 5. The medium was gelled with agar (5 g/l Plant Agar). All the components of the medium were added before autoclavation. The following types of culture vessels were tested: V1: Eco 2 Box with vented lids, V2: Magenta GA7 vessels, V3 : 720 ml jars with polypropylene lids without vents, V4 : 320 ml jars with polypropylene lids without vents, V5: 720 ml jars with metal caps with vents equipped with anti-microbial filter made of autoclavable plastic sponge (Fig.1.) 20 microcuttings were inoculated into each vessel, with the exception of variant V2, where 16 microcuttings were inoculated/vessel.

V1 V2 V3 V4 V5

Fig. 1. *Vaccinium corymbosum*, cv. `Blue Crop` in various culture vessels

The cultures were incubated in the growth room in artificial light ensured by fluorescent tubes. Light intensity was of about 2500 Luxes and temperature was of 24-26^{o} C.

Results and Discussions. The highest proliferation rate (4.03 shoots/explant) was obtained in the 720 ml jars with vented metal lids equipped with filters made of plastic sponge. The shoots were vigorous and bright green in colour. In variant number 3 (720 ml jars with polypropylene lids without vents) the proliferation rate was close (3.88 shoots/plantlet). It should be mentioned that cultivar `Blue Crop` is difficult to propagate *in vitro*, a higher proliferation rate can be obtained by using zeatin as growth regulator, but in the present experiment zeatin was replaced with 2-Isopentenyladenine and vitamin C in order to reduce costs.

The significance of differences regarding the proliferation rates in blueberry cultivar `Blue Crop` in the 5 types of culture vessels, evaluated by Duncan's test (Tab. 1) confirms that, among the types of vessels, the 720 ml jars with vented metal lids equipped with filters made of plastic sponge were the most effective for the micropropagation of this highbush blueberry cultivar if 2-Isopentenyladenine is used as growth regulator.

Tab. 1

The significance of differences regarding proliferation rates, evaluated by Duncan's test

Variant	Average number of shoots/vesel	Classification
V2	44.13	A
V1	47.50	A
V4	51.38	A
V3	77.75	B
V5	80.63	B

Conclusion. In the process of *in vitro* propagation of highbush blueberry cultivar `Blue Crop`, the culture vessels influence proliferation rate as well as the quality of the shoots obtained. The most effective vessels were the 720 ml jars with metal caps with vents equipped with anti-microbial filters made of autoclavable plastic sponge. These ensured the highest proliferation rate and the shoots were vigorous, long, bright green in color.

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