

In vitro propagation of *Vaccinium corymbosum* L.

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Abstract

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The production of *Vaccinium* sp. has been a growing worldwide interest after understanding their importance for diet and human health.

The aim of the work was direct regeneration, propagation and rooting of axillary buds isolated from five highbush blueberry cultivars ‘Bluecrop’, ‘Bluegold’, ‘Bluejay’, ‘Spartan’ and ‘Patriot’ and adapt of suitable protocol, developed and applied for *Vaccinium corymbosum* L. cultivars in our laboratory.

For micropropagation, a basal cultural medium WPM was used, supplemented with 3.0 mg/L zeatin and 2.0 mg/l 2-iP, pH 4.2. The highest proliferation capacity was reported for cv. ‘Bluegold’ after fourth passage of sub cultivation (4.4 shoots per explant) and the largest length of the shoots was reported for the cv. ‘Bluejay’ (3.9 cm).

The rooting ability was established on the same basal medium (WPM) with half-reduced salt concentration enriched with 1.0 mg/L IAA, pH 4.2. The high rooting percentage (33.3%) and the highest average number of roots (3.9) was reported for cv. ‘Spartan’ and ‘Patriot’.

The results demonstrate that regeneration potential is genotyp dependent.

Keywords: *Vaccinium corymbosum* L.; *in vitro*; micropropagation; rooting

Abbreviations: Woody Plant Medium (WPM); zeatin (4- Hydroxy -3-methylbut-2-enylamino) purine; 2iP (N⁶ – isopentenyladenine); IAA (Indole-3-acetic acid); IBA (indole-3-butyric acid)

Introduction

Small berry fruits are an economically crops in many countries. The interest to them has recently increased because they are rich sources of vitamins, anti-oxidants and other valuable compounds.

The application of plant tissue culture methods for *in vitro* propagation is a major technology for production of healthy, strong and genetically identical plants. Highbush blueberry (*Vaccinium corymbosum* L.) is one of them, which belongs to *Ericaceae* family.

The choice of blueberry cultivars for objective of the research has a scientific and commercial advantage.

A number of studies have examined *in vitro* cultivation of blueberry (George & Sherrington, 1984; Smagula & Lyrene, 1984; Zimmerman, 1991; Galletta & Ballington, 1996; Deb-nath, 2003; Rowland & Hammerschlag, 2005). A great number of researchers have focused their research on the cultural medium suitable for the rapid propagation of this species. Some of them recommend different variants of modified and agar-gelled WPM medium (Reed, 1991; Gadjosova et al., 2006; Doina & Fira, 2007; Fira et al., 2008), while others offer AN artificial cultural medium (Anderson, 1980; Grout & Reed, 1986; Ostrolucká et al., 2004; Gadjosova et al., 2006; Gadjosova et al., 2009). The appropriate cytokinin choice determines the successful micropropagation of highbush

blueberry. The most commonly used cytokines, which are applied exogenously during the proliferation phase, are zeatin and 2-iP (Chandler & Draper, 1986; Eccher & Noe, 1989; Reed & Esquivel, 1991; Ostrolucka et al., 2004; Gadjosova et al., 2006; Doina & Fira, 2007; Fira et al., 2008; Gadjosova et al., 2009; Litwińczuk, 2013, etc.).

Fira et al. (2008) reported that the using of TDZ in medium induced slow growth and low multiplication rate, and therefore they do not recommend the use of this growth regulator for micropropagation of the highbush blueberry.

Highbush blueberry can be rooted successfully on *ex vitro* and *in vitro* conditions.

Ex vitro rooting can be conducted without auxin treatment (Gonzalez et al., 2000; Morison et al., 2000) or by preliminary immersion in IBA solution (1 g/l IBA in 50% ethanol) - Zimmerman and Broome (1980); immersion in 0.8 mg/l IBA (Ostrolucká et al., 2004; Gadjosova et al., 2009) or 3.0 g/l (Litwińczuk, 2013). Rooting in *ex vitro* conditions could reduce costs (Zimmerman, 1987), although the process is often slower than *in vitro* rooting (Wolfe et al., 1983). *In vitro* rooting has been found to reduce disease risk and overcome environmental stress (Debnath, 2006).

Material and Methods

Planting material

The objective of this study was to evaluate five high-bush blueberry cultivars (CVs): Bluecrop, Bluegold, Bluejay, Spartan and Patriot regarding their in-vitro behavior.

The selected cultivars are distinguished by good shoot formation ability, moderate to strong growth, very good fruitfulness and adaptability in various natural and climatic conditions average to large-sized fruit (Leposavić, 2014).

The axillary buds were separated from annual shoots. Donor plants were cultivated in isolation facilities where they were grown in individual containers, a sterile soil substrate covered with a fine mesh to prevent further contamination by vectors (aphids and nematodes).

Working protocols for sterilization and micropropagation of the cv. Toro have been used, published in previous article (Georgieva et al., 2016).

Micropropagated plants were grown in growth chambers with controlled conditions. The maintained temperature was $22 \pm 25^\circ\text{C}$, a 16/8 day/night photoperiod and illumination of 2000-3000 lx. The subculturing period was 30 days.

The adaptation of the rooted plants was carried out in a sterile, acid peat substrate, under polyethylene at high air humidity, which were gradually reduced, and a temperature of $20-22^\circ\text{C}$. The transfer of microplants from *in vitro* to *in vivo* conditions was successful. About 80-90% of them survived.

The following parameters are reported: proliferation capacity, length of *in vitro* shoots, rooting %, average length and number of roots.

The data are processed by a variational-statistical method (Lidanski, 1988).

Results and Discussion

The results indicate that the five cultivars of highbush blueberry can be successfully micropropagated by axillary organogenesis on WPM cultural medium enriched with 3.0 mg/l zeatin and 2.0 mg/l 2-iP and pH 4.2. The propagation potential is dependent on the type, cytokinin concentration, culture medium, subculture passage, seasonality, type of explant, genotypic characteristics of the cultivar, etc. The proliferation coefficient of the individual subculturing passages is different (Figure 1). However, there are no mathematically proven differences between the different passages of the varieties, but there is a good statistical difference between the studied genotypes. High variability is observed in the cv's Bluecrop, Bluegold, Spartan and Patriot (Table 1).

In this experiment, the multiplication factor is within 1.2 (cv. Bluecrop -V passage) to 4.4 (cv. Bluegold - IV passage). In two cv's (Spartan and Patriot) the maximum propagational potential was recorded on the VI passage from subcultivation, respectively 2.9 and 2.5. Similar to our results are recorded by Yavorska et al. (2016), which reported a micropropagational factor of 2.21 in cv. Bluecrop and 4.38 in cv. Bluegold on WPM cultural medium enriched with 0.5 mg/l zeatin and 4 mg/2-iP. Ostrolucka et al. (2004) found that a zeatin level of 2.0 mg/l was appropriate for the micropropagation of four blueberry cultivars, as cv. Bluecrop the multiplication index was 3.94 using AN cultural medium. Gadjosova et al. (2006), reported that the best plant response

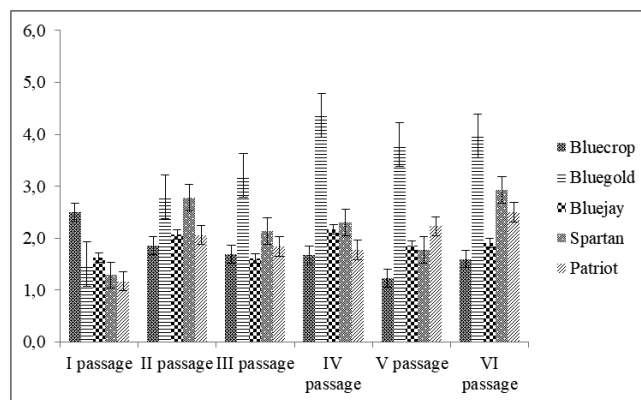


Fig. 1. Multiplication potential of studied cultivars of *p. Vaccinium* (±SE)

Table 1. Statistical analysis of the multiplication potential

Multiplication potential					
Cultivars	Bluecrop	Bluegold	Bluejay	Spartan	Patriot
St Dev	0.42	1.03	0.23	0.62	0.46
Amplitude min - max	1.23-2.50	1.5-4.37	1.61-2.17	1.29-2.93	1.17-2.50
CV	23.86	31.40	12.30	28.18	23.83
LSD 0.05	P > 0.05				
Passages LSD 0.01	4.03				

for the five cultivars of highbush blueberries was found for morphogenesis and multiplication in a low application of zeatin (0.5 mg/l). Our results, as well as the results of Ruzich et al. (2012) convincingly prove that zeatin and 2-iP are essential for axillary organogenesis in highbush blueberries. Zeatin stimulates shoot growth and is better for the effective multiplication in *Vaccinium* sp. (Eccher & Noe, 1989; Rowland & Ogden, 1992; Ostrolucká et al., 2002; Ondrušková et al., 2003; Ostrolucká et al., 2004; Ruzich et al., Fira et al., 2008; Ruzich et al., 2012).

In two cv. (Spartan and Patriot) of the 5 genotypes studied, the maximum propagational potential was recorded in the sixth subculturing passage, respectively 2.9 and 2.5 *in vitro* shoots.

The length of the newly formed shoots was in the range of 1.1 cm (cv.Bluecrop- IV passage to 3.9 cm (cv. Bluejoy- I

passage) (Figure 2). For all genotypes included in the present study, the maximum values of this index of the first passage of the preliminary propagations were registered.

There is no statistically significant difference between the passages, but a very good evidence was found among the studied cultivars. High variations in values of the cultivars are recorded (Table 2).

All cultivars included in the study were successfully rooted on WPM cultural medium with a half-reduced salt concentration and 1 mg/l IAA addition (Figure 4). The quality of the root system is determined by the length and average number of roots per *in vitro* plant. The highest rooting percentage values in the present experimental protocol were reported for cv's Spartan and Patriot - 33.3% (Figure 3). Satisfactory rooting was recorded in cv. Bluejay - 8.8%. Similar results are reported by Sedlak & Paprstein (2009), who reported 70% rooting for cv. Berkeley and 61% in cv. 'Blue-

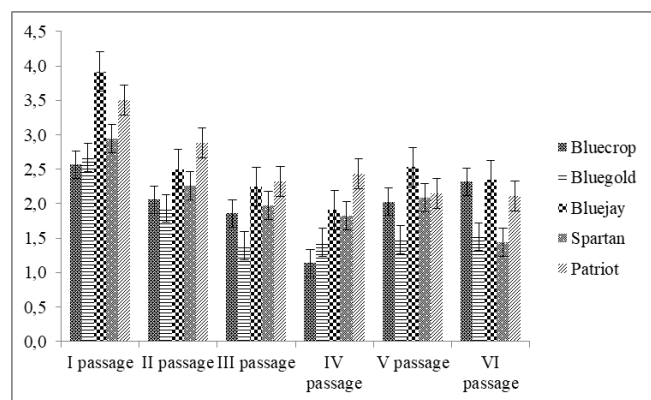


Fig. 2. Length of the shoots of different passages of subculturing (±SE)

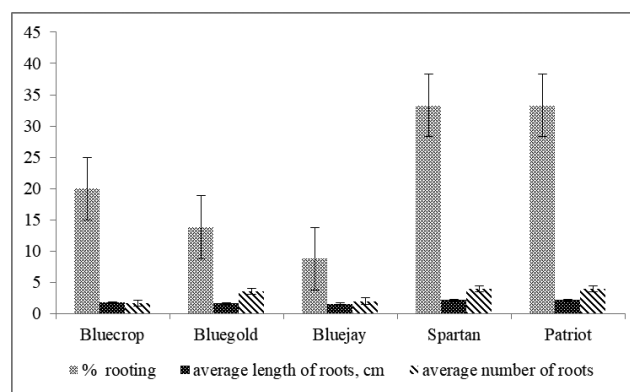


Fig. 3. Rooting potential of plants of *Vaccinium* (±SE)

Table 2. Statistical analysis of shoot lengths

Length of shoots					
Cultivars	Bluecrop	Bluegold	Bluejay	Spartan	Patriot
St Dev	0.49	0.50	0.70	0.50	0.53
Amplitude min - max	1.14-2.57	1.39-2.67	1.91-3.92	1.44-2.94	2.11-3.50
CV	24.5	28.74	27.13	23.92	20.62
LSD 0.05	P > 0.05				
Passages LSD 0.001	4.49				

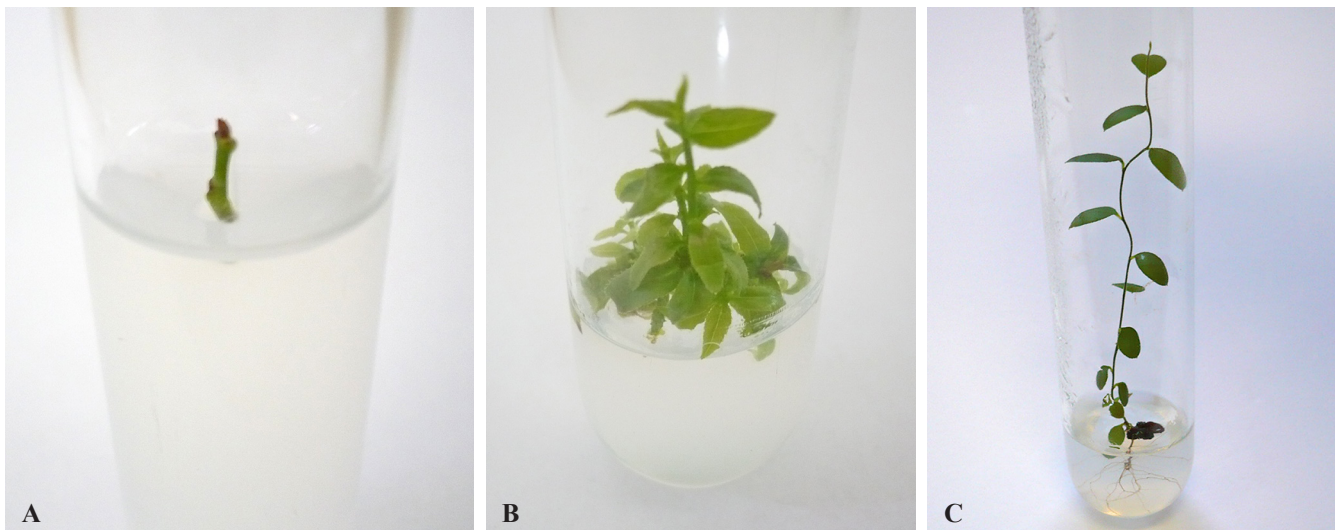


Fig. 4. A. Introduction into *in vitro* culture of cv. Spartan:

B. Multiplication capacity of cv. Spartan after 30 days of cultivation on WPM cultural medium containing 3.0 mg/l zeatin and 2.0 mg/l 2-iP; C. Rooting potential of cv. Spartan on WPM, with half-reduced salt concentration enriched with 1.0 mg/l IAA, pH 4.2

crop' on WPM medium supplemented with 1.0 mg/l IBA. Similarly, Ruzić et al. (2012) received 39.1% rooting in cv. Bluecrop and 81.8% in cv. Goldtraube on AN cultural medium enriched with 0.8 mg/l IBA and 4 g/l activated carbon.

The highest average root length of *in vitro* plants was observed in cv. Patriot - 2.2 cm. For the other genotypes of highbush blueberry, they were in the range of 1.6-2.2 cm (Figure 3).

The highest average number of roots is found in cv. Spartan and cv. Patriot genotypes - 3.9.

The experimental protocol can be optimized and used for the accelerated propagation and production of virus-free planting material of *Vaccinium* species for commercial purposes.

Conclusions

The propagation of a large number of plant species through *in vitro* technology has great potential, especially for species with recalcitrant behavior. The five cultivars of highbush blueberry were successfully propagated by *in vitro* conditions, on WPM cultural medium enriched with 3.0 mg/l zeatin and 2.0 mg/l 2-iP, pH - 4.2.

The multiplication potential of the studied cultivars differs for the individual passages of the study. The highest proliferation capacity was recorded in cv. Bluegold cultivar on the fourth passage of subcultivation - 4.4 numbers/explant.

The length of the shoots is specific for the cultivar. The largest length of the shoots was reported for cv. Bluejay - 3.9 cm.

The risogenetic capacity was established on WPM cultural medium with half-reduced salt concentration, supplemented with 1.0 mg/l IAA. Rooting ability is genotypically dependent. The highest rate of rooting was observed in cv's Spartan and Patriot - 33.3%. The best results relation to the average number of roots were recorded in the genotypes of cv's Spartan and Patriot - 3.9. The largest length of microplants was recorded in cv. Patriot - 2.3 cm.

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