Efficient micropropagation protocol of three cultivars of highbush blueberry (Vaccinium corymbosum L.)

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Abstract

Highbush blueberry (Vaccinium corymbosum L.) is increasingly farmed for its nutritional and health benefits, but high yield and fruit quality require proper planting material. Modified Murashige and Skoog (MW), Anderson’s Rhododendron (AN), and Woody Plant Medium (WPM) were compared for in vitro organogenesis and rooting of three highbush blueberries ‘Elizabeth’, ‘Meader’, and ‘Liberty’. All media contained 0.1 mg L⁻¹ zeatin applied with a combination of IBA, IAA, and GA₃. The results showed that MW medium is more suitable for in vitro multiplication of ‘Elizabeth’ and ‘Meader’, and WPM medium for ‘Liberty’. However, medium supplemented with a low concentration of IBA (≤ 0.4 mg L⁻¹) and 0.1 mg L⁻¹ zeatin increased the shoot regeneration rate of highbush blueberries multiplied in vitro. The rooting capability was studied by using WPM and AN medium with IBA and IAA with zeatin. The highest rooting rate (85%) and acclimatization (70%) were achieved in ‘Liberty’, and the lowest was in ‘Elizabeth’ (33.3% and 50%, respectively) and ‘Meader’ (33.3% and 43.8%, respectively). Rooted plantlets developed good quality roots and were transplanted into peat:perlite (4:1) substrates and acclimatized in a greenhouse under controlled conditions. We developed a complete micropropagation protocol for cvs. ‘Meader’, ‘Elizabeth’ and ‘Liberty’ blueberry. This protocol can be used for the production of certified vegetative material or different biotechnological purposes.

Keywords: acclimatization; highbush blueberry; in vitro; plant growth regulators; rooting

Abbreviations: AN, Anderson’s Rhododendron medium; GA₃, Gibberellic Acid; IAA, indole-3-acetic acid; IBA, indole-3-butyric acid; MS, Murashige and Skoog medium; WPM, The McCown Woody Plant medium; MW, mixture of equal parts of MS and WPM media; TDZ, tidiazuron, 2iP, 2-isopentenyladenine.

Introduction

The highbush blueberry (Vaccinium corymbosum L.) belongs to the Ericaceae family. Highbush blueberry is a crop with an increasing trend in global consumption and cultivation volume (Podymiak, 2015;
Brazelton and Young, 2017; Ochmian et al., 2020). World production of highbush blueberry fruit was 655.0 metric tonnes (MT), an increase of about 14% compared to 2014. Poland ranks second in Europe and seventh in the world in terms of highbush blueberry fruit production (Brazelton and Young, 2017). The largest producers are the USA, Chile and Canada. In Europe, production is concentrated in Spain, Poland and Germany (Brazelton, 2013; Brazelton and Young, 2017). Blueberry fruits are valued for their high content of health-promoting components such as polyphenols, low calorie content, taste and nutritional value (Brazelton, 2013; Brazelton and Young, 2017). Highbush blueberry is a plant that requires acidic soils (pH 3.8-5.5), well aerated, with stable groundwater levels and high humus content (Ochmian et al., 2020; Figiel-Kroczyńska et al., 2022). Despite the worldwide increase in blueberry cultivation, the availability of suitable soils for this species remains a problem (Ochmian et al., 2018).

Due to the rapid increase in blueberry growing area, blueberry seedlings should be produced quickly and in large quantities. Traditionally, blueberry is propagated by coniferous, semi-coniferous and deciduous cuttings, by rhizomatous or rhizomatous cuttings of selected clones. Problems in this production are the very low rooting percentage for many genotypes, the long time to propagation and commercialization of newly obtained plants, and phytosanitary.

Tissue culture propagation techniques can be used as a system for effective plant production of virus-free plants which are genetically identical. There are many reports in the literature on in vitro propagation of blueberry (Sedlák and Paprštein, 2009; Ružić et al., 2012; Cüce and Sökmen, 2017; Kruczek et al., 2021). However, all aforesaid attempts showed great variations in terms of basal media as well as plant growth hormones, growth conditions, explant types, sampling, and physiological condition of the explants (Ružić et al., 2012; Krupa-Malkiewicz et al., 2017; Kruczek et al., 2020). Therefore, the results presented in current study are not broadly applicable, because the effectiveness of the medium and morphogenesis of Vaccinium in vitro plants dependent on genotype or even cultivar (Sedlák and Paprštein, 2009).

The aim of the presented study was to determine the best media for in vitro shoot proliferation and rooting of three highbush blueberry cultivars grown in Poland.

Materials and Methods

Characteristics of the area of research and characteristics of cultivars

Actively growing young nodal steam (about 10 cm) were collected from five-year-old Vaccinium corymbosum L. plants, from the bushes grown on organic farm specializing in the cultivation of the highbush blueberry, located about 60 km east of Szczecin (the North-Western part of Poland) between April and May 2018. The first three steps of propagation (initiation, multiplication, rooting) were conducted in the laboratory of the Department of Plant Genetics, Breeding and Biotechnology at the West Pomeranian University of Technology in Szczecin, Poland. The last stage – acclimatisation, was conducted on the same production plantation from which the research material originated and lasted from 2020 to 2021.

Plant material

The plants material consisted three cultivars of highbush blueberry: ‘Elizabeth’, ‘Liberty’ and ‘Meader’. After defoliation, stems were washed with running tap water with a detergent (Ludwik washing-up liquid) for 1 h and then surface-sterilized with 70% (v/v) ethanol for 30 s. Next explants were incubated in 7.5% (v/v) sodium hypochlorite (NaOCl) for 10 minutes and 0.2% (v/v) mercury sulphate (HgSO₄) for 10 minutes. Explants were rinsed with sterile deionized water three times under laminar flow hood. The stems were further cut as two-node stem explants (about 1-2 cm) and cultured on initiation medium WPM (Lloyd and McCown, 1980) with the addition of 0.1 mg L⁻¹ zeatin. Initial medium was selected based on preliminary experiments (unpublished data).
**Multiplication and culture conditions**

An axillaries bud of highbush blueberry was taken from initial *in vitro* culture. The shoot explants were transferred to MW medium (mixture of equal parts of MS – Murashige and Skoog (1962) and WPM media) with the addition of IBA in a concentration of 0.2 and 0.4 mg L\(^{-1}\) (MW 1 and MW 2, respectively); WPM medium with the addition of IBA in a concentration of 0.2 and 0.4 mg L\(^{-1}\) (WPM 1 and WPM 2, respectively) and WPM + 0.2 mg L\(^{-1}\) IBA + 0.4 mg L\(^{-1}\) GA\(_3\) (WPM 3); AN medium (Anderson, 1984) with the addition of IBA in a concentration of 0.2 and 0.4 mg L\(^{-1}\) (AN 1 and AN 2, respectively), and AN + 0.4 mg L\(^{-1}\) IBA + 0.4 mg L\(^{-1}\) GA\(_3\) (AN 3). Each medium combination also contained the addition of 0.1 mg L\(^{-1}\) zeatin (Table 1). After 35 days, explants were removed and washed with deionized distilled water, shoot length, number of new shoots, fresh and dry mass were measured as well as shoot regeneration rate (%). Dry mass of explants was determined after drying in the hot-air oven at 70 °C for 24 hours.

**Table 1.** Multiplication mediums with different plant growth regulators

<table>
<thead>
<tr>
<th>Medium</th>
<th>mg L(^{-1}) IBA</th>
<th>mg L(^{-1}) GA(_3)</th>
<th>mg L(^{-1}) zeatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>AN* 1</td>
<td>0.2</td>
<td>-</td>
<td>0.1</td>
</tr>
<tr>
<td>AN 2</td>
<td>0.4</td>
<td>-</td>
<td>0.1</td>
</tr>
<tr>
<td>AN 3</td>
<td>0.4</td>
<td>0.4</td>
<td>0.1</td>
</tr>
<tr>
<td>WPM 1</td>
<td>0.2</td>
<td>-</td>
<td>0.1</td>
</tr>
<tr>
<td>WPM 2</td>
<td>0.4</td>
<td>-</td>
<td>0.1</td>
</tr>
<tr>
<td>WPM 3</td>
<td>0.4</td>
<td>0.4</td>
<td>0.1</td>
</tr>
<tr>
<td>MW 1</td>
<td>0.2</td>
<td>-</td>
<td>0.1</td>
</tr>
<tr>
<td>MW 2</td>
<td>0.4</td>
<td>-</td>
<td>0.1</td>
</tr>
</tbody>
</table>

AN* – Anderson Rhododendron; WPM – Woody Plant Media; MW – a mixture of equal parts of MS – Murashige and Skoog and WPM media

**In vitro and ex vitro rooting**

Axillary shoots height 2.5 cm were rooted in AN medium (AN A and AN B) and WPM medium (WPM A and WPM B), each supplemented with 0.1 mg L\(^{-1}\) zeatin and IBA or IAA at concentration of 0.5 or 1.0 mg L\(^{-1}\) (Table 2). After 35 days of culture, rooting percentage (%), the length of the shoots and roots, the number of new shoots, roots and leaves, as well as fresh and dry mass were recorded.

**Table 2.** Rooting medium with different plant growth regulators

<table>
<thead>
<tr>
<th>Medium</th>
<th>mg L(^{-1}) IBA</th>
<th>mg L(^{-1}) zeatin</th>
<th>mg L(^{-1}) IAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>AN* A</td>
<td>0.5</td>
<td>0.1</td>
<td>-</td>
</tr>
<tr>
<td>AN B</td>
<td>1.0</td>
<td>0.1</td>
<td>-</td>
</tr>
<tr>
<td>WPM A</td>
<td>-</td>
<td>0.1</td>
<td>0.5</td>
</tr>
<tr>
<td>WPM B</td>
<td>-</td>
<td>0.1</td>
<td>1.0</td>
</tr>
</tbody>
</table>

AN* – Anderson Rhododendron; WPM – Woody Plant Media

Explants were initially selected under an *in vitro* culture, and then made adaptive to greenhouse conditions in May 2020. The plantlets with well-developed shoots and roots were transferred into round black pots of diameter 7 cm, filled with a mixture of 4 L peat, 1 L perlite, 1 L water and 1 mL systemic multi-site fungicide (Previcur Energy 840 SL, Bayer). Peat consisted 66.9% organic matter, EC 0.24 mS/cm, volume weight 0.35 kg dm\(^{-1}\), full water capacity 85.2% vv and pH in KCl 3.44. It was characterized by optimal content for blueberry (Komosa, 2007). The pots were placed on 60-cm high tables in the greenhouse at 20 °C/15 °C day/night temperature cycles and light intensity of 110 mol m\(^{-2}\) s\(^{-1}\) during the experimental period. The temperature inside the greenhouse was controlled by vents that opened automatically. After six weeks, the plants were transferred to the biggest pots (one plant per pot) diameter of 10 cm and 400 mL capacity and filled with the same substrate as previously. The number of plants that were successfully acclimatised was counted.
and the acclimatisation rate (%) was calculated. The plants at any stage of acclimisation were watered and sprayed twice a week with a fungicide (Switch 62.5 WG; Syngenta).

After two-month, plants were transferred (July 2020) to a blueberry plantation, where they were kept in the currently prevailing weather conditions in an open space.

**Plant culture conditions**

*In vitro* culture was conducted in 300 mL flask filled with 30 mL of medium. All media were supplemented with 30 g L$^{-1}$ sucrose (Chempur, Poland) and 100 mg L$^{-1}$ myo-inositol (Duchefa, The Netherlands) and were solidified with 8 g L$^{-1}$ agar (Biocorp, Poland). pH of all the media was adjusted to 5.8 prior to autoclaving at 121 °C and 0.1 Mpa. All cultures were incubated in a growth room at a temperature of 25 ± 2 °C under 16 hours photoperiod with a photosynthetic flux density (PPFD) of 40 µmol m$^{-2}$ s$^{-1}$ provided by Narva (Germany) emitting daylight cool white and 60-70% humidity. Each combination included 32 shoots (4 explants per flask in eight replications).

**Statistical analysis**

All statistical analyses were performed using Statistica 13.0 (StatSoft, Cracow, Poland). Statistical significance of the differences between means was determined by testing the homogeneity of variance and normality of distribution, followed by ANOVA with Tukey’s post hoc test. The significance was set at $p<0.05$.

**Results**

**Multiplication**

Significant differences in multiplication parameters were observed between media with different hormonal compositions (Table 3). In our study, among the combinations of multiplication medium tested, WPM media and MW media yielded the best shoot regeneration rate from 80 to 100% (Table 3). It was found that the WPM 3 medium for multiplication with the addition of GA$_3$ was the best for cultivar ‘Liberty’. In case of cultivars ‘Elizabeth’ and ‘Meader’ the best results in multiplication were achieved when MW 2 and MW 1, respectively were used. Significantly worse *in vitro* multiplication results of selected cultivars of blueberry were found on different modifications of AN medium. Moreover, it was observed that shoots multiplied on AN medium was showed red colouration of leaves and stems and their leaves were small and more abundant, whereas shoots multiplied on MW and WPM medium were light green and had large and green coloured leaves as observed for ‘Elizabeth’ and ‘Meader’ (Figure 1). Furthermore, ‘Liberty’ plantlets regenerated more than 20% better than ‘Elizabeth’ and ‘Meader’.

Among the cultivars tested, the ‘Liberty’ multiplied much better than ‘Elizabeth’ and ‘Meader’. The plantlets of the ‘Liberty’ were 21 and 37% higher than ‘Elizabeth’ and ‘Meader’, respectively. No significant differences were observed between the number of shoots for ‘Elizabeth’ and ‘Meader’. While ‘Liberty’ developed 53% more new shoot per plant in comparison to the other cultivars tested. The greater height and bushiness of ‘Liberty’ plantlets translated into their higher mass. The plants had 19.60 and 27.33% higher dry mass, compared to ‘Elizabeth’ and ‘Meader’, respectively.
Table 3. The influence of the various medium on the morphological traits and regeneration rate in vitro of *V. corymbosum* 'Elizabeth', 'Liberty' and 'Meader' after 35 days of cultivating

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>AN1</th>
<th>AN2</th>
<th>AN3</th>
<th>WPM1</th>
<th>WPM2</th>
<th>WPM3</th>
<th>MW1</th>
<th>MW2</th>
<th>MEAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elizabeth</td>
<td>1.41b-f</td>
<td>1.49b-g</td>
<td>1.83f-j</td>
<td>1.27a-d</td>
<td>1.59c-h</td>
<td>1.78f-i</td>
<td>1.80f-i</td>
<td>1.91g-k</td>
<td>1.62B</td>
</tr>
<tr>
<td>Meader</td>
<td>0.86a</td>
<td>0.98ab</td>
<td>1.23a-c</td>
<td>1.34b-e</td>
<td>1.56c-h</td>
<td>1.81f-j</td>
<td>1.89g-k</td>
<td>1.94c-h</td>
<td>1.43A</td>
</tr>
<tr>
<td>Liberty</td>
<td>1.67d-h</td>
<td>1.81f-i</td>
<td>1.71e-i</td>
<td>2.10k</td>
<td>2.23jk</td>
<td>2.27k</td>
<td>1.91g-k</td>
<td>1.94c-h</td>
<td>1.96C</td>
</tr>
<tr>
<td>MEAN</td>
<td>1.34A</td>
<td>1.54AB</td>
<td>1.60B</td>
<td>1.57B</td>
<td>1.83C</td>
<td>1.98C</td>
<td>1.87C</td>
<td>1.82C</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number of new shoots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivar</td>
</tr>
<tr>
<td>Elizabeth</td>
</tr>
<tr>
<td>Meader</td>
</tr>
<tr>
<td>Liberty</td>
</tr>
<tr>
<td>MEAN</td>
</tr>
</tbody>
</table>

| Fresh mass (mg) |
|----------------
| Cultivar | AN1 | AN2 | AN3 | WPM1 | WPM2 | WPM3 | MW1 | MW2 | MEAN |
| Elizabeth | 40.90bc | 41.40b-d | 48.69c-g | 43.20c-e | 49.00c-e | 52.70fg | 50.80c-e | 54.30f-i | 48.56B |
| Meader | 30.70a | 32.70ab | 35.89ab | 45.78c-g | 48.10c-g | 51.41fg | 55.95g | 50.10c-g | 43.89A |
| Liberty | 50.24d-g | 52.28fg | 49.00c-g | 67.24j | 68.10j | 70.12j | 60.28hi | 64.98ij | 60.40C |
| MEAN | 43.82A | 43.89A | 45.51A | 53.24B | 59.41C | 56.36C | 57.73C | 59.30C |

| Dry mass (mg) |
|---------------
| Cultivar | AN1 | AN2 | AN3 | WPM1 | WPM2 | WPM3 | MW1 | MW2 | MEAN |
| Elizabeth | 13.30a-c | 12.90a-c | 10.98ab | 10.30ab | 11.05a-c | 15.05a-c | 10.52ac | 15.40a-c | 12.22A |
| Meader | 9.65a | 9.75a | 9.95a | 1.68a-c | 10.75ab | 14.83a-c | 15.70a-c | 14.95a-c | 12.84A |
| Liberty | 15.14a-c | 16.74bc | 14.54a-c | 18.22c | 18.34c | 18.58c | 16.78bc | 17.86c | 17.03B |
| MEAN | 13.51AB | 14.33AB | 11.43A | 13.77AB | 15.03AB | 15.80B | 16.20B | 16.67B |

<table>
<thead>
<tr>
<th>Shoot regeneration rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivar</td>
</tr>
<tr>
<td>Elizabeth</td>
</tr>
<tr>
<td>Meader</td>
</tr>
<tr>
<td>Liberty</td>
</tr>
<tr>
<td>MEAN</td>
</tr>
</tbody>
</table>

*Means followed by different letters in columns are significantly different at the 5% level according to Tukey’s multiple ranges.*
Figure 1. *In vitro* multiplication of highbush blueberry ‘Elizabeth’ (a), ‘Liberty’ (b), and ‘Meader’ (c) on different multiplying medium

*In vitro and ex vitro rooting*

The most important characteristics indicating the success of *in vitro* rooted plants are the number of roots, root length, and their quality as well as *ex vitro* rooting rate. The maximum mean number of roots (2.14) with the longest root (0.77 cm) was observed on the WPM B medium supplemented with 0.1 mg L⁻¹ zeatin and 1.0 mg L⁻¹ IAA (Table 4). Good rhizogenesis was correlated with the highest mean fresh mass of plantlets (60.33 mg). Moreover, ‘Liberty’ explants developed more than 2.5 times longer roots in comparison to other cultivars tested (Table 4 and Figure 2). Conversely, the highest shoots length of these plantlets (1.77 cm) was lower than ‘Elizabeth’ and ‘Meader’ by 12 and 11%, respectively. It was observed, that ‘Elizabeth’ explants had 259 and 143% a smaller number of roots than cvs. Meader and Liberty, respectively. However, their length was not statistically different from that of the roots of the cultivar ‘Meader’. In addition, plantlets of cultivar ‘Meader’ and ‘Liberty’ did not produce roots at both modifications of AN medium. Very weak rhizogenesis was observed only in case of ‘Elizabeth’ explants (Figure 3). The highest *in vitro* rooting rate (85%) was obtained for cv. Liberty on WPM A (WPM+0.5 mg L⁻¹ IAA+0.1 mg L⁻¹ zeatin) and WPM B medium (WPM+1.0 mg L⁻¹ IAA+0.1 mg L⁻¹ zeatin), the lowest - for both ‘Elizabeth’ and ‘Meader’ rooted on WPM A medium (Figure 3). When WPM A and WPM B medium were used, the roots’ structure was well developed – the roots were longer, thicker and better branched (Figure 2). Therefore, for acclimatization to greenhouse conditions only plantlets with well-developed roots and the best rhizogenesis rooted on WPM A and WPM B medium were selected.
Table 4. In vitro rooting capacity of *V. corymbosum* ‘Elizabeth’, ‘Liberty’, and ‘Meader’

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Medium</th>
<th>Shoot length (cm)</th>
<th>Number of roots</th>
<th>Root length (cm)</th>
<th>Fresh mass (mg)</th>
<th>Dry mass (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AN A</td>
<td>AN B</td>
<td>WPM A</td>
<td>WPM B</td>
<td>MEAN</td>
</tr>
<tr>
<td>Elizabeth</td>
<td></td>
<td>2.01d</td>
<td>1.56a-c</td>
<td>1.90b-d</td>
<td>1.27a</td>
<td>1.70A</td>
</tr>
<tr>
<td>Meader</td>
<td>2.01c-d</td>
<td>1.56a-c</td>
<td>1.90b-d</td>
<td>1.27a</td>
<td>1.70A</td>
<td>-</td>
</tr>
<tr>
<td>Liberty</td>
<td>1.69a-c</td>
<td>1.77a-d</td>
<td>1.53a-c</td>
<td>1.43ab</td>
<td>1.61A</td>
<td>2.60</td>
</tr>
<tr>
<td>MEAN</td>
<td>1.89B</td>
<td>1.67AB</td>
<td>1.76AB</td>
<td>1.43A</td>
<td>2.14B</td>
<td>0.02A</td>
</tr>
</tbody>
</table>

Means followed by different letters in columns are significantly different at the 5% level according to Tukey’s multiple ranges.

Figure 2. In vitro rooting of highbush blueberry ‘Elizabeth’ (a), ‘Liberty’ (b) and ‘Meader’ (c) on WPM B medium
The percentage of plants acclimatized to the greenhouse conditions varied from 70% (for plantlets of cv. ‘Liberty’ from WPM B medium) to 30% (for plantlets of cv. ‘Meader’ from WPM A medium) (Figure 4). In the case of IAA concentrations used, plant survivability was lower by a 15 – 20% when 0.1 mg L\(^{-1}\) IAA (WPM A) was applied. Moreover, a positive correlation was observed between the in vitro rooting rate and the acclimatization rate. However, among the tested cultivars highbush berry ‘Liberty’ showed the highest percent of adapted plantlets (70%) with the best developed roots (Figure 5).
Due to the great variability within genus *Vaccinium*, some species and cultivars still require further research to optimize multiplication media (Vescan *et al.*, 2012; Fan *et al.*, 2017). The addition of zeatin, in preliminary experiments to develop the optimum composition of medium for the initiation of highbush blueberry (data unpublish), showed a positive effect on plant growth. Zeatin at concentration 0.1 mg L\(^{-1}\) was the most favourable for shoot regeneration in *V. corymbosum* cultivars. As a result, it was decided to add to the medium zeatin at each stage of micropropagation. As suggested by many authors (Tetsumura *et al.*, 2008; Ružič *et al.*, 2012; Mohamed *et al.*, 2018; Schuchovski and Biasi, 2019) the most frequently media used for *in vitro*
multiplication of highbush blueberry are AN and WPM, while MS was suggested as very effective medium for *Vaccinium* species by Cappelletti *et al.* (2016). This study also confirmed that WPM and MW (mixture of equal parts of MS and WPM media) achieves the most efficient multiplication of blueberry plants. However, multiplication rate depends not only on the medium but also on the response of individual species, cultivars, and even the original position of the explant from which nodal segments were excised for micropropagation (Cüce and Sökmen, 2017). Positive results in terms of blueberry regeneration efficiency were obtained culturing ‘Elizabeth’ and ‘Meader’ cultivars in a MW medium supplemented with IBA 0.4 mg L\(^{-1}\) or 0.2 mg L\(^{-1}\) (respectively) and zeatin 0.1 mg L\(^{-1}\). Best regeneration efficiency was obtained culturing ‘Liberty’ shoots in a WPM medium supplemented with IBA 0.4 mg L\(^{-1}\) with GA\(_3\) 0.4 mg L\(^{-1}\) and zeatin 0.1 mg L\(^{-1}\). The same tendency in positive influences of 0.5 mg L\(^{-1}\) zeatin combined with low concentration of IBA on length of axial shoots in highbush blueberry cultivars was observed by Litwińczuk and Wadas (2008), and Ružić *et al.* (2012). A much higher concentration of zeatin (2 mg L\(^{-1}\)) for the regeneration of shoots of highbush blueberry cv. ‘Berkley’ was successfully applied by Ostrolucká *et al.* (2007). Other responses of the growth regulators were observed by Vescan *et al.* (2012), indicated that 5 mg L\(^{-1}\) of 2iP (2-isopentenyladenine) was very good alternative to expensive zeatin for increasing efficiency and lowering cost *in vitro* culture establishment for highbush blueberry cv. ‘Elliot’. Also, Cappalletti *et al.* (2016) reported that TDZ (tidiazuron) is more efficient in shoot regeneration of blueberry cv. ‘Duke’ compared to zeatin.

Based on the results obtained, it can be concluded that the propagation medium is important for rooting of blueberry shoots. On the well-developed blueberry shoots, the roots also developed well. According to Mohamed *et al.* (2018) for *Vaccinium* species L. IBA and IAA are frequently used for *in vitro* root initiation and increasing root number and length even alone or in combination with each other. The process of rhizogenesis stimulated by the presence of auxin in the medium supports the elongation of root hair cells by importing auxin into non-root forming epidermal cells. Cellular auxin levels regulate the expression of genes that determine root formation. In our study, rooting capacity of shoots varied greatly among tested blueberry cultivars. WPM medium with the addition of IAA and 0.1 mg L\(^{-1}\) zeatin was the most effective for root induction. The rooting rate in cultivar Liberty (85%), along with the others rooting parameters, was significantly higher than in those obtained for ‘Meader’ and ‘Elizabeth’. However, all cultivars tested in the study did not developed root on AN medium. Our findings are in agreement with previous report. Meiners *et al.* (2007) observed that the addition of 0.5 mg L\(^{-1}\) with activated charcoal (AC) gave the highest *in vitro* rooting success of different *Vaccinium* species. Also, Cüce and Sökmen (2017) concluded that as far as the rooting process was concerned, IBA and IAA concentrations increased the root formation, whereas NAA treatments did not give positive response. In contrast, Ostrolucká *et al.* (2009) used AN medium supplemented with IBA and AC for *in vitro* rooting of *V. corymbosum* and *V. vitis-idaea* microshoots with the highest rooting success (85-95%). Sedlák and Paprštein (2009) achieved 70% rooting rate for cv. Berkeley, 61% for ‘Bluecrop’ and only 9% for cv. ‘Spartan’.

**Conclusions**

The presented results confirm that the process of *in vitro* rhizogenesis of *Vaccinium* sp. depending on the plant species, the growth conditions of the mother plant and the physiological conditions of the explant. The *in vitro* regeneration protocol of highbush blueberry developed in this paper could help optimize shoot propagation system for other *Vaccinium* species and can be useful, for example, to produce certified vegetative material or biotechnological purposes.
Authors’ Contributions


Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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