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Research Article

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 GA_3. 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; GA_3, 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;

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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 hormons, growth conditions, explant types, sampling, and physiological condition of the explants (Ružić *et al*., 2012; Krupa-Małkiewicz *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 ($HgSO_4$) 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

Medium	mg L^{-1} IBA	mg L^{-1} GA_3	mg L^{-1} zeatin
AN* 1	0.2	-	0.1
AN 2	0.4	-	0.1
AN 3	0.4	0.4	0.1
WPM 1	0.2	-	0.1
WPM 2	0.4	-	0.1
WPM 3	0.4	0.4	0.1
MW 1	0.2	-	0.1
MW 2	0.4	-	0.1

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

Medium	mg L^{-1} IBA	mg L^{-1} zeatin	mg L^{-1} IAA
AN* A	0.5	0.1	-
AN B	1.0	0.1	-
WPM A	-	0.1	0.5
WPM B	-	0.1	1.0

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 acclimatisation 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

Cultivar	Medium								
	Shoot length (cm)								
	AN1	AN2	AN3	WPM1	WPM2	WPM3	MW1	MW2	MEAN
Elizabeth	1.41b-f*	1.49b-g	1.83f-j	1.27a-d	1.59c-h	1.78f-i	1.80f-i	1.91g-k	1.62B
Meader	0.86a	0.98ab	1.23a-c	1.34b-e	1.56c-h	1.81f-j	1.89g-k	1.56c-h	1.43A
Liberty	1.67d-h	1.81f-i	1.71e-i	2.10i-k	2.23jk	2.27k	1.91g-k	1.94h-k	1.96C
MEAN	1.34A	1.54AB	1.60B	1.57B	1.83C	1.98C	1.87C	1.82C	
	Number of new shoots								
	AN1	AN2	AN3	WPM1	WPM2	WPM3	MW1	MW2	MEAN
Elizabeth	1.00a	1.13a	1.75a-c	1.80a-c	2.25b-e	2.38c-f	2.63c-g	2.75c-h	1.95A
Meader	1.13ab	1.25a-c	1.13ab	1.90a-c	2.13a-e	2.75c-h	2.88c-h	2.00a-d	1.94A
Liberty	2.00a-d	2.20a-e	1.80a-c	3.20e-h	3.50f-h	3.80gh	3.10d-h	3.90h	2.94B
MEAN	1.42A	1.64A	1.58A	2.30B	2.69BC	3.04C	2.88C	2.96C	
	Fresh mass (mg)								
	AN1	AN2	AN3	WPM1	WPM2	WPM3	MW1	MW2	MEAN
Elizabeth	40.90bc	41.40b-d	48.69c-g	43.20c-e	49.00c-g	52.70fg	50.80e-g	54.30f-i	48.56B
Meader	30.70a	32.70ab	35.89ab	45.78c-g	48.10c-g	51.41fg	55.95g-i	50.10c-g	43.89A
Liberty	50.24d-g	52.28fg	49.00c-g	67.24j	68.10j	70.12j	61.22hi	64.98ij	60.40C
MEAN	43.82A	43.89A	45.51A	53.24B	59.41C	56.36C	57.73C	59.30C	
	Dry mass (mg)								
	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	15.25a-c	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	
	Shoot regeneration rate (%)								
	AN1	AN2	AN3	WPM1	WPM2	WPM3	MW1	MW2	MEAN
Elizabeth	0	12.50	62.50	80.00	100	87.50	100	100	67.81
Meader	12.50	25.00	12.50	90.00	100	87.50	100	87.50	64.38
Liberty	70.00	80.00	70.00	100	100	100	90	100	88.75
MEAN	27.50	39.17	48.33	90.00	100.00	91.67	96.67	95.83	

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

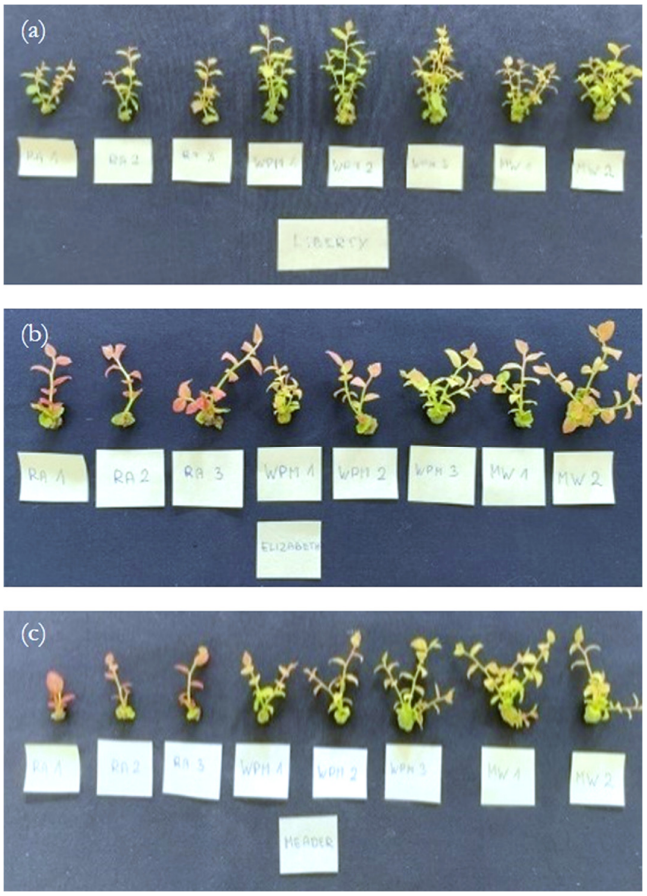

Figure 1. *In vitro* multiplication of highbush blueberry 'Elizabeth' (a), 'Liberty' (b), and 'Meader' (c) on different multiplying medium

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 then 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'

Cultivar	Medium				
	Shoot length (cm)				
	AN A	AN B	WPM A	WPM B	MEAN
Elizabeth	2.01d*	1.56a-c	1.90b-d	1.27a	1.70A
Meader	1.98c-d	1.72a-d	1.85b-d	1.52a-c	1.74A
Liberty	1.69a-d	1.77a-d	1.53a-c	1.43ab	1.61A
MEAN	1.89B	1.67AB	1.76AB	1.43A	
	Number of roots				
	AN A	AN B	WPM A	WPM B	Mean
Elizabeth	0.06	0.18	0.83	0.82	0.41A
Meader	-	-	2.05	2.95	1.49B
Liberty	-	-	2.60	2.00	1.17B
MEAN	0.02A	0.07A	1.91B	2.14B	
	Root length (cm)				
	AN A	AN B	WPM A	WPM B	MEAN
Elizabeth	0.20	0.35	0.33	0.60b	0.43A
Meader	-	-	0.31	0.55	0.44A
Liberty	-	-	0.84b	1.43c	1.08B
MEAN	0.20A	0.35A	0.49A	0.77B	
	Fresh mass (mg)				
	AN A	AN B	WPM A	WPM B	MEAN
Elizabeth	30.01a	45.48	36.92a	63.51b	42.77A
Meader	33.10a	27.73a	39.33a	62.57b	42.47A
Liberty	38.12a	40.07ab	42.61ab	50.39ab	42.80A
MEAN	32.84A	37.50A	39.26A	60.33B	
	Dry mass (mg)				
	AN A	AN B	WPM A	WPM B	MEAN
Elizabeth	8.20a	10.20a	8.98a	15.80b	10.79A
Meader	8.70a	7.78a	9.50a	15.93b	10.48A
Liberty	9.26a	9.64a	10.04a	10.63a	9.98A
MEAN	8.86A	9.31A	9.18A	13.24B	

*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

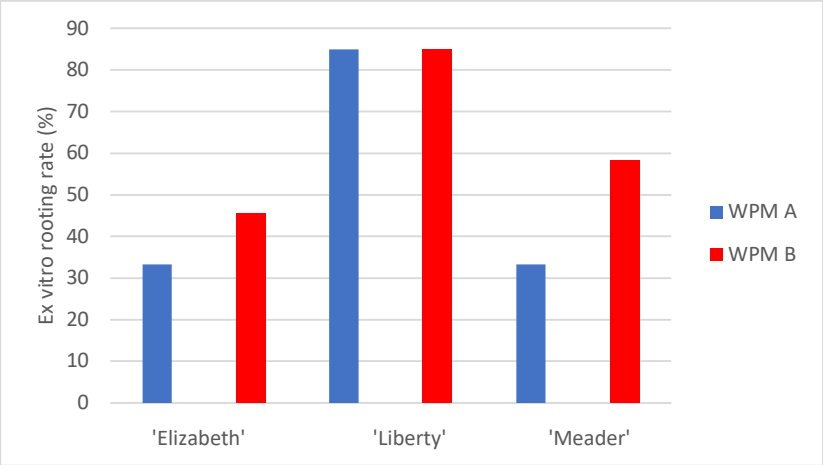

Figure 3. *Ex vitro* rooting rate (%) of *V. corymbosum* 'Elizabeth', 'Liberty' and 'Meader'

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).

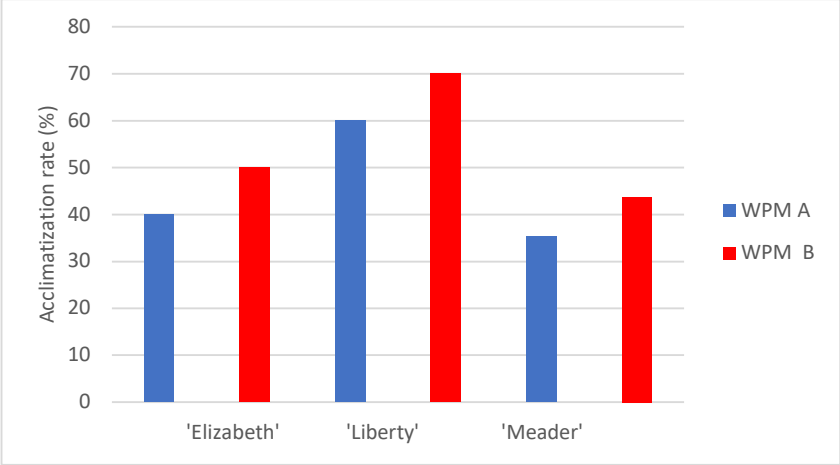

Figure 4. Acclimatization rate (%) of *V. corymbosum* 'Elizabeth', 'Liberty', and 'Meader'

Figure 5. Acclimatization of highbush blueberry 'Elizabeth' (a), 'Liberty' (b and d) and 'Meader' (c) at different stages

Discussion

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; Ružic *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 '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 proces 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

Conceptualization: M K-M; Data curation M F-K, M K-M; Formal analysis: M F-K, M K-M, I O; Funding acquisition: M F-K, M K-M, I O; Investigation: M F-K, M K-M; Methodology: M F-K, M K-M; Supervision: I O; Writing original draft: M F-K, M K-M, I O; Writing review and editing: M K-M; All authors read and approved the final manuscript.

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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