COMPARISON OF THE EFFECTIVENESS OF CHITOSAN AND AUXIN ON *IN VITRO* AND *EX VITRO* ROOTING OF *Vaccinium corymbosum* L.

Marcelina Krupa-Małkiewicz[1,a,*], Ireneusz Ochmian[2,b]

[1] – Department of Plant Genetics, Breeding and Biotechnology, West Pomeranian University of Technology Szczecin, Słowackiego 17 Str., 71-434 Szczecin, Poland
[a] – ORCID: 0000-0002-4333-9122
[2] – Department of Horticulture, West Pomeranian University of Technology Szczecin, Słowackiego 17 Str., 71-434 Szczecin, Poland
[b] – ORCID: 0000-0002-3606-1927
[*]corresponding author: mkrupa@zut.edu.pl

Abstract

*A highly efficient micropropagation process can be achieved by using proper conditions for root initiation and growth of regenerated shoots. The efficiency of auxin and chitosan on in vitro rhizogenesis of the highbush blueberry (*Vaccinium corymbosum *L.) cv. Liberty was developed. After the multiplication stage, explants were transferred to Woody Plant Medium (WPM) supplemented with 0.1 mg dm^{-3} zeatin and 1.0 or 2.0 mg dm^{-3} of the auxin indole-3-butyric acid (IBA), or chitosan with a molecular weight of 800 kDa at a concentration of 20 or 40 ppm. Among the tested combinations, the maximum mean shoot and root length (6.28 and 1.73 cm, respectively) were recorded for WPM with 0.1 mg dm^{-3} zeatin and 2.0 mg dm^{-3} IBA. The higher chitosan concentration in the WPM medium increased the length of shoots and roots. The highest percentage (100%) of rooted plants was obtained on WPM with the addition of 0.1 mg dm^{-3} zeatin and 40 ppm chitosan. Moreover, leaves of the highbush blueberries from this medium were brighter (L* from 22% to 36%) and greener (a* -2.70 and -4.56, respectively) compared with the leaves of plants grown on WPM containing IBA.*

Keywords: *plant acclimatisation, biopolymer, rhizogenesis, medium, highbush blueberry, Cie L*a*b**

Received: 27.02.2024
Accepted: 24.05.2024

1. Introduction

The blueberry (genus *Vaccinium* L., family Ericaceae Juss.) is a very popular fruit. Its production and consumption have increased globally, characterised in particular by the expansion of production activities into new geographic areas [1]. Poland ranks second in Europe and seventh in the world in blueberry production [2]. The leader in highbush blueberry plantations in terms of cultivated area is the Masovian Voivodeship. According to Cüce and Sökmen [3], the genus *Vaccinium* is more variable than other members of the Ericaceae family. These genetic variations manifest in different growth habits, fruiting times and responses to environmental conditions. *In vitro* culture conditions, including medium composition, need to be adapted to the specific needs of each cultivar to optimise growth and development. Cultivars differ in their ability to cope with the stress associated with tissue culture. Researchers typically perform extensive trials to determine the optimal conditions for each cultivar, including testing different medium compositions and environmental conditions. This ensures that each cultivar grows robustly and healthily, ultimately leading to successful acclimatisation and adaptation to field conditions.

Blueberries have low endogenous hormone levels, and enrichment of the environment with appropriate auxins and organic supplements is a prerequisite for the formation of a good root system [4]. According to Debnath and McRae [5], *Vaccinium macrocarpon* Ait. shoots rooted well *in vitro* in the same media used for shoot proliferation, but without any growth regulators. Tetsumura et al. [6] described that the rooting of shoots of three cultivars of *Vaccinium corymbosum* L. was affected by the multiplication media, namely Murashige and Skoog (MS) [7], Woody Plant Medium (WPM) [8] and a mixture of equal parts of MS and WPM (MW). However, they noticed that the superiority in rooting percentage varied with the cultivar. In other related studies, Ružic et al. [9] examined the effect of the auxin indole-3-butyric acid (IBA) and active charcoal on induction of rhizogenesis in three cultivars of *V. corymbosum*, and Erst et al. [10] investigated the effect of IBA and indole-3-acetic acid (IAA) on *in vitro* rhizogenesis of *Vaccinium uliginosum* L. These researchers confirmed that rooting capacity of shoots varied widely among the tested cultivars.

As is apparent from the literature, the blueberry species *V. corymbosum* and *V. virgatum* root well on propagation medium or on medium without the addition of plant growth regulators. The process of root development can be divided broadly into two primary phases: initiation of roots and their subsequent elongation. During the initiation phase *in vitro*, specific cells undergo dedifferentiation to create root meristems, which then proceed to elongate. Each phase, root initiation and root elongation, has different optimal conditions for plant growth regulators, especially auxin [3]. It is important to consider the potential benefits of using naturally derived, biologically active substances that have a minimal environmental impact and can support both phases of rooting. Due to its characteristics, chitosan is viewed as a biomaterial of interest for plant growth [11, 12]. There are many reports about its positive effects on the growth and yield of plants such as *Dendrobium phalaenopsis* [13], *V. corymbosum* [14], *Lycium chinense* [15], *Vitis vinifera* L. [16] and *Lonicera caerulea* L. [17]. The mechanism of action of chitosan in plant systems is not yet completely clear. According to Lopez-Moya et al. [18], chitosan promotes the accumulation of auxins (mainly IAA) in the root apex of plants, causing strong changes in root cell morphology. Chitosan treatment can induce several biochemical and molecular changes that may be responsible for improving plant growth including rooting, as shown in the examples above [19–21].

The present study compared the effectiveness of chitosan with a molecular weight of 800 kDa (CH_8) and IBA on *in vitro* rooting and *ex vitro* acclimatisation of *V. corymbosum* cv. Liberty explants.

2. Materials and Methods

2.1. Plant Material

V. corymbosum cv. Liberty explants were obtained from the Laboratory of Tissue Culture, West Pomeranian University of Technology, Szczecin, Poland. The shoots with auxiliary buds explants were sub-cultured several times. The stem segments of blueberry explants were cut into several small pieces (approximately 17–20 mm long) and removed from the leaf blades.

2.2. *In Vitro* Rooting Conditions

In a laminar flow cabinet, shoot explants were initiated on Woody Plant Medium (Duchefa Biochemie B.V., the Netherlands) with the addition of 0.1 mg dm^{-3} zeatin (Zea) and CH_8 at a concentration of 20 and 40 ppm, or on WPM supplemented with 0.1 mg dm^{-3} Zea and 1.0 or 2.0 mg dm^{-3} IBA. The pH of each medium was adjusted to 5.7–5.8. Zea, CH_8 and IBA were added to the media before autoclaving.

All media were supplemented with 3% (w/v) sucrose (Chempur, Poland), 0.8% (w/v) agar (Biocorp, Poland) and 100 mg dm^{-3} myo-inositol (Duchefa Biochemie B.V., The Netherlands). Then, they were heated and 30 ml was poured into a 450-ml flask. Next, they were autoclaved at 121°C (0.1 MPa) for the appropriate time (based on the volume of medium in the vessel). Cultures were incubated in a growth room at 24 ± 2°C under a 16-h photoperiod with a photosynthetic photon flux density (PPFD) of 40 µmol m^{-2} s^{-1}. Each combination included 32 shoots (8 replicates with 4 explants per flask). After 35 days, the explants were removed and wash with deionised distilled water, and the shoot and root length (cm), the number of new shoots and the colour were assessed. The explants were weighed to determine the fresh mass (g).

2.3. Chitosan

CH_8 was obtained from the Center of Bioimmobilisation and Innovative Packaging Materials, West Pomeranian University of Technology, Szczecin, Poland [22]. It was prepared using the free-radical degradation process. After purification (filtration), it was characterised using high-performance liquid chromatography (SmartLine Knauer, Germany; Tessek Separon HEMA-BIO 40 column, Tessek, Czech Republic). The average degree of deacetylation of the product was 85%.

2.4. *Ex Vitro* Acclimatisation

Explants with developed roots from all combinations of the experiment were removed from the culture vessels and then washed thoroughly with deionised water to remove residual medium. The explants were transferred to plastic pots (10-cm diameter) containing sterile soil (a mixture of 90% peat and 10% perlite). The pots were covered with agro-textile fabric to ensure 90% humidity and incubated in the growth room at 22°C and 73% relative humidity. After acclimatisation for 6 weeks, the percentage survival of the plants was determined.

2.5. Determination of Colour

The leaves from the middle part of the shoot were evaluated using a CM-700d spectrophotometer (Konica Minolta, Japan). Measurements were made in CIE $L^*a^*b^*$ system [23]. The 10° observer type and D65 illuminant were applied. Colour was measured in triplicate for each experimental combination. *a** ranges from green (-*a**) to red (+*a**). *b** describes the colour in the range from yellow (+*b**) to blue (-*b**). *L**, which represents monochromaticity, ranges from 0 (black) to 100 (white).

2.6. Statistical Analysis

The results were evaluated using the Statistica v.12 software. The results were submitted to variance analysis (ANOVA) followed by Tukey's test. A p value ≤ 0.05 was considered to indicate a statistically significant difference.

3. Results and Discussion

A successful micropropagation system requires the proper conditions for root initiation and growth of regenerated shoots. Healthy roots enable seedlings to establish in the soil and promote normal growth and development. There have been many reports of successful *in vitro* and *ex vitro* rooting of blueberries. Efficient *in vitro* rhizogenesis is generally strongly influenced by the presence of auxins in the medium [10, 24]. Ostrolucká et al. [25] achieved rooted *V. corymbosum* and *Vaccinium vitis-idaea* L. microshoots on Anderson's medium supplemented with 0.8 mg dm^{-3} IBA. Meiners et al. [24] examined IBA and naphthaleneacetic acid (NAA) for their effect on root formation of the *Vaccinium* cultivars Ozarkblue and Red Pearl; they noticed that only IBA was suitable for root induction. Tetsumura et al. [6] compared MS, WPM and MW and observed that the rooting ability of blueberry plants is strictly dependent on the cultivar as well as the multiplication medium. The effectiveness of chitosan on the rooting of highbush blueberry plants remains incompletely investigated. Chitosan is a biopolymer that is completely safe for the environment. It shows positive effects on the growth of many plants, including those propagated *in vitro*. According to Veraplakon et al. [26], chitosan increased the survival percentage and growth rate of *ex vitro* lantana seedlings. Kruczek et al. [15] reported that 20 ppm of chitosan with a molecular weight of 330 kDa was optimal for the initiation of goji explant rhizogenesis *in vitro*. Krupa-Małkiewicz and Ochmian [16] confirmed that 10 ppm of chitosan with a molecular weight of 330 kDa stimulated rhizogenesis of winegrape explants.

Table 1 provides the mean shoot length, number of new shoots, fresh and dry mass of *V. corymbosum* cv. Liberty. WPM containing 0.1 mg dm^{-3} Zea and 2.0 mg dm^{-3} IBA provided the best stimulation of shoot length and root development: compared with the other culture media used, highbush blueberry explants grown on this medium showed longer shoots and roots (6.28 and 1.73 cm, respectively). Good rhizogenesis was correlated with the highest mean fresh and dry mass of the plantlets (0.91 and 0.14 g, respectively). However, there were no significant differences in the number of new explants, which ranged from 1.25 to 1.92. Of the two chitosan concentrations applied in the experiment, 40 ppm was the most efficient in stimulating root growth (Figure 1). Explants grown on WPM containing 40 ppm CH_8 had 44% and 67% longer shoots and roots, respectively, compared with explants grown on WPM containing 20 ppm CH_8. The inclusion of 40 ppm CH_8 also led to the highest number of new explants (1.92 per plant).

Table 1. The effects of different medium compositions on the morphological traits of *Vaccinium corymbosum* vs. Liberty plants under *in vitro* condition after 35 days of culture (n = 32 shoots per treatment).

Medium	Shoot length [cm]	Number of new explants	Root length [cm]	Fresh mass [g]	Dry mass [g]
WPM + 0.1 mg dm^{-3} Zea + 1.0 mg dm^{-3} IBA	2.89^{b}	1.25^{a}	0.88^{ab}	0.88^{a}	0.12^{a}
WPM + 0.1 mg dm^{-3} Zea + 2.0 mg dm^{-3} IBA	6.28^{a}	1.83^{a}	1.73^{a}	0.91^{a}	0.14^{a}
WPM + 0.1 mg dm^{-3} Zea + 20 ppm CH_8	2.57^{b}	1.45^{a}	0.16^{b}	0.16^{a}	0.02^{b}
WPM + 0.1 mg dm^{-3} Zea + 40 ppm CH_8	3.56^{b}	1.92^{a}	0.19^{b}	0.28^{a}	0.04^{b}

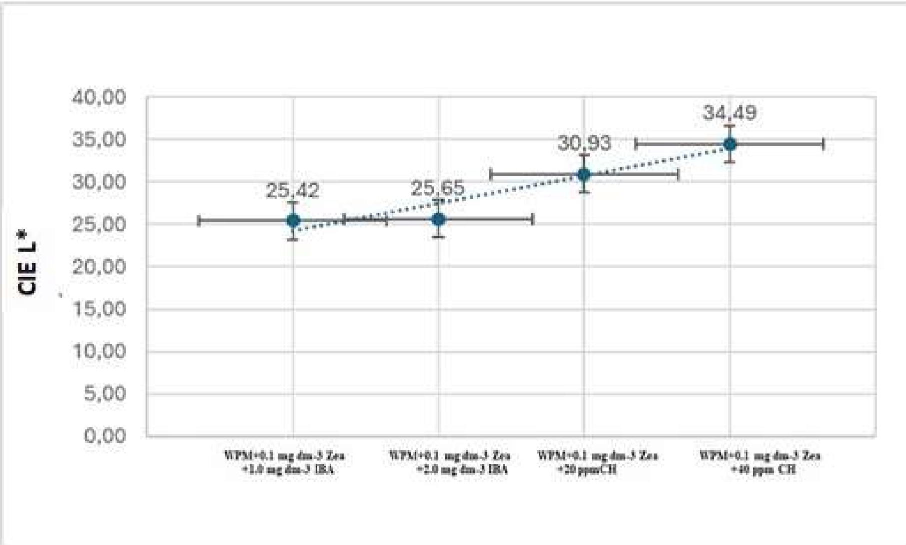
Note. In each column, means with different superscript letters are significantly different according to Tukey's multiple range test ($p < 0.05$). Abbreviations: CH_8, chitosan with a molecular weight of 800 kDa; IBA, indole-3-butyric acid; WPM, Woody Plant Medium; Zea, zeatin.

Figure 1. Shoot explants of *Vaccinium corymbosum* L. cv. Liberty after growth for 6 weeks on Woody Plant Medium (WPM) containing (a) 0.1 mg dm^{-3} zeatin (Zea) + 1.0 mg dm^{-3} indole-3-butyric acid (IBA); (b) 0.1 mg dm^{-3} Zea + 2.0 mg dm^{-3} IBA; (c) 0.1 mg dm^{-3} Zea + 20 ppm chitosan with a molecular weight of 800 kDa (CH_8); or (d) 0.1 mg dm^{-3} Zea + 40 ppm CH_8.

Many authors [27–29] have suggested that the contents of photosynthetic pigments in leaves are closely correlated to their colour. The colour of the leaves was analysed in the transmitted mode using the photocolourimetric method in the CIE $L*a*b*$ system [23]. $L*$ is related to the physiological attributes of visual response [30]. In the present study, the leaves of the explants grown on WPM containing IBA were similar. $L*$ was 25.42 and 25.65 (Figure 2a). However, the leaves of the explants grown on WPM containing 20 or 40 ppm CH_8 were brighter, as indicated by the 36% and 22% higher $L*$ values, respectively, compared with the explants grown on WPM containing IBA. Moreover, the explants grown on WPM containing 20 or 40 ppm CH_8 had greener leaves ($a* = –2.70$

and –4.56, respectively) compared with the leaves of the explants grown on WPM containing IBA (Figure 2b). When higher concentrations of IBA or CH_8 were used in the medium, *b**, which indicates chromaticity, was higher (Figure 2b). Therefore, for the subsequent acclimatisation to greenhouse conditions, only plantlets with well-developed roots and the best rhizogenesis rooted on WPM supplemented with IBA and WPM supplemented with CH_8 were selected.

(a)



(b)

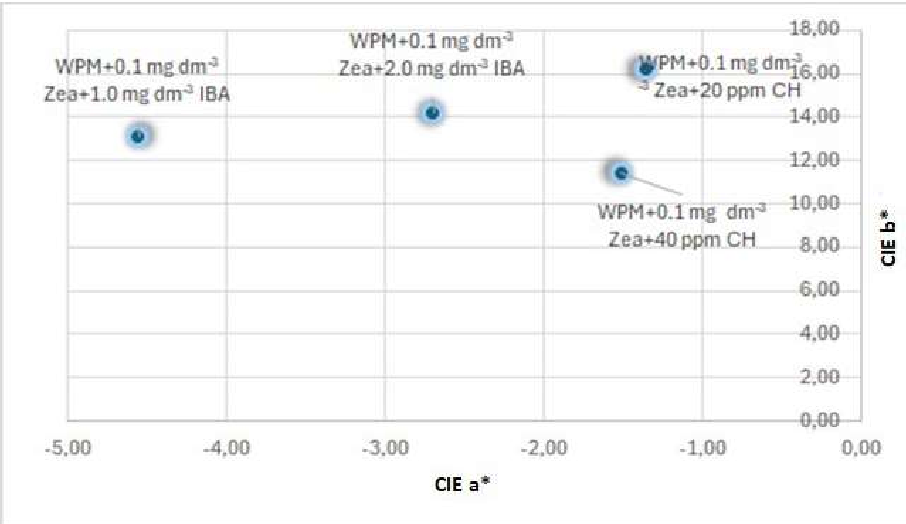


Figure 2. The effect of different medium compositions on the colour of *Vaccinium corymbosum* cv. Liberty leaves (n = 32 shoots per treatment) based on the CIE *L*a*b** system – (a) *L**, the lightness coefficient and (b) *a** (green colour) and *b** (yellow colour) – at the end of the experiment. Abbreviations: CH_8, chitosan with a molecular weight of 800 kDa; IBA, indole-3-butyric acid; WPM, Woody Plant Medium; Zea, zeatin.

The *ex vitro* rooting rate of *V. corymbosum* cv. Liberty was the highest on WPM containing 0.1 mg dm^{-3} Zea and 2.0 mg dm^{-3} IBA or 40 ppm CH_8 (98% and 100%, respectively) (Figure 3a, b). The lowest rooting efficiency (80%) was observed on WPM containing 0.1 mg dm^{-3} Zea and 20 ppm CH_8 (Figure 4). Ružić et al. [9] obtained a similar *ex vivo* rooting rate for the Goldtraube (91.8%) and Berkeley (66.7%) cultivars. Ostrolucká et al. [31] used Anderson's Rhododendron medium (AN) containing 0.8 mg dm^{-3} IBA and 8 g dm^{-3} charcoal and recorded a rooting percentage of 95%, 90% and 80% for the blueberry cultivars Berkeley, Bluecrop and Brigitta, respectively. In contrast, Erst et al. [10] found that the optimal technique of *in vitro* rooting of *V. uliginosum* involved treating microshoots with an auxin solution for 24 h, followed by transfer to media that did not contain growth regulator. They obtained a 100% rooting percentage for explants of the Golubaya rossyp and Naktarnaya cultivars.

(a)

(b)

Figure 3. The photographs show *Vaccinium corymbosum* cv. Liberty plants (a) after being acclimatised for 35 days and (b) after transfer to the greenhouse (b). Abbreviations: CH_8, chitosan with a molecular weight of 800 kDa; IBA, indole-3-butyric acid; WPM, Woody Plant Medium; Zea, zeatin.

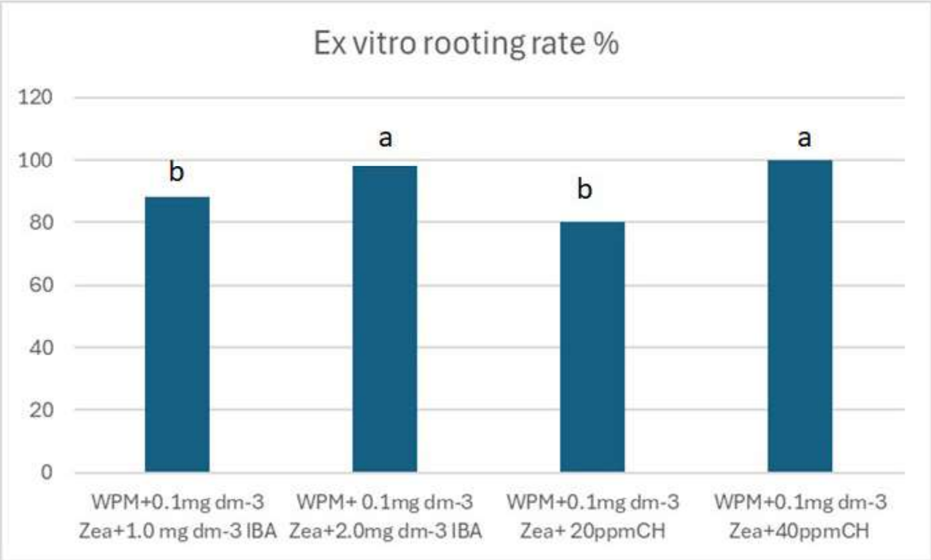


Figure 4. The *ex vitro* rooting rate of *Vaccinium corymbosum* cv. Liberty plants. Abbreviations: CH_8, chitosan with a molecular weight of 800 kDa; IBA, indole-3-butyric acid; WPM, Woody Plant Medium; Zea, zeatin.

4. Conclusions

The obtained results enrich the knowledge on *in vitro* and *ex vitro* rooting of *V. corymbosum* cv. Liberty. Based on the results of this experiment, the addition of CH_8 to WPM resulted in a rooting rate similar to that of the auxin IBA. However, the addition of 40 ppm chitosan to WPM resulted in longer shoots, more new shoots per plant and longer roots compared with the lower chitosan concentration (20 ppm). Moreover, the addition of a higher concentration of IBA to WPM (2.0 mg dm^{-3}) resulted in more plants with longer roots. The addition of 40 ppm CH_8 had a positive effect on the percentage of rooted plants. Research into the development of an optimal rooting protocol for *V. corymbosum* should improve plant survival at later stages of adaptation to *ex vitro* conditions.

5. Acknowledgements

This work was supported by the West Pomeranian University of Technology, Szczecin (grant number 518-07-014-3171-03/18).

6. References

[1] Brazelton C; (2013) World blueberry acreage & production. North American Blueberry Council, Folsom.
[2] Brazelton C, Young K; (2017) World blueberry statistics and global market analysis. Spring Preview for GBC. 2017. International Blueberry Organization.
[3] Cüce M, Sökmen A; (2017) *In vitro* production protocol of *Vaccinium uliginosum* L. (Bog bilberry) growing in the Turkish flora. Turkish J Agric Forest 41(4), 294–304. **DOI:**10.3906/tar-1704-19

[4] Georgieva M, Kondakova V; (2021) *In vitro* propagation of *Vaccinium corymbosum* L. Bulgarian J Agric Sci 27(2), 323–327.

[5] Debnath SC, McRae KB; (2001) An efficient *in vitro* shoot propagation of cranberry (*Vaccinium macrocarpon* Ait.) by axillary bud proliferation. In Vitro Cell Dev Biol-Plant 37, 243–249. **DOI:**10.1079/IVP2000135

[6] Tetsumura T, Matsumoto Y, Sato M, Honsho C, Yamashita K, Komatsu H, Sugimoto Y, Kunitake H; (2008) Evaluation of basal media for micropropagation of four highbush blueberry cultivars. Sci Hort 119, 72–74. **DOI:**10.1016/j.scienta.2008.06.028

[7] Murashige T, Skoog F; (1962) A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiol Plant 15, 473–497.

[8] Lloyd G, McCown B; (1980) Commercially feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot-tip culture. Comb Proc Int Plant Propag Soc 30, 421–427.

[9] Ružić D, Vujović T, Libiakova G, Cerović R, Gajdošova A; (2012) Micropropagation *in vitro* of highbush blueberry (*Vaccinium corymbosum* L.). J Berry Res 2(2), 97–103. **DOI:**10.3233/JBR-2012-030

[10] Erst AA, Gorbunov AB, Erst AS; (2018) Effect of concentration, method of auxin application and cultivation conditions on *in vitro* rooting of bog blueberry (*Vaccinium uliginosum* L.). J Berry Res 8(1), 41–53. **DOI:**10.3233/JBR-170253

[11] Alenazi MM, El-Ebidy AM, El-Shehaby OA, Seleiman MF, Aldhuwaib KJ, Abdel-Aziz HM; (2024) Chitosan and chitosan nanoparticles differentially alleviate salinity stress in *Phaseolus vulgaris* L. plants. Plants 13(3), 398. **DOI:**10.3390/plants13030398

[12] Malerba M, Cerana R; (2016) Chitosan effects on plant systems. Int J Mol Sci 17, 996. **DOI:**10.3390/ijms17070996

[13] Nge KL, Nwe N, Chandrkrachang S, Stevens WF; (2006) Chitosan as a growth stimulator in orchid tissue culture. Plant Sci 170(6), 1185–1190. **DOI:**10.1016/j.plantsci.2006.02.006

[14] Cabrera G, González D, Silva C, Bernardo Y, Gidekel M, Osorio J, Taboada E, Gutierrez A, Cabrera JC; (2010) Effect of chitosan on growth and development of highbush blueberry plants. Adv Chitin Sci 12, 191–195.

[15] Kruczek A, Krupa-Małkiewicz M, Ochmian I; (2021) Micropropagation, rooting, and acclimatization of two cultivars of goji (*Lycium chinense*). Not Bot Horti Agr Cluj-Napca 49(2), 12271. **DOI:**10.15835/nbha49212271

[16] Krupa-Małkiewicz M, Ochmian I; (2023) Chitosan and *meta*-topolin supplementation of the multiplication media of *Vitis vinifera* L. Prog Chem Appl Chitin Deriv 28, 46–55. **DOI:**10.15259/PCACD.27.011

[17] Ochmian I, Krupa-Małkiewicz M; (2023) The effects of applying chitosan of different molecular weights on the growth and quality of kamchatka berries (*Lonicera caerulea* L.): part 1. Prog Chem Appl Chitin Deriv 28, 119–127. **DOI:**10.15259/PCACD.28.011

[18] Lopez-Moya F, Escudero N, Zavala-Gonzalez EA, Esteve-Bruna D, Blázquez MA, Alabadí D, Lopez-Llorca LV; (2017) Induction of auxin biosynthesis and WOX5 repression mediate changes in root development in *Arabidopsis* exposed to chitosan. Sci Rep 7(1), 16813. **DOI:**10.1038/s41598-017–16874-5

[19] Sopalun K, Thammasiri K, Ishikawa K; (2010) Effects of chitosan as the growth stimulator for *Grammatophyllum speciosum in vitro* culture. Int J Innov Res Sci Eng Technol 4(11), 828–830.

[20] Krupa-Małkiewicz M. Smolik B; (2019) Alleviative effects of chitosan and ascorbic acid on *Petunia* × *atkinsiana* D. Don under salinity. Eur J Hortic Sci 84(6), 359–365. **DOI:**10.17660/eJHS.2019/84.6.5

[21] Coelho N, Romano A; (2022) Impact of chitosan on plant tissue culture: recent applications. Plant Cell Tissue Org Cult 148, 1–13. **DOI:**10.1007/s11240-021-02156-6

[22] Bartkowiak A; (2001) Binary polyelectrolyte microcapsules based on natural polysaccharides. Szczecin University of Technology, Szczecin.

[23] Hunterlab; (2012) Measuring Colour using Hunter L, a, b versus CIE 1976 L*a*b*. http://www.hunterlab.com/an-1005b.pdf.

[24] Meiners J, Schwab M, Szankowski I; (2007) Efficient *in vitro* regeneration systems for *Vaccinium species.* Plant Cell Tissue Org Cult 89, 169–176. **DOI:**10.1007/s11240-007-9230-7

[25] Ostrolucká MG, Libiaková G, Ondrußková E, Gajdoßová A; (2004) *In vitro* propagation of *Vaccinium species.* Acta Univ Latviensis 676, 207–676.

[26] Veraplakorn V, Kudan S; (2021) Chitosan elicitor stimulation of *in vitro* growth and *ex vitro* acclimatization of *Lantana camara* L. Agr Nat Resour 55, 431–439. **DOI:**10.34044/j.anres.2021.55.3.13

[27] Pacewicz K, Gregorczyk A; (2009) Porównanie ocen zawartości chlorofilu chlorofilometrami SPAD-502 i N-Tester. Folia Pomer Univ Technol Stetin Agric Aliment Pisc Zootech 269, 49–46. [In Polish]

[28] Ochmian I, Kubus M, Dobrowolska A; (2013) Description of plants and assessment of chemical properties of three species from the *Amelanchier* genus. Dendrobiology 70, 59–64. **DOI:**10.12657/denbio.070.006

[29] Krupa-Małkiewicz M, Calomme M; (2021) Actisil application affects growth, flowering, and biochemical parameters in petunia *in vitro* and greenhouse. Plant Cell Tiss Org Cult 146, 449–459. **DOI:**10.1007/s11240-021-02078-3

[30] Wrolstad RE, Durst RW, Lee L; (2005) Tracking color and pigment changes in anthocyanin products. Trends Food Sci Technol 16, 423–428. **DOI:**10.1016/j.tifs.2005.03.019

[31] Ostrolucká MG, Gajdošova A, Ondruškova E, Libiakova G; (2009) *In vitro* propagation of several *Vaccinium corymbosum* L. and *Vaccinium vitis-idaea* L. cultivars. Latvian J Agron/Agronomija Vestis 12, 75–80.