

IN VITRO PROPAGATION OF THREE CULTIVARS OF BLUE HONEYSUCKLE (*LONICERA CAERULEA* L.)

Ana-Maria RADOMIR, Ramona STAN, Dorin Ioan SUMEDREA

National Research and Development Institute for Biotechnology in Horticulture Ștefănești-Argeș,
37 Bucharest-Pitești Road, 117715, Ștefănești, Argeș, Romania

Corresponding author email: dsunedrea@yahoo.com

Abstract

Lonicera caerulea L. (blue honeysuckle) is a valuable nutraceutical species belonging to the Caprifoliaceae family. This study aimed to develop efficient micropropagation protocols for three blue honeysuckle cultivars: 'Loni', 'Cera', and 'Kami'. The use of 8% calcium hypochlorite for disinfection of explants and a plain Murashige and Skoog (MS) medium for their inoculation proved effective in inducing regenerative processes. In the initiation phase, 'Loni' had the best regenerative potential of the three cultivars studied (between 40 ± 10 and $73.33 \pm 11.55\%$ explants that developed shoots). The highest multiplication rate (4.00 ± 0.10 shoots/explant) and the highest average shoot length (7.89 ± 0.50 cm) were obtained for the 'Cera' cultivar on MS medium supplemented with 1 mg/L BAP and 1 mg/L IAA. The most effective culture medium for in vitro shoot rooting proved to be $MS\frac{1}{2}$ medium supplemented with 2 mg/L IBA. The 'Loni' cultivar showed the best rooting potential (rooting rate $93.34 \pm 5.77\%$, number of roots/shoot 4.33 ± 0.15 , and average root length 2.27 ± 0.06 cm). In vitro regenerated plants were efficiently acclimatized to ex vitro conditions. In vitro rooting can be successfully replaced with in vivo rooting simultaneously with acclimatization, thus shortening the technological flow of obtaining planting material through micropropagation and considerably reducing the unit price of the plant.

Key words: 'Loni', 'Cera', 'Kami', micropropagation, plant growth regulators.

INTRODUCTION

Currently, the World Health Organization is concerned about the nutrition of the population, especially the lack of dietary fiber, vitamins, and minerals in people's diets (McLennan, 2018; Waterlander et al., 2018). Among foods, fruits and vegetables are of major importance in a healthy diet. In addition to having excellent taste qualities, they are natural sources of bioactive substances with prophylactic and therapeutic properties. Therefore, the demand for these foods is constantly increasing (Chang et al., 2019; Sharma and Lee, 2021).

Among the fruits that contain bioactive compounds with particularly valuable nutritional and therapeutic properties are the fruits of *Lonicera caerulea* L. (blue honeysuckle) (Celli et al., 2014; Caprioli et al., 2016). Blue honeysuckle belongs to the Caprifoliaceae family, genus *Lonicera*, which includes about 200 species (Svarcova et al., 2007; Miyashita et al., 2009). *L. caerulea* fruits contain large amounts of vitamins (ascorbic acid, niacin, and tocopherols), minerals (potassium, calcium, and phosphorus), phenolic

compounds (protocatechuic, gentisic, ellagic, ferulic, caffeic, chlorogenic, and coumaric acid), and anthocyanins (cyanidin 3-glucoside, peonidin 3-glucoside, cyanidin 3-rutinoside, malvidin 3-arabinoside, petunidin 3-glucoside, and petunidin 3-rutinoside) (Palikova et al., 2008; Wang et al., 2016; Rupasinghe et al., 2018; Grobelna et al., 2019; Grobelna et al., 2020). The health-promoting properties of blue honeysuckle berries include protective effects against cardiovascular and neurodegenerative diseases, osteoporosis, type 2 diabetes, anemia, as well as antimicrobial, anticancer, and anti-inflammatory activity (Park et al., 2005; Kula et al., 2013; Celli et al., 2014; Caprioli et al., 2016; Wang et al., 2016; Cory et al., 2018; Grobelna et al., 2019; Gawroński et al., 2020). These berries can be included in the group of so-called "superfruits" (Bojarska et al., 2019).

Honeysuckle species are traditionally propagated by cuttings. This method, although generally successful, depends largely on the genotype, the age of the mother plant, and the vegetation period (Hui et al., 2012). In contrast, propagation by seeds does not guarantee the regeneration of genetically uniform plants. Also,

Lonicera sp. seeds have a low germination rate and a long germination time.

Intensive honeysuckle cultivation technologies aim to increase production and find the best methods for obtaining high-quality planting material (Debnath, 2007). Thus, to meet the increasing demand for *L. caerulea* fruits and planting material, tissue culture techniques are used as an alternative to traditional propagation methods. *In vitro* multiplication is an effective method to obtain a large number of healthy plants in a relatively short time (Clapa et al., 2023). However, the *in vitro* regenerative potential of plants depends on a number of biotic (species, genotype, and type of explant) and abiotic (culture medium composition, especially plant growth regulators) factors (Gahan and George, 2008). Therefore, there is a need to develop new *in vitro* propagation technologies or optimize existing technologies, especially for less commercialized and less studied species and cultivars.

Research on the *in vitro* multiplication of the species *L. caerulea* is quite limited. In addition, due to the different results obtained, no general recommendations can be made regarding the *in vitro* propagation of this species, each cultivar having a different behavior in *in vitro* culture. Considering the above, the aim of this study was to develop efficient micropropagation protocols for three blue honeysuckle cultivars: 'Loni', 'Cera', and 'Kami'.

MATERIALS AND METHODS

The two major pathways for *in vitro* plant regeneration are organogenesis and somatic embryogenesis. Of these, direct organogenesis is considered the most suitable method for clonal plant multiplication due to the stability and genetic uniformity of the regenerants, but also because the multiplication rate can be maintained at a high level over several subcultures. Starting from this premise, in the research within the present study we opted to implement this method for the *in vitro* propagation of the *L. caerulea* species.

The *in vitro* propagation technique involves four stages: culture initiation, shoot multiplication, shoot rooting, and acclimatization of *in vitro* regenerated shoots to *ex vitro* conditions.

In vitro culture initiation stage

The explants used for initiating *in vitro* cultures consisted of apical and axillary buds taken from shoots harvested from *L. caerulea* mother plants (cultivars 'Loni', 'Cera', and 'Kami') selected from a phytosanitary point of view and for the authenticity of the variety. The studied cultivars were obtained at the Research Institute for Fruit Growing Pitesti, Romania.

The shoots were defoliated, fragmented and first washed with tap water, after which they were sterilized with calcium hypochlorite (CaCl_2O_2) in different concentrations: 6%, 8%, and 10%. The sterilization time was 15 minutes. Subsequently, three rinses with sterile distilled water were performed to remove traces of sterilizing agent.

After disinfection of the plant material, the explants were sampled and inoculated under aseptic conditions on MS culture medium (Murashige and Skoog, 1962) without growth regulators.

At this stage of the experiment, as well as in subsequent stages, the culture medium was supplemented with 40 g/L glucose, 32 mg/L NaFeEDTA, and 7 g/L agar.

The culture media were sterilized by autoclaving at 120°C for 20 min. Before autoclaving, the pH of the medium was adjusted to 5.6-5.8 with 1N KOH or 1N HCl.

The cultures were maintained in the growth chamber under controlled conditions of temperature (22-24°C), photoperiod (16 hours light/8 hours dark), and light intensity (2500 lx). Four weeks after the initiation of cultures, the following parameters were calculated: the percentage of contaminated explants, the percentage of uncontaminated explants that developed shoots, and the percentage of uncontaminated explants that did not develop shoots.

The experiments were organized in 3 repetitions. For each experimental variant, respectively for each repetition, 10 explants were used.

In vitro multiplication stage

The apexes and nodal fragments resulting from the division of regenerated shoots during the *in vitro* culture initiation phase were inoculated on a full-strength MS medium supplemented with different types of cytokinins (BAP -

benzylaminopurine, KIN - kinetin, 2iP - 2 isopentyl adenine) at two concentrations (1 and 3 mg/L) in combination with 1 mg/L IAA (indoleacetic acid) or without the addition of auxin. MS medium without growth regulators was used as a control (Table 1).

Table 1. Experimental variants - *in vitro* multiplication of *L. caerulea* shoots

Variant	Phytohormones (mg/L)			
	BAP	KIN	2iP	IAA
V0	-	-	-	-
V1	1	-	-	-
V2	3	-	-	-
V3	-	1	-	-
V4	-	3	-	-
V5	-	-	1	-
V6	-	-	3	-
V7	1	-	-	1
V8	3	-	-	1
V9	-	1	-	1
V10	-	3	-	1
V11	-	-	1	1
V12	-	-	3	1

The subculturing of the biological material on fresh medium was performed at an interval of four weeks.

Four weeks after subcultivation, the number of shoots per explant and shoot length were evaluated as growth parameters.

The experiments were organized in 3 repetitions. For each experimental variant, respectively for each repetition, 10 explants were used.

***In vitro* rooting stage**

Shoots regenerated on the propagation medium were individualized and cultivated on several variants of rooting medium in which the type of auxin (IBA, NAA, IAA) and its concentration (0.5, 1, 2 mg/L) were varied. The MS_{1/2} medium (Murashige and Skoog, 1962) without auxins was used as a control (Table 2).

The development of the rooting process at the level of *L. caerulea* shoots was evaluated, four weeks after the initiation of the experiment, by calculating the following parameters: rooting rate, number of roots/shoot, and average root length.

The experiments were organized in 3 repetitions. For each experimental variant, respectively for each repetition, 30 explants were used.

Table 2. Experimental variants - *in vitro* rooting of *L. caerulea* shoots

Variant	Auxins (mg/L)		
	IBA	NAA	IAA
V0	-	-	-
V1	0.5	-	-
V2	1	-	-
V3	2	-	-
V4	-	0.5	-
V5	-	1	-
V6	-	2	-
V7	-	-	0.5
V8	-	-	1
V9	-	-	2

Acclimatization stage

Acclimatization of in vitro rooted shoots

The *in vitro* rooted shoots were carefully removed from the culture vessels. Their roots were washed in running tap water to remove the agarized medium and eliminate possible sources of phytopathogenic agents. They were then planted in a substrate consisting of a mixture of peat, manure, and perlite in a ratio of 2:1:1 and Jiffy peat pellets.

The plants were placed under glass jars or covered with plastic bags to provide a high-humidity environment and eliminate the risk of dehydration. Two to three weeks after transfer to *ex vitro* conditions, when the plants began to grow, the humidity was gradually reduced by uncovering the cultures, until the plants adapted to natural living conditions. The appearance of the first *ex vitro* leaves marked the end of the acclimatization phase.

Four weeks after the start of the experiment, the acclimatization rate was calculated.

The experiments were organized in 3 repetitions. For each experimental variant, respectively for each repetition, 10 plants were used.

***Ex vitro* rooting and acclimatization of in vitro regenerated shoots**

Shoots of 3-4 cm derived from the propagation medium were transferred to two types of rooting substrate: perlite and Jiffy peat pellets. The shoots were washed with water to remove remaining agar traces, which may favor the occurrence of infections when transferring the shoots to *in vivo* conditions. They were either treated or not with a rooting stimulator (Radi Stim no. 1 - biopreparation for rooting herbaceous cuttings).

During the experiment, the shoots were maintained at a temperature of 22-25°C and high atmospheric humidity (85-90%) by covering them with polyethylene film or jars. Airing and spraying with water were carried out daily, and when the shoots formed new leaves, the cultures were uncovered. After the cultures were uncovered, the photosynthesis process intensified, so that along with rooting, the shoots were also acclimatized.

Four weeks after the start of the experiment, the rooting/acclimatization rate was calculated.

The experiments were organized in 3 repetitions. For each experimental variant, respectively for each repetition, 10 plants were used.

The acclimatized plants were then transplanted into pots in a peat-based mixture for further fortification and development.

Data analysis

Statistical interpretation of data was performed using SPSS 10 for Windows program. Differences between treatments for each cultivar were analyzed with One-way ANOVA - Tukey's HSD posthoc test, being considered significant at $P < 0.05$. Values shown are means $\pm$ standard deviation (SD).

RESULTS AND DISCUSSIONS

In vitro culture initiation stage

Culture initiation is a basic technological sequence in *in vitro* plant propagation.

One of the essential conditions on which the success of initiating a cell culture depends is the selection of appropriate sterilizing agents for the disinfection of explants. The method of sterilization of biological material varies depending on the origin of the material, the physiological state of the plant, and the type of organ. In general, the selection of a sterilizing agent depends on its effectiveness and the impact on the subsequent development of the explant.

Currently, the most effective preparations are based on mercury, but their toxicity inhibits the further development of plantlets (Mayorova et al., 2016). For example, when 0.10% mercuric chloride (HgCl_2) solution was used to sterilize honeysuckle explants (cultivars 'Cheliabinka' and 'Duet'), the regeneration rate was only 65.9% and 64.9% respectively (Dziedzic, 2008).

Increasing the concentration to 0.15% resulted in 95% sterile explants, of which 67.5% developed further (Sedlák and Paprštejn, 2007). When 0.2% mercuric sulfate (HgSO_4) was used, 54.43% explants proliferated, and 4.57% did not develop at all (Krupa-Malkiewicz and Ochmian, 2014). Lisoformin 3000 has also been successfully used for sterilization of explants of 14 species of ornamental and fruit crops (Bludneva et al., 2013). Other sterilizing agents, such as sodium and calcium hypochlorite or hydrogen peroxide did not provide a satisfactory yield of sterile explants. Thus, finding effective and less toxic sterilizing agents to obtain an aseptic honeysuckle culture is a necessity.

In the present study, calcium hypochlorite (CaCl_2O_2) was used to sterilize the explants. To optimize the disinfection parameters of the initial biological material, different concentrations of calcium hypochlorite (6%, 8%, and 10%) were tested.

The observations made highlighted the fact that the inclusion of the *L. caerulea* species in the *in vitro* culture system does not pose any particular problems, the use of calcium hypochlorite for sterilizing the explants proving effective.

Overall, for the 3 cultivars studied, the percentage of explants contaminated with fungal and/or bacterial infections after the first four weeks of culture ranged between 10.00 ± 10.00 and $53.33 \pm 5.77\%$. This ranged between 10.00 ± 10.00 and $43.33 \pm 5.77\%$ for the 'Loni' cultivar, between 16.67 ± 11.55 and $53.33 \pm 5.77\%$ for the 'Cera' cultivar, and between 10.00 ± 10.00 and $53.33 \pm 5.77\%$ for the 'Kami' cultivar (Table 3).

The lowest contamination rate was recorded when using 10% CaCl_2O_2 , but in this case, the percentage of explants that did not develop shoots was higher compared to that recorded when using 8% CaCl_2O_2 .

Considering that when calcium hypochlorite at a concentration of 8% was used as a disinfectant the explants had the best regenerative potential, we can conclude that this sterilization method was the most effective.

Another important aspect in the initiation stage of *in vitro* cultures is selecting the optimal type of explant to obtain a high percentage of explants that start to grow. In the case of the 3 cultivars studied ('Loni', 'Cera', and 'Kami'), following comparative analyses that targeted the

morphogenetic competence of explants of different origins (apical buds and axillary buds), the results obtained revealed that both types of explants used had the ability to regenerate shoots under *in vitro* conditions, but apical buds had the best morphogenetic potential. The percentage of explants that showed regenerative processes recorded values between 33.33 ± 5.77 and $73.33 \pm 11.55\%$ when using apical buds as explants and between 30.00 ± 10.00 and $63.33 \pm 5.77\%$ when using axillary buds (Table 3). The morphogenetic capacity of the explants also differed depending on the cultivar. Of the 3 cultivars studied, ‘Loni’ had the best regenerative potential (between 40.00 ± 10.00 and $73.33 \pm 11.55\%$ of explants developed shoots), followed by ‘Kami’ (between 36.67 ± 5.77 and $66.67 \pm 20.82\%$ of explants developed

shoots) and ‘Cera’ (between 30.00 ± 10.00 and $63.33 \pm 15.28\%$ of explants developed shoots) (Table 3).

The use of MS basal medium without growth hormones favored the production of plantlets that were subsequently used to test the morphogenetic response of *L. caerulea* explants on nutrient media with different hormonal formulas (Figure 1 a).

Research by Isac and Mladin (2013) showed that in the case of the cultivars ‘Loni’ and ‘Cera’, the best initiation medium was the mixture of Quoirin and Lepoivre (QL) mineral salts (Quoirin and Lepoivre, 1977) and Linsmaier and Skoog (LS) vitamins (Linsmaier and Skoog, 1965), containing 0.1 mg/L GA₃ (gibberellic acid) and 1 mg/L IBA.

Table 3. Influence of explant type and sterilization method on the *in vitro* regenerative potential of *L. caerulea* explants

Cultivar	Explant type	Concentration of CaCl ₂ O ₂ (%)	Contaminated explants (%)	Uncontaminated explants that have developed shoots (%)	Uncontaminated explants that have not developed shoots (%)
‘Loni’	apical buds	6	43.33 ± 5.77^b	53.33 ± 5.77^{ab}	3.33 ± 5.77^a
		8	20.00 ± 17.32^{ab}	73.33 ± 11.55^c	6.67 ± 5.77^a
		10	13.33 ± 11.55^{ab}	46.67 ± 5.77^{ab}	40.00 ± 10.00^{bc}
	axillary buds	6	43.33 ± 11.55^b	40.00 ± 10.00^a	16.67 ± 5.77^{ab}
		8	16.67 ± 5.77^{ab}	63.33 ± 5.77^{bc}	20.00 ± 10.00^{abc}
		10	10.00 ± 10.00^a	46.67 ± 5.77^{ab}	43.33 ± 15.28^c
‘Cera’	apical buds	6	53.33 ± 5.77^c	40.00 ± 0.00^{ab}	6.67 ± 5.77^a
		8	26.67 ± 15.28^{abc}	63.33 ± 15.28^c	10.00 ± 10.00^a
		10	20.00 ± 10.00^{ab}	33.33 ± 5.77^{ab}	46.67 ± 5.77^{bc}
	axillary buds	6	46.67 ± 5.77^{bc}	30.00 ± 10.00^a	23.33 ± 5.77^a
		8	20.00 ± 10.00^{ab}	53.33 ± 5.77^{bc}	26.67 ± 5.77^{ab}
		10	16.67 ± 11.55^a	33.33 ± 5.77^{ab}	50.00 ± 10.00^c
‘Kami’	apical buds	6	53.33 ± 5.77^c	43.33 ± 5.77^a	3.33 ± 5.77^a
		8	26.67 ± 23.09^{abc}	66.67 ± 20.82^a	6.67 ± 5.77^a
		10	13.33 ± 11.55^{ab}	40.00 ± 10.00^a	46.67 ± 5.77^c
	axillary buds	6	46.67 ± 5.77^{bc}	36.67 ± 5.77^a	16.67 ± 5.77^{ab}
		8	16.67 ± 15.28^{ab}	56.67 ± 11.55^a	26.67 ± 5.77^b
		10	10.00 ± 10.00^a	40.00 ± 0.00^a	50.00 ± 10.00^c

Values shown are means $\pm$ SD. Different lowercase letters indicate significant differences between treatments for each cultivar, according to Tukey’s HSD test ($P < 0.05$).

***In vitro* multiplication stage**

The multiplication phase spans several subcultures lasting approximately 4 weeks. The number of subcultures should be limited, any extension of this phase requiring additional checks on the genetic stability of the material. The number of passages should not exceed 10-15 (Kushnir and Sarnatska, 2005). In this phase, the main goal is to obtain as many plantlets as possible, while maintaining the genetic stability of the material. Increasing the

propagation rate can be achieved by stimulating axillary budding, by stimulating shoot elongation, or by both methods simultaneously. At this stage, the composition of the culture medium, especially the phytohormonal balance, plays a decisive role. High multiplication rates are generally achieved by increasing the amount of cytokinins in the culture medium or by a hormonal balance tilted in favor of cytokinins. Phytohormones are considered the most important endogenous substances for

modulating physiological and molecular responses, a critical requirement for plant survival. They act either at their site of synthesis or elsewhere in the plant after their transport (Al-Taey, 2017). Among them, cytokinins are known to significantly improve plant growth by inducing cell division, regulating cellular metabolism, and activating several specific enzymes (Al-Taey and Saadoon, 2012; Al-Taey et al., 2018a; Al-Taey et al., 2018b).

In our experiments, in order to multiply the shoots, the apexes and nodal fragments resulting from the portioning of the regenerated shoots in the initiation phase of *in vitro* cultures were inoculated on MS culture medium (Murashige and Skoog, 1962) supplemented with different types of cytokinins (BAP, KIN, and 2iP) in combination with IAA or without the addition of auxin.

The results obtained showed that shoot regeneration was influenced both by the composition of the culture medium, namely the phytohormonal balance, and by the cultivar. Statistical interpretation of the results obtained using the SPSS 10 program highlighted the fact that, in the case of the 3 cultivars studied ('Loni', 'Cera', and 'Kami'), supplementing the nutrient medium with phytohormones induced an increase compared to the control in both the number of shoots/explant and the length of the shoots, in most variants the increases being significant. Of the three cytokinins used, the most effective in terms of shoot regeneration was BAP, followed by KIN and 2iP, respectively. Increasing the concentration of cytokinins in the nutrient medium from 1 mg/L to 3 mg/L led to a weaker morphogenetic response. The addition of auxin (IAA) to the culture medium supplemented with cytokinins had a positive effect on regenerative processes, regardless of the type or concentration of cytokinin used (Table 4).

The variant in which the culture medium was supplemented with 1 mg/L BAP and 1 mg/L IAA led to the best morphogenetic response, but this was different depending on the cultivar. Of the 3 cultivars studied, 'Cera' presented the best regenerative potential, followed by 'Loni' and 'Kami', respectively. The control variant without phytohormones recorded the lowest values in terms of the number of shoots/explant and shoot length (Table 4).

Table 4. Influence of type, concentration, and combination of phytohormones on the number of shoots/explant and shoot length in the species *L. caerulea*

Variant	Cultivar					
	'Loni'		'Cera'		'Kami'	
	No. of shoots/explant	Shoot length (cm)	No. of shoots/explant	Shoot length (cm)	No. of shoots/explant	Shoot length (cm)
V0	1.00 ± 0.00 ^a	1.28 ± 0.06 ^a	1.00 ± 0.00 ^a	1.42 ± 0.06 ^a	1.00 ± 0.00 ^a	1.19 ± 0.06 ^a
V1	3.87 ± 0.12 ^g	7.04 ± 0.14 ^{ij}	3.93 ± 0.15 ^g	7.70 ± 0.49 ^{hi}	2.47 ± 0.12 ^g	3.46 ± 0.12 ^h
V2	1.60 ± 0.10 ^{bc}	3.01 ± 0.19 ^c	1.73 ± 0.12 ^{bc}	2.99 ± 0.13 ^{dc}	1.47 ± 0.15 ^{bc}	2.44 ± 0.18 ^d
V3	2.93 ± 0.15 ^c	6.47 ± 0.18 ^h	3.07 ± 0.15 ^c	6.87 ± 0.23 ^g	2.17 ± 0.06 ^{efg}	3.09 ± 0.11 ^{fg}
V4	1.20 ± 0.10 ^a	2.09 ± 0.30 ^{cd}	1.23 ± 0.06 ^a	2.16 ± 0.12 ^{bc}	1.13 ± 0.12 ^a	2.06 ± 0.09 ^c
V5	2.27 ± 0.12 ^d	4.78 ± 0.05 ^f	2.40 ± 0.20 ^d	5.52 ± 0.22 ^f	1.87 ± 0.06 ^{de}	2.57 ± 0.08 ^{de}
V6	1.10 ± 0.10 ^a	1.52 ± 0.11 ^{ab}	1.13 ± 0.06 ^a	1.54 ± 0.06 ^{ab}	1.03 ± 0.06 ^a	1.44 ± 0.09 ^{ab}
V7	3.93 ± 0.06 ^g	7.20 ± 0.16 ⁱ	4.00 ± 0.10 ^g	7.89 ± 0.50 ⁱ	2.87 ± 0.06 ^h	3.96 ± 0.14 ⁱ
V8	1.87 ± 0.06 ^c	3.04 ± 0.07 ^c	1.97 ± 0.15 ^c	3.25 ± 0.26 ^c	1.77 ± 0.06 ^{cd}	2.43 ± 0.08 ^d
V9	3.47 ± 0.12 ^f	6.72 ± 0.15 ^{hi}	3.53 ± 0.21 ^f	7.07 ± 0.12 ^{gh}	2.20 ± 0.20 ^{fg}	3.22 ± 0.12 ^{gh}
V10	1.30 ± 0.10 ^{ab}	2.41 ± 0.11 ^d	1.37 ± 0.15 ^{ab}	2.43 ± 0.14 ^{cd}	1.27 ± 0.06 ^{ab}	2.32 ± 0.12 ^{cd}
V11	2.43 ± 0.15 ^d	5.82 ± 0.13 ^g	2.53 ± 0.15 ^d	6.08 ± 0.18 ^f	2.07 ± 0.15 ^{def}	2.84 ± 0.14 ^{ef}
V12	1.13 ± 0.12 ^a	1.79 ± 0.17 ^{bc}	1.17 ± 0.06 ^a	1.82 ± 0.13 ^{abc}	1.07 ± 0.06 ^a	1.68 ± 0.11 ^b

Values shown are means ± SD. Different lowercase letters indicate significant differences between treatments for each cultivar, according to Tukey's HSD test (P<0.05).

From a qualitative point of view, the biological material regenerated *in vitro* had normal morphology, without aspects of vitrification, necrosis or callus differentiation (Figure 1 b).

Most studies on *in vitro* culture of blue honeysuckle have involved micropropagation by axillary budding in the presence of BAP (Sedlák and Paprštein, 2007; Dziedzic, 2008; Krupa-Małkiewicz and Ochmian, 2014) or the mixture of 2iP and 3-hydroxybenzyladenine (meta-topolin) (Gabryszewska et al., 2016). In research conducted by Isac and Mladin (2013) on two cultivars of *L. caerulea* ('Loni' and 'Cera') the highest multiplication rate was obtained using 1 mg/L BAP and 0.05 mg/L GA₃. In contrast, Fira et al. (2014) obtained a high propagation rate by supplementing the culture medium with 0.7 mg/L *N*-(2-chloro-4-pyridyl)-*N'*-phenylurea (CPPU).

***In vitro* rooting stage**

For effective *in vitro* propagation protocols, it is essential that shoots root in high proportions and that regenerated plants successfully acclimatize to *ex vitro* conditions. Rooting is a difficult process in recalcitrant species, in the absence of roots the survival rate of acclimatized plants being reduced.

It is known that, in *in vitro* cultures, auxins are responsible for stimulating root development and cell elongation. However, high concentrations of auxins added to nutrient media result in callus development and inhibition of root system development (Yankovskaya et al., 2011).

Thus, in order to select the optimal composition of the nutrient medium for the rooting of *L. caerulea* shoots, in the present study several medium variants were tested in which the type of auxin (IBA, NAA, IAA) and its concentration (0.5, 1, 2 mg/L) were varied.

The results obtained showed that *in vitro* rooting of shoots was influenced by both the composition of the culture medium, namely the type and concentration of auxin, and the cultivar.

Statistical interpretation using the SPSS 10 program of the obtained results revealed that supplementing the MS½ nutrient medium with

auxins had a stimulatory effect on the rooting rate, number of roots/shoot, and average root length, with significant increases in all variants. The highest values of these parameters were obtained when the culture medium was supplemented with IBA, followed by NAA and IAA respectively. In the case of all auxins used, increasing their concentration led to an increase in the rooting rate, number of roots/shoot, and average root length (Table 5). These results are consistent with those reported by Hartmann et al. (2002).

The variant in which the culture medium was supplemented with 2 mg/L IBA led to the best response in terms of the rhizogenesis process, but this was different depending on the cultivar. Of the 3 cultivars studied, 'Loni' presented the best rooting potential, followed by 'Cera' and 'Kami'.

In the case of all three *L. caerulea* cultivars studied ('Loni', 'Cera', and 'Kami'), shoots did not form roots on the control variant without auxin. These results are consistent with the results reported by Krupa-Mańkiewicz et al. (2017) and Wojtania et al. (2018) who, on auxin-free medium, obtained low rooting rates (4-8% in the 'Wojtek' cultivar and 40% in the 'Zojka' cultivar) and a weak root system.

Table 5. Influence of auxin type and concentration on the *in vitro* rooting capacity of *L. caerulea* shoots

Variant	Cultivar								
	'Loni'			'Cera'			'Kami'		
	Rooting rate (%)	No. of roots/shoot	Root length (cm)	Rooting rate (%)	No. of roots/shoot	Root length (cm)	Rooting rate (%)	No. of roots/shoot	Root length (cm)
V0	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a
V1	86.67±3.34 ^{efg}	3.97±0.15 ^{de}	2.03±0.06 ^{cf}	80.00±6.67 ^{cde}	3.67±0.15 ^{de}	1.83±0.06 ^{cf}	73.33±6.67 ^{de}	2.67±0.15 ^{cde}	1.33±0.06 ^{cf}
V2	90.00±3.33 ^{fg}	4.27±0.12 ^{ef}	2.10±0.10 ^{fg}	83.33±3.34 ^{de}	3.97±0.12 ^{ef}	1.90±0.10 ^{ef}	73.34±5.77 ^{de}	2.97±0.12 ^{de}	1.40±0.10 ^{cf}
V3	93.34±5.77 ^g	4.33±0.15 ^f	2.27±0.06 ^g	86.67±3.34 ^e	4.03±0.15 ^f	2.07±0.06 ^f	76.67±3.34 ^e	3.03±0.15 ^e	1.57±0.06 ^f
V4	76.67±6.67 ^{bcd}	3.63±0.15 ^d	1.73±0.12 ^{cd}	73.33±8.82 ^{bcd}	3.33±0.15 ^d	1.57±0.15 ^{cd}	66.67±3.34 ^{bcd}	2.37±0.21 ^c	1.07±0.15 ^{cd}
V5	80.00±6.67 ^{cdef}	3.77±0.12 ^d	1.87±0.06 ^{de}	76.67±3.34 ^{bcd}	3.47±0.12 ^d	1.67±0.06 ^{de}	70.00±5.77 ^{cde}	2.47±0.12 ^c	1.17±0.06 ^{de}
V6	83.33±6.67 ^{defg}	3.90±0.10 ^d	1.93±0.12 ^{def}	80.00±6.67 ^{cde}	3.60±0.10 ^d	1.73±0.06 ^{de}	73.33±3.34 ^{de}	2.60±0.10 ^{cd}	1.23±0.06 ^{de}
V7	66.67±3.34 ^b	2.77±0.12 ^b	1.47±0.06 ^b	63.33±6.67 ^b	2.47±0.12 ^b	1.30±0.10 ^b	56.67±3.34 ^b	1.47±0.12 ^b	0.80±0.10 ^b
V8	70.00±0.00 ^{bc}	2.90±0.10 ^{bc}	1.53±0.06 ^{bc}	66.67±6.67 ^{bc}	2.60±0.10 ^{bc}	1.33±0.06 ^{bc}	60.00±0.00 ^{bc}	1.60±0.10 ^b	0.83±0.06 ^{bc}
V9	73.33±3.34 ^{bcd}	3.13±0.15 ^c	1.60±0.10 ^{bc}	70.00±0.00 ^{bcd}	2.83±0.15 ^c	1.40±0.10 ^{bc}	63.33±3.34 ^{bcd}	1.83±0.15 ^b	0.90±0.10 ^{bc}

Values shown are means ± SD. Different lowercase letters indicate significant differences between treatments for each cultivar, according to Tukey's HSD test (P<0.05).

The above-mentioned results confirm that, in the *in vitro* culture of *L. caerulea*, auxins play an important role in achieving high rooting rates (Karhu, 1997; Sedlák and Paprštejn, 2007; Dziedzic, 2008; Krupa-Mańkiewicz et al., 2017). Similar results have also been reported in other plant species (Soliman et al., 2014).

The results obtained show that IBA at a concentration of 2 mg/L was the most effective auxin for *in vitro* rooting of *L. caerulea* shoots. These results are consistent with those reported by Sedlák and Paprštejn (2007), who obtained a 100% rooting rate for the 20/1 and 'Altaj' genotypes on MS medium with macro- and micronutrients reduced to one-third

supplemented with 2.5 mg/L IBA. In studies conducted by Zapolsky et al. (2018) and Kadhim et al. (2019), the highest rooting percentage was obtained using IBA at concentrations of 1 and 1.5 mg/L. Isac and Mladin (2013) obtained a rooting rate of 89.6% for the 'Loni' cultivar on Murashige and Skoog (MS) nutrient medium supplemented with 1 mg/L IBA and 162 mg/L PG (phloroglucinol). According to Dziedzic (2008), WPM medium (Woody Plant Medium) (Lloyd and McCown, 1981) supplemented with 2 mg/L IBA and 5 mg/L IAA was more effective for inducing rhizogenesis in the cultivars 'Czelabinka' (96% rooted shoots) and 'Duet' (92% rooted shoots) compared to MS medium. The superior efficiency of IBA compared to NAA and IAA in the rooting process was also demonstrated by Anuradha et al. (2016) on the strawberry cultivar 'Ofra' and by Koubouris and Vasilakakis (2006) on the *Prunus* species. This may be due to the higher stability of IBA (Nissen and Sutter, 1990). However, there are studies in which the presence of auxins in the culture medium stimulated unwanted callus formation at the base of rooted shoots, which blocked the formation of new roots and decreased the survival rate of blue honeysuckle transferred under *ex vitro* conditions (Karhu, 1997; Wojtania et al., 2018). On the other hand, according to Dziedzic (2008), Osburn et al. (2009), and Hui et al. (2012), the acclimatization of blue honeysuckle plants regenerated by *in vitro* culture depends largely on the genotype and not on the auxin concentration in the culture medium.

Acclimatization stage

The acclimatization is the final stage of microclonal propagation. For effective micropropagation protocols, it is essential that *in vitro* regenerated plants successfully acclimatize to *ex vitro* conditions.

Acclimatization of *in vitro* rooted shoots

The experiments conducted led to the conclusion that the nutrient substrate played an important role in the successful acclimatization of *L. caerulea* vitroplants, the best results being obtained when Jiffy peat pellets were used (100 ± 0.00% acclimatized plants in the case of the 'Loni' cultivar, 93.33 ± 5.77% acclimatized plants in the case of the 'Cera' cultivar, and 86.67 ± 5.77% acclimatized plants in the case of

the 'Kami' cultivar). When using the mixture of peat, manure, and perlite in a ratio of 2:1:1, the percentage of acclimatized plants was lower (96.67 ± 5.77% acclimatized plants in the case of the 'Loni' cultivar, 90.00 ± 10.00% acclimatized plants in the case of the 'Cera' cultivar, and 80.00 ± 10.00% acclimatized plants in the case of the 'Kami' cultivar), this substrate however ensuring good fortification of the acclimatized plants. Regardless of the nutrient substrate used, the highest acclimatization rate was recorded for the 'Loni' cultivar, followed by the 'Cera' cultivar and the 'Kami' cultivar (Table 6).

Table 6. Influence of nutrient substrate on the acclimatization capacity of *in vitro* rooted plants to *ex vitro* conditions

Cultivar	Nutritive substrate	Acclimatization rate (%)
'Loni'	peat, manure, and perlite (2:1:1)	96.67 ± 5.77
	peat pellets (Jiffy type)	100.00 ± 0.00
'Cera'	peat, manure, and perlite (2:1:1)	90.00 ± 10.00
	peat pellets (Jiffy type)	93.33 ± 5.77
'Kami'	peat, manure, and perlite (2:1:1)	80.00 ± 10.00
	peat pellets (Jiffy type)	86.67 ± 5.77

Values shown are means ± SD.

Similar results, namely high acclimatization rates, were obtained in other studies on the propagation of the species *L. caerulea* by micropropagation (Wojtania et al., 2020; Liu et al., 2023).

Ex vitro rooting and acclimatization of *in vitro* regenerated shoots

Observations made four weeks after the start of the experiments demonstrated that, in the studied species, the *in vivo* rooting process took place under optimal conditions, with favorable results. During this period, the shoots showed intense development of the root system, forming a significant number of roots (Figures 1 e and f). Research on the influence of rooting stimulants and rooting substrate on the *in vivo* rhizogenesis process in *L. caerulea* shoots has highlighted the fact that the composition of the substrate influenced the rooting of the shoots, but rooting stimulants played an important role in rooting success, in their absence the shoots rooted in a lower percentage. Treating the base of the shoots with rooting stimulants promotes cell division and the formation of adventitious roots, which allows the rooting period to be shortened and the percentage of rooted shoots to be increased.

The use of Radi Stim as a rooting stimulator and peat pellets as a substrate led to the best results, achieving rooting of $100.00 \pm 0.00\%$ of shoots in the case of the 'Loni' and 'Cera' cultivars and $86.67 \pm 5.77\%$ in the case of the 'Kami' cultivar. When the shoots were treated with rooting stimulator and perlite was used as the rooting substrate, the percentage of rooted plants *in vivo* was $96.67 \pm 5.77\%$ in the case of the 'Loni' cultivar, $93.33 \pm 5.77\%$ in the case of the 'Cera' cultivar, and $83.33 \pm 5.77\%$ in the case of the 'Kami' cultivar. The lowest values in terms of *in vivo* shoot rooting rate were obtained in the absence of rooting stimulants and when using perlite as a substrate ($86.67 \pm 5.77\%$ for the 'Loni' cultivar, $83.33 \pm 5.77\%$ for the 'Cera' cultivar, and $80.00 \pm 0.00\%$ for the 'Kami' cultivar). Of the 3 cultivars studied, the 'Loni' cultivar showed the best rooting potential, followed by the 'Cera' cultivar and the 'Kami' cultivar (Table 7).

High rates of *ex vitro* rooting and acclimatization were also obtained in other studies on *in vitro* propagation of the *L. caerulea* species (max. 96% for the cultivar 'Wojtek' and max. 88% for the cultivar 'Zojka') (Wojtania et al., 2020). Regarding the nutrient substrate, peat or a mixture of peat and perlite/vermiculite has been shown to be a better substrate for the rooting and *ex vitro* growth of blue honeysuckle shoots compared to a mixture of peat and sand (Karhu, 1997; Dziedzic, 2008; Krupa-Małkiewicz et al., 2017; Wojtania et al., 2020).

Table 7. The influence of the culture substrate and the rooting stimulator on the *in vivo* rooting capacity of *L. caerulea* shoots simultaneously with acclimatization

Cultivar	Rooting substrate	Rooting stimulator	Rooting/ acclimatization rate (%)
'Loni'	Perlite	-	86.67 ± 5.77^a
	Perlite	Radi Stim no. 1	96.67 ± 5.77^{ab}
	peat pellets (Jiffy type)	-	93.33 ± 5.77^{ab}
	peat pellets (Jiffy type)	Radi Stim no. 1	100.00 ± 0.00^b
'Cera'	Perlite	-	83.33 ± 5.77^a
	Perlite	Radi Stim no. 1	93.33 ± 5.77^a
	peat pellets (Jiffy type)	-	90.00 ± 10.00^a
	peat pellets (Jiffy type)	Radi Stim no. 1	100.00 ± 0.00^a
'Kami'	Perlite	-	80.00 ± 0.00^a
	Perlite	Radi Stim no. 1	83.33 ± 5.77^a
	peat pellets (Jiffy type)	-	83.33 ± 5.77^a
	peat pellets (Jiffy type)	Radi Stim no. 1	86.67 ± 5.77^a

Values shown are means $\pm$ SD. Different lowercase letters indicate significant differences between treatments for each cultivar, according to Tukey's HSD test ($P < 0.05$).

Simultaneously with the *in vivo* rooting of the shoots, their acclimatization to *ex vitro* conditions was also performed. The acclimatized plants were subsequently fortified in pots in a peat-based mixture. It should be noted that the *in vitro* regenerated plants retained the morphological characteristics of the donor plants (Figures 1 g and h).

Figure 1 shows aspects of the main stages of *in vitro* culture of *L. caerulea*.



Figure 1. Aspects of *in vitro* culture of *L. caerulea*: a - explants in the initiation phase; b - shoot propagation; c - *in vitro* rooted shoots; d, e - *in vivo* rooting of shoots in perlite; f - *in vivo* rooting of shoots in Jiffy peat pellets; g, h - acclimatized plants fortified in pots

CONCLUSIONS

The use of 8% calcium hypochlorite for disinfection of explants and a plain MS medium for their inoculation proved effective in inducing regenerative processes in the three *L. caerulea* cultivars studied: 'Loni', 'Cera', and 'Kami'. The highest number of shoots per explant and the highest average shoot length were obtained on MS medium supplemented with 1 mg/L BAP and 1 mg/L IAA. The most effective culture medium for *in vitro* shoot rooting proved to be MS½ medium supplemented with 2 mg/L IBA. *In vitro* regenerated plants were efficiently acclimatized to *ex vitro* conditions. *In vitro* rooting can be successfully replaced with *in vivo* rooting simultaneously with acclimatization, thus shortening the technological flow of obtaining planting material through micropropagation and considerably reducing the unit price of the plant. The results obtained show that micropropagation is an effective method of producing large quantities of healthy blue honeysuckle planting material in a short time.

ACKNOWLEDGEMENTS

This work was carried out with the support of the Ministry of Research, Innovation and Digitalization through the Nucleu Program 37N/2023, project PN 23.41.01.02.

REFERENCES

- Al-Taey, D. K. A., & Saadoon, A. H. (2012). Effect of treatment of kinetin to reduce the salinity damage by drainage water irrigation on the growth and nitrate accumulation in the leaves of spinach, *Spinacia oleracea* L. *Euphrates J. Agric. Sci.*, 4(4), 11-24.
- Al-Taey, D. K. A. (2017). Mitigation of salt stress by organic matter and GA₃ on growth and peroxidase activity in pepper (*Capsicum annum* L.). *Adv. Natural and Appl. Sci.*, 11(10), 1-11.
- Al-Taey, D. K. A., Mijwel, A. K., & Al-Azawy, S. S. (2018a). Study efficiency of poultry litter and kinetin in reduced effects of saline water in *Vicia faba*. *Res. J. Pharm. and Tech.*, 11(1), 294-300. <http://dx.doi.org/10.5958/0974-360X.2018.00054.9>
- Al-Taey, D. K. A., Al-Azawi, S. S. M., Al-Shareefi, M. J. H., & Al-Tawaha, A. R. (2018b). Effect of saline water, NPK and organic fertilizers on soil properties and growth, antioxidant enzymes in leaves and yield of lettuce (*Lactuca sativa* var. Parris Island). *Res. Crops*, 19(3), 441-449. <https://doi.org/10.31830/2348-7542.2018.0001.14>
- Anuradha, Sehrawat, S. K., Vijayluxmi, & Bhat Sandhya (2016). Effect of growth regulators on *in vitro* root formation in strawberry. *Res. Environ. Life Sci.*, 9(11), 1316-1318.
- Bludneva, E. A., Kritskaya, T. A., & Kashin, A. S. (2013). The use of clonal micropropagation for mass production of planting material of ornamental and fruit crops in the botanical garden of Saratov state university. *Bulletin of the Botanic Gardens of Saratov State University*, 11, 119-131.
- Bojarska, J. E., Markuszewski, B., Majewska, K. M., Józefowicz, W., & Bieniek, A. (2019). Preliminary studies on the storage of blue honeysuckle (*Lonicera caerulea* L.) berries. In 4th International Conference "Effects of Pre- and Post-harvest Factors on Health Promoting Components and Quality of Horticultural Commodities", Skierniewice, Poland.
- Caprioli, G., Iannarelli, R., Innocenti, M., Bellumori, M., Fiorini, D., Sagratini, G., Vittori, S., Buccioni, M., Santinelli, C., Bramucci, M., Quassinti, L., Lupidi, G., Vitali, L., Petrelli, D., Beghelli, D., Cavallucci, C., Bistoni, O., Trivisonno, A., & Maggi, F. (2016). Blue honeysuckle fruit (*Lonicera caerulea* L.) from eastern Russia: Phenolic composition, nutritional value and biological activities of its polar extracts. *Food Funct.*, 7(4), 1892-1903. <https://doi.org/10.1039/C6FO00203J>
- Celli, G. B., Ghanem, A., & Brooks, M. S. L. (2014). Haskap berries (*Lonicera caerulea* L.) - A critical review of antioxidant capacity and health-related studies for potential value-added products. *Food Bioprocess Technol.*, 7, 1541-1554. <https://doi.org/10.1007/s11947-014-1301-2>
- Chang, S. K., Alasalvar, C., & Shahidi, F. (2019). Superfruits: Phytochemicals, antioxidant efficacies, and health effect - A comprehensive review. *Crit. Rev. Food Sci. Nutr.*, 59(10), 1580-1604. <https://doi.org/10.1080/10408398.2017.1422111>
- Clapa, D., Radomir, A. M., Peticila, A. G., & Harta, M. (2023). Evaluation of biomass production of *Stevia rebaudiana* Bertoni using classical *in vitro* culture and temporary immersion bioreactor system. *Scientific Papers. Series B, Horticulture*, 67(1), 558-565.
- Cory, H., Passarelli, S., Szeto, J., Tamez, M., & Mattei, J. (2018). The Role of polyphenols in human health and food systems: A mini-review. *Front. Nutr.*, 5, 87. <https://doi.org/10.3389/fnut.2018.00087>
- Debnath, S. C. (2007). Strategies to propagate *Vaccinium* nuclear stocks for the Canadian berry industry. *Can. J. Plant Sci.*, 87, 911-922.
- Dziedzic, E. (2008). Propagation of blue honeysuckle (*Lonicera caerulea* var. *kamtschatica* Pojark.) in *in vitro* culture. *J. Fruit Orn. Plant Res.*, 16, 93-100.
- Fira, A., Clapa, D., Cristea, V., & Plopa, C. (2014). *In vitro* propagation of *Lonicera kamtschatica*. *Agricultura - Știință și practică*, 1-2, 90-99.
- Gabryszevska, E., Górąj-Koniarska, J., Orlikowska, T., Malinowski, T., Markiewicz, M., & Wojtania, A. (2016). Metodyka inicjacji i stabilizacji kultur oraz namnażania pędów jagody kamczackiej (*Lonicera caerulea* L. var. *kamtschatica* Sevest.) *in vitro*. *Research Institute of Horticulture, Skierniewice, Poland*, 13 p.

- Gahan, P. B., & George, E. F. (2008). Adventitious regeneration. In *Plant Propagation by Tissue Culture*; George, E. F., Hall, M. A., De Klerk, G.-J., Eds.; Springer: Dordrecht, The Netherlands, pp. 355-366.
- Gawroński, J., Żebrowska, J., Pabich, M., Jackowska, I., Kowalczyk, K., & Dyduch-Siemńska, M. (2020). Phytochemical characterization of blue honeysuckle in relation to the genotypic diversity of *Lonicera* sp. *Appl. Sci.*, 10(18), 6545. <https://doi.org/10.3390/app10186545>
- Grobelna, A., Kalisz, S., & Kieliszek, M. (2019). Effect of processing methods and storage time on the content of bioactive compounds in blue honeysuckle berry purees. *Agronomy*, 9(12), 860. <https://doi.org/10.3390/agronomy9120860>
- Grobelna, A., Kalisz, S., Kieliszek, M., & Giurgulescu, L. (2020). Blue honeysuckle berry (*Lonicera caerulea* L.), as raw material, is particularly predisposed to the production of functional foods. *Carpathian J. Food Sci. Technol.*, 12(3), 144-155. <https://doi.org/10.34302/crpjfst/2020.12.3.12>
- Hartmann, H. T., Kester, D. E., & Geneve, R. L. (2002). *Plant Propagation Principles and Practices*, 7th edn. Perntice Hall, Inc. New Jersey, USA.
- Hui, J. X., Wen, S. C., Hua, Z. Y., & Ming, L. X. (2012). Comparative study on different methods for *Lonicera japonica* Thunb. micropropagation and acclimatization. *J. Med. Plants Res.*, 6(27), 4389-4393. <https://doi.org/10.5897/JMPR011.1715>
- Isac, V., & Mladin, Gh. (2013). Micropropagation of two blue honeysuckle cultivars released in Romania. *Acta Hortic.*, 981, 453-459. <https://doi.org/10.17660/ActaHortic.2013.981.72>
- Kadhim, Z. K., AL-Shareefi, J. H. M., & Lateef, S. M. (2019). Effect of growth regulators on *in vitro* micropropagation of blue honeysuckle (*Lonicera caerulea* L.). *Res on Crops*, 20(3), 635-641. <http://dx.doi.org/10.31830/2348-7542.2019.093>
- Karhu, S. T. (1997). Rooting of blue honeysuckle microshoots. *Plant Cell, Tissue Organ Cult.*, 48, 153-159. <https://doi.org/10.1023/A:1005768117246>
- Koubouris, G., & Vasilakakis, M. (2006). Improvement of *in vitro* propagation of apricot cultivar 'Bebecou'. *Plant Cell, Tissue Organ Cult.*, 85, 173-180. <https://doi.org/10.1007/s11240-005-9066-y>
- Krupa-Malkiewicz, M., & Ochmian, I. (2014). Propagation of blue honeysuckle (*Lonicera caerulea* L.) in *in vitro* culture. *J. Basic Appl. Sci.*, 10, 164-169. <https://doi.org/10.6000/1927-5129.2014.10.22>
- Krupa-Malkiewicz, M., Ochmian, I., Smolik, M., & Ostrowska, K. M. (2017). Comparison of propagation method in *in vitro* and *in vivo* condition of *Lonicera caerulea* L. *Folia Pomer. Univ. Technol. Stetin.*, 334(42)2, 79-88. <https://doi.org/10.21005/AAPZ2017.42.2.09>
- Kula, M., Majdan, M., Radwańska, A., Nasal, A., Hałas, R., Glód, D., Matkowski, A., & Krauze-Baranowska, M. (2013). Chemical composition and biological activity of the fruits from *Lonicera caerulea* var. *edulis* 'Wojtek'. *Acad. J. Med. Plants*, 8, 141-148. <https://doi.org/10.15413/ajmp.2013.0134>
- Kushnir, H. P., & Sarnatska, V. V. Microclonal propagation of plants. K.: Naukova dumka, 2005:271 p.
- Linsmaier, E. M., & Skoog, F. (1965). Organic growth factor requirements of tobacco tissue cultures. *Physiol Plant*, 18, 100-127. <https://doi.org/10.1111/j.1399-3054.1965.tb06874.x>
- Liu, Y., Zhan, Y., Fu, Q., Li, S., Sun, X., Wang, Y., Yu, M., Qin, D., Huo, J., & Zhu, C. (2023). Plant Regeneration via Somatic Embryogenesis and Indirect Organogenesis in Blue Honeysuckle (*Lonicera caerulea* L.). *Horticulturae*, 9(9), 996. <https://doi.org/10.3390/horticulturae9090996>
- Lloyd, G., & McCown, B. H. (1981). Woody Plant Medium (WPM) - A Mineral Nutrient Formulation for Microculture of Woody Plant Species. *HortScience*, 16, 453.
- Mayorova, O. Yu., Hrytsak, L. R., Mazur, D. S., Vitrova, S. A., & Drobyk, N. M. (2016). Microclonal propagation and rooting *in vitro* of rare *Gentiana acaulis* L. species. *Faktory eksperymentalnoi evoliutsii orhanizmv*, 19, 162-166.
- McLennan, A. K. (2018). Book review: Food Health. Nutrition, technology, and public health. (Research methods for anthropological studies of food and nutrition (Ed. by Jane Chrzan, John Brett). *Anthropos*, 113, 710-711.
- Miyashita, T., Ohashi, T., Shibata, F., Araki, H., & Hoshino, Y. (2009). Plant regeneration with maintenance of the endosperm ploidy level by endosperm culture in *Lonicera caerulea* var. *emphyllolachyx*. *Plant Cell, Tissue Organ Cult.*, 98, 291-301. <https://doi.org/10.1007/s11240-009-9562-6>
- Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.*, 15, 473-497. <https://doi.org/10.1111/j.1399-3054.1962.tb08052.x>
- Nissen, S. J., & Sutter, E. G. (1990). Stability of IAA and IBA in nutrient medium to several tissue culture procedures. *Hort. Sci.*, 25(7), 800-802.
- Osburn, L. D., Yang, X., Li, Y., & Cheng, Z. M. (2009). Micropropagation of Japanese honeysuckle (*Lonicera japonica*) and Amur honeysuckle (*L. maackii*) by shoot tip culture. *J. Environ. Hortic.*, 27(4), 195-199. <https://doi.org/10.24266/0738-2898-27.4.195>
- Palikova, I., Heinrich, J., Bednar, P., Marhol, P., Kren, V., Cvak, L., Valentova, K., Ruzicka, F., Simanek, V., & Ulrichova, J. (2008). Constituents and Antimicrobial Properties of Blue Honeysuckle: A Novel Source for Phenolic Antioxidants. *J. Agric. Food Chem.*, 56(24), 11883-11889. <https://doi.org/10.1021/jf8026233>
- Park, E., Kum, S., Wang, C., Park, S. Y., Kim, B. S., & Schuller-Lewis, G. (2005). Anti-inflammatory activity of herbal medicines: inhibition of nitric oxide production and tumor necrosis factor- α secretion in an activated macrophage-like cell line. *Am. J. Chinese Med.*, 33(3), 415-424. <https://doi.org/10.1142/S0192415X05003028>
- Quoirin, M., & Lepoivre, P. (1977). Improved media for *in vitro* culture of *Prunus* sp. *Acta Hortic.*, 78, 437-442. <https://doi.org/10.17660/ActaHortic.1977.78.54>

- Rupasinghe, H. P. V., Arumuggam, N., Amararathna, M., & De Silva, A. B. K. H. (2018). The potential health benefits of haskap (*Lonicera caerulea* L.): Role of cyanidin-3-Oglucoside. *J. Funct. Foods*, 44, 24-39. <https://doi.org/10.1016/j.jff.2018.02.023>
- Sedláč, J., & Paprštein F. (2007). *In vitro* propagation of blue honeysuckle. *Hort. Sci.*, 34(4), 129-131. <https://doi.org/10.17221/1871-HORTSCI>
- Sharma, A., & Lee, H.-J. (2021). *Lonicera caerulea*: An updated account of its phytoconstituents and health-promoting activities. *Trends Food Sci. Technol.*, 107, 130-149. <https://doi.org/10.1016/j.tifs.2020.08.013>
- Soliman, H. I. A., Metwali, E. M. R., & Almaghrabi, O. A. H. (2014). Micropropagation of *Stevia rebaudiana* Betroni and assessment of genetic stability of *in vitro* regenerated plants using inter simple sequence repeat (ISSR) marker. *Plant biotechnology*, 31(3), 249-256. <https://doi.org/10.5511/PLANTBIOTECHNOLOGY.14.0707A>
- Svarcova, I., Heinrich, J., & Valentova, K. (2007). Berry fruits as a source of biologically active compounds: The case of *Lonicera caerulea*. *Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czech Repub.*, 151(2), 163-174. <https://doi.org/10.5507/bp.2007.031>
- Wang, Y., Zhu, J., Meng, X., Liu, S., Mu, J., & Ning, C. (2016). Comparison of polyphenol, anthocyanin and antioxidant capacity in four varieties of *Lonicera caerulea* berry extracts. *Food Chem.*, 197, 522-529. <https://doi.org/10.1016/j.foodchem.2015.11.006>
- Waterlander, W. E., Ni Mhurchu, C., Eyles, H., Vandevijvere, S., Cleghorn, C., Scarborough, P., Swinburn, B., & Seidell, J. (2018). Food Future: Developing effective food systems interventions to improve public health nutrition. *Agric. Syst.*, 160, 124-131. <https://doi.org/10.1016/j.agsy.2017.01.006>
- Wojtania, A., Matysiak, B., Góraj-Koniarska, J., Kiszczak, W., Kucharska, D., & Kowalska, U. (2018). Ukorzenianie i aklimatyzacja mikrosadzonek truskawki, maliny, jagody kamczackiej i czosnku w warunkach *ex vitro* – raport z badań wykonanych w 2018 roku. *Research Institute of Horticulture, Skierniewice, Poland*, 16 p.
- Wojtania, A., Markiewicz, M., & Góraj-Koniarska, J. (2020). *Ex vitro* Rooting, Acclimatization and Genetic Stability of *Lonicera caerulea* var. *kamtschatica*. *J. Hortic. Res.*, 28(2), 61-70. <https://doi.org/10.2478/johr-2020-0019>
- Yankovskaya, M. B., Shornikov, D. G., Muratova, S. A., & Solovykh, N. V. (2011). Preservation and propagation of valuable forms of berries and ornamental plants by biotechnology methods. *Vestnik IrGSKhA*, 4(44), 160-166.
- Zapolsky, Y., Medvedeva, T., Natalchuk, T., & Bublyk, M. (2018). Propagation of edible honeysuckle (*Lonicera edulis* Turcz) in *in vitro* conditions. *Agric. sci. pract.*, 5(2), 18-26. <https://doi.org/10.15407/agrisp5.02.018>