Some clonal micropropagation features of *Lonicera caerulea* L. cultivars *in vitro*

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Abstract. *Lonicera caerulea* L. is one of the early temperate berry crops. Honeysuckle is a deciduous shrub up to 2.5 m high with edible blue fruits rich in vitamins and polyphenols. The work is devoted to the improvement of *in vitro* propagation method of some *Lonicera caerulea* cultivars. The differences in regeneration and formation of axillary shoots were revealed depending on the mineral medium composition. The optimal mineral composition of the nutrient medium for clonal micropropagation of the studied genotypes was determined. Cultivation on of Quoirin and Lepoivre medium contributed to the maximum reproduction coefficient (14.3) and height of microshoots (5.1 cm). The optimal concentration of auxin at the rooting stage has been established. The highest percentage of rooted microshoots (91%) was noted on a nutrient medium with 0.3 mg/l indoleacetic acid.

1 Introduction

Lonicera caerulea L. is one of the high-vitamin and early-bearing berry crops cultivated in northern latitudes [1, 2]. Blue honeysuckle fruits contain a large amount of biologically active substances, macro- and microelements (Mg, Na, K, Mn, Cu, Si, I, etc.) [3, 4]. They are characterized by capillary-strengthening, hepato- and cardioprotective and antioxidant effects due to which they are used in cosmetology, food and medical industries [4, 5, 6].
Currently, clonal micropropagation of honeysuckle is the most effective of vegetative propagation methods. In vitro cultivation of *L. caerulea* makes it possible to obtain a large amount of genetically homogeneous, self-rooted planting material in a short time. The selection of the mineral medium composition at the multiplication stage and of auxins at the rooting stage are part of the optimization of the in vitro cultivation technology for this crop [7].

Murashige and Skoog (MS) medium is most often used for clonal micropropagation of honeysuckle [8]. At the same time there are works on the *Lonicera* cultivation on other media: Osburn L.D. and others noted in their studies that Driver and Kuniyuki (DKW) medium was optimal for successful in vitro propagation of *L. japonica* while MS medium was optimal for *L. maacki* [9]; Mihaljević I. observed the maximum reproduction rate of *L.*

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caerulea cultivars when cultivated them on DKW medium [10]; Makarov S.S. got best results on Quoirin and Lepoivre (QL) medium [11].

The correct choice of the type of auxin and its concentration is of great importance for effective rooting under in vitro conditions [12]. IAA (β-indoleaceticacid) is most often used to induce rhizogenesis. However, in the works of Y.S. Zapolsky [13] and Z.K. Kadhim [14] on the rooting of blue honeysuckle the maximum percentage of rooting was observed when using IBA (β-indolebutyricacid) at a concentration of 1.0 and 1.5 mg/l.

The scientific literature contains conflicting data on the choice of the optimal mineral medium composition and auxin for the cultivation of the genus *Lonicera* representatives, which makes research in this direction relevant.

The aim of our work is to study the effect of the mineral medium composition and auxin concentrations on the regeneration of *L. caerulea* cultivars at the multiplication and rooting stages.

2 Materials and methods

The experiment was carried out in the Laboratory of Plant Biotechnology of Tsitsin Main Botanical Garden of Russian Academy of Sciences (MBG RAS).

The objects of the study were honeysuckle cultivars Goluboj Desert, Gordost' Bakchara, Diana, Lebedushka, Lyuliya, and Yugana.

At the multiplication stage the following nutrient media were used in the experiment: MS (Murashige and Skoog, 1962), QL (Quoirin and Lepoivre medium, 1977) [17], DKW (Driver and Kuniyuki medium, 1984) [18], B5 (Gamborg, 1968) [19], and WPM (Woody Plant Medium, 1980) [20] with the addition of 6-benzylaminopurine (6-BAP) at a concentration of 0.8 mg/L. MS medium was taken as a control as it is most commonly used for clonal micropropagation of honeysuckle.

After 30 days of cultivation the height of microshoots was measured, the number of microshoots and the number of internodes on them were counted, and the reproduction coefficient was calculated.

At the rooting stage half-strength MS medium was used with the addition of growth regulators IAA and IBA at concentrations of 0.3, 0.5, and 1.0 mg/L. ½ MS without the addition of auxins was taken as a control. After 14 and 28 days the percentage of rooted regenerants was calculated.

The experiments were repeated in triplicate, each with 10 explants.

The nutrient medium was sterilized using pressurized saturated steam (P=101 kPa) at a temperature of 120°C for 20 minutes. Vessels for cultivation, instruments, and equipment were sterilized according to generally accepted methods [16].

The regenerants were cultivated at light intensity of 1.5...2.0 klux, 16-hour photoperiod and temperature of 25±2°C. Parts of microshoots containing 2-3 metamers were used as explants.

The obtained data were processed according to the generally accepted methods of ANOVA statistical analysis [21] using Microsoft Office Excel 2010 software.

3 Results and discussion

One of the largest in vitro genetic bank of *L. caerulea* representatives in Russia has been formed at MBG RAS which currently includes 34 genotypes.

Previously, all cultivars were conditionally divided into groups according to the their reproduction coefficients: low (3-5); medium (5-8); above average (8-10); high (>10) [22].

The experiment involved genotypes with high and medium reproduction coefficient. In addition the studied cultivars were characterized by different morphology of microshoots: Gordost' Bakchara, Lyuliya, and Diana had thinned microshoots, Goluboj Desert, Lebedushka, and Yugana had normal ones.

The reproduction coefficient and the height of microshoots are the most significant parameters for assessing the morphogenetic potential of plants at the multiplication stage. The reproduction coefficient is one of the key indicators in determining the effectiveness of in vitro cultivation. The height of microshoots is also an equally important factor in cultivation since the size of microshoots is of great importance for the successful rooting of honeysuckle cultivars [23]. Well-formed microshoots with an optimal length of internodes and developed axillary buds facilitate the process of microcutting.

At the stage of micropropagation, a significant effect of mineral medium composition on the reproduction coefficient (64%) was found while the interaction of factors (22%) and the plant genotype (9%) had a lesser effect. A significant effect of the genotype (47%) and the mineral medium composition (28%) on the height of microshoots was found. (Fig. 1).

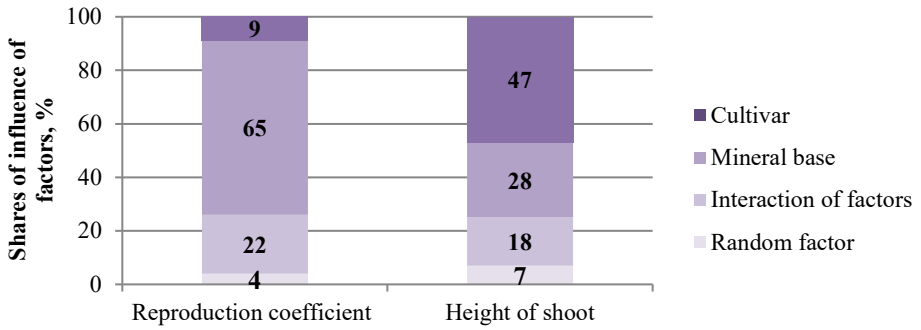

Fig. 1. Shares of influence of factors on the reproduction coefficient and height of microshoots in *L. caerulea* cultivars.

When cultivating on QL (14.3) and B5 (14.5) media the maximum reproduction coefficients were noted that exceeded reproduction rate on the control medium (12.1) (fig. 2). This is consistent with the results of studies by S. S. Makarov and others [11] where the highest reproduction coefficient was noted on the QL medium.

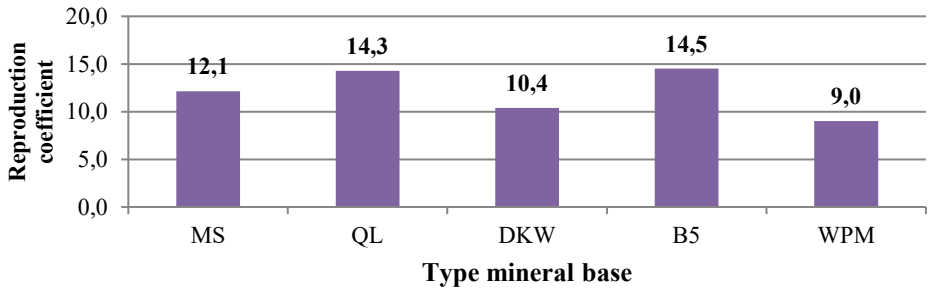

Fig. 2. The reproduction coefficients of *L. caerulea* cultivars depending on the mineral medium composition (LSD_{05}=0,38).

According to the height of microshoots the studied cultivars can be divided into two groups: with a height of 3.5 to 4.5 (Diana - 3.6 cm, Lyulia - 3.7 cm, Yugana - 4.2 cm) and

with a height of 4.6 to 5.6 cm (Goluboj Desert - 5.4 cm, Gordost' Bakchara- 5.6 cm, Lebedushka - 5.3 cm) (Fig. 3).

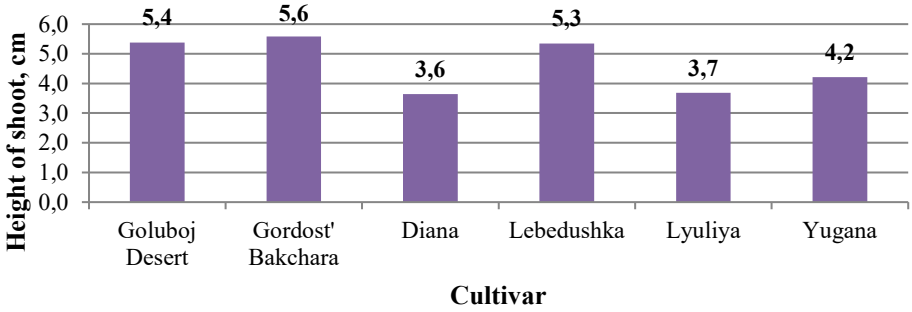

Fig. 3. The height of microshoots of different *L. caerulea* cultivars (LSD_{05}=0,27).

Cultivars Goluboj Desert, Gordost' Bakchara and Lebedushka were characterized by the maximum height of microshoots on the QL medium (6.2 cm, 6.5 cm, and 6.3 cm respectively). The maximum height of microshoots in cultivars Diana (4.4 cm), Lyuliya (4.6 cm) and Yugana (4.7 cm) was noted at DKW medium. Osburn L.D. and others [9] observed a similar result during in vitro propagation of *L. japonica*. The minimum height of microshoots was noted on B5 and WPM media (Table 1).

Table 1. The height of microshoots of *L. caerulea* cultivars depending on the mineral medium composition, cm (LSD_{05}=0,79).

Cultivar	**Mineral medium composition**				
	MS	QL	DKW	B5	WPM
Goluboj Desert	5,8	**6,2**	5,9	4,0	4,4
Gordost' Bakchara	5,7	**6,5**	6,4	4,3	5,2
Diana	3,5	3,2	**4,4**	3,1	3,1
Lebedushka	6,0	**6,3**	5,8	3,9	3,8
Lyuliya	3,4	3,7	**4,6**	2,6	2,7
Yugana	4,1	**4,7**	**4,7**	3,3	3,4

Based on the values of both factors (height of microshoots and reproduction coefficient) QL medium is optimal for using at the multiplication stage for the most studied genotypes of *L. caerulea*.

The rooting stage is of great importance when cultivating plants under in vitro conditions. Selection of the optimal type of auxin and its concentration increases the efficiency of rooting. The highest percentage of rooting was obtained on the medium with the addition of IAA (86%) while the percentage of rooting on medium with IBA and on the control was 76% (Fig. 4).

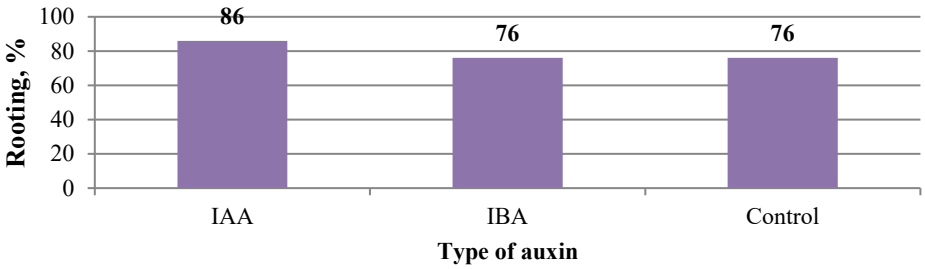

Fig. 4. Rooting of *L. caerulea* cultivars depending on the auxin, %.

The optimal choice of auxin concentration at the rooting stage contributes to the most effective manifestation of the root-forming potential. When comparing the studied concentrations the maximum rooting of honeysuckle microshoots (91%) was revealed on a medium with the addition of IAA at a concentration of 0.3 mg/l (fig. 5).

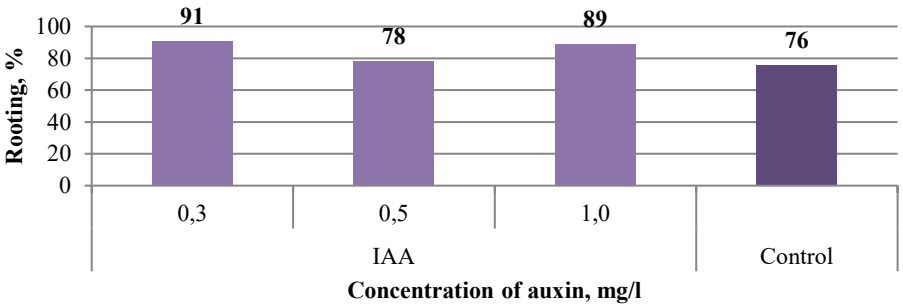

Fig. 5. Rooting of *L. caerulea* cultivars depending on concentration of IAA, %.

4 Conclusion

When comparing the shares of the influence of the mineral medium composition and the genotype the dominant effect of the first factor (64%) on the reproduction coefficient of honeysuckle cultivars was established. On the contrary, the height of microshoots was mostly influenced by the genotype factor (47%). The optimal mineral medium composition for clonal micropropagation of *L. caerulea* was QL: the maximum values of the reproduction coefficient (14.3) and the height of microshoots (5.1 cm) were noted in the most studied cultivars.

The timing of root formation and the degree of development of the root system depends on growth regulators and their concentration. The use of 0.3 mg/L IAA (the percentage of rooting was 91%) at the rooting stage was more effective than other auxin types and concentrations.

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