Improvement of clonal micropropagation technique of promising Lonicera caerulea L. cultivars

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Abstract. The work is focused on enhancement of in vitro propagation technique of valuable Lonicera caerulea L. cultivars. The impact of some nutrient medium components at main cultivation stages of the genus Lonicera L. representatives was studied. Some cultivars showed the positive effect of substituting sucrose for glucose in the medium at the multiplication stage (Diana (30 or 40 g L⁻¹), Moskovskaya 23 (20 g L⁻¹) and Yugana (40 g L⁻¹)). When studying the influence of different auxin types on honeysuckle rhizogenesis, Solovey and Yugana preferred addition of 1.0 mg L⁻¹ IBA into the medium. During the research of influence of different auxin concentrations on L. caerulea rooting the increased number of rooted microshoots of Goluboy Desert was demonstrated with the increasing of IAA concentration (from 0.5 to 3.0 mg L⁻¹). Meanwhile, Diana and Yugana better rooted on the medium containing 1.0 mg L⁻¹ IAA but Zolushka reached its highest rooting percentage on the medium supplemented with 0.5 mg L⁻¹ IAA. The assessment of second-year plants undergone the whole cycle of clonal micropropagation was carried out 85% of these plants began to bear fruits.

1. Introduction

Lonicera L. genus (Honeysuckle) belongs to Caprifoliaceae family and includes about 200 species [1-2], widespread mostly in temperate regions of the Northern hemisphere [3]. Lonicera caerulea L. is claimed to be one of the highest in vitamins [4-6] and early ripening berry crops. In recent years L. caerulea as a berry crop became increasingly popular.

Tsitsin Main Botanical Garden of Russian Academy of Sciences (MBG RAS) in vitro gene bank of plants was formed in 1996 and it has been replenished until now. The in vitro bank contains 153 species and 1157 varieties and cultivars, belonging to 183 genera and 61 families. One tenth of the horticultural crop in vitro collection is represented by L. caerulea cultivars (30 cultivars) reflecting the most significant achievements of leading breeding centers. According to 2021 data the Russian State Registry of protected selection achievements includes 128 cultivars of edible honeysuckle [7].

Since the popularity of L. caerulea is rapidly increasing, the demand for berries and planting material of this crop at large horticulture companies and local farms is also increasing. Unlike the conventional propagation methods of honeysuckle (cuttings, etc.), in vitro propagation is able to obtain large amount of high-quality planting material in short period of time.

A lot of research institutes conduct the works on clonal micropropagation of honeysuckle. The micropropagation protocols for various Lonicera species have been published [8-11]. However, they are not always suited for the large-scale production of honeysuckle planting material because the culture conditions for this crop vary depending on the cultivar [12].
Therefore, the improvement of clonal micropropagation technique of valuable *L. caerulea* cultivars for obtaining large amount of genetically uniform plants is the aim of our research [13-14].

2. Materials and methods

The work was carried out in the Plant Biotechnology Laboratory of MBG RAS.

The following model honeysuckle cultivars were used in the research: Indigo Gem, Gzhelka, Goluboy desert, Diana, Dlinnoplodnaya, Zolushka, Knyaginya, Leonya, Moskovskaya 23, Solovey, Yugana.

The research methods were based on the generally accepted techniques of plant tissue culture [15] and techniques developed in the Laboratory of Plant Biotechnology of MBG [16].

For introduction into in vitro culture, the samples growing in the field collection of MBG were used as starting material. The primary explants were lateral buds of young shoots. As a sterilization scheme used: 2% Fundazole solution with an exposure of 10-15 minutes, 70% ethanol (C\(_2\)H\(_6\)O) for 30 seconds and 7% calcium hypochlorite (Ca(ClO)\(_2\)) added with 1-2 drops Tween-20 for 5-7 minutes depending on physiological condition of the plant.

The nutrient medium Murashige-Skoog (MS) [18] supplemented with 30 g L\(^{-1}\) of sucrose, 6.8 g L\(^{-1}\) agar and 0.5 mg L\(^{-1}\) 6-Benzylaminopurine (6-BAP) was used at the initiation stage. In some cases, it was necessary to cultivate explants on the medium supplemented with 0.25 mg L\(^{-1}\) Gentamicin for 10 days.

The modified MS medium supplemented with carbohydrates (glucose or sucrose) in concentrations of 20, 30 or 40 g L\(^{-1}\) and with 0.5 mg L\(^{-1}\) BAP (6-Benzylaminopurine) was used at the multiplication stage. MS medium supplemented with 30 g L\(^{-1}\) sucrose was used as control. After 5 weeks of subculture the following indicators were measured and calculated: height of shoot, cm; number of nodes; reproduction coefficient (Rc).

The half-strength MS medium supplemented with IAA (β- indole acetic acid) in concentrations of 0.5, 1.0, or 3.0 mg L\(^{-1}\) or with 1.0 mg L\(^{-1}\) IBA (β- indole butyric acid) was used at the rooting stage. MS medium supplemented with 1.0 mg L\(^{-1}\) IAA was used as control. After 3 weeks of subculture the following indicators were measured and calculated: length of roots, cm; rooting percentage.

The nutrient medium was sterilized using pressurized saturated steam (P=101 kPa) at 120°C for 20 minutes. The process of planting explants on a medium was carried out in a laminar box in accordance with the rules for working with sterile material [15].

The regenerants were cultivated under the following conditions: light intensity of 1.5-2.0 klux, 16-h photoperiod and temperature of 25±2°C.

For the adaptation of the regenerants to ex vitro condition the mix of peat, sand, topsoil and leavening agent (perlite) in 1:1:1:1 ratio. After 4 weeks of adaptation the height of shoots was measured.

All the experiments were repeated in triplicate, each with 10 explants.

ANOVA Test [18] was used for the data analysis. All the statistical analyses were performed using Microsoft Office Excel 2010.

3. Results and Discussion

As most researchers said the main role in development and optimization of plant clonal micropropagation techniques is played by plant genetic features, type of explant, nutrient medium composition and culture conditions [19-21].

The cultivars of in vitro gene bank were divided into 4 groups according to the morphogenetic potential (figure 1):

1. With low reproduction coefficient (Rc=3-5) (Aurora, Indigo Jem, Borealis Beauty, Borealis Blizzard);
2. With average reproduction coefficient (Rc=5-8) (Bakcharskiy Velikan, Galochka, Kamchadalka, Knyaginya, Leningradskiy Velikan, Morena, Ptashka);
3. With high reproduction coefficient (Rc=8-10) and normal microshoots (Vostorg, Gzhelka, Goluboy Desert, Gordost Bakchara, Lebedushka, Leonya, Moskovskaya 23, Solovey, Yugana);

4. With high reproduction coefficient (Rc>10) and thin microshoots (Volkhova, Diana, Dlinnoplodnaya, Zolushka).

The composition of the culture medium determines the in vitro growth of plants. Carbohydrate is a very important component in medium and its addition is essential for in vitro growth and development of plants. The regeneration potential of *Lonicera caerulea* depends on carbon source of the medium. When analyzing the impact of carbohydrate and its concentration on the reproduction coefficient we observed positive effect of substituting sucrose for glucose in medium composition on some cultivars: Diana (on 30 g L\(^{-1}\) and 40 g L\(^{-1}\)), Moskovskaya 23 (on 20 g L\(^{-1}\)) and Yugana (on 40 g L\(^{-1}\)) (figure 2).

**Figure 1.** The morphogenetic potential of *Lonicera caeruleae* cultivars in vitro: 1 – Borealis Blizzard; 2 – Kamechadalka; 3 – Moskovskaya 23; 4 – Zolushka
Thereby it was established the genetic features of studied cultivars should be considered during the selection of carbon source in medium composition for honeysuckle reproduction.

Rooting stage is the important part of clonal micropropagation. Correct choice of auxin and its concentration is attached considerable importance for effective rooting in vitro [22]. When studying the impact of auxins on induction of root formation cultivars Solovey and Yugana showed better rooting on the medium supplemented with 1.0 mg L$^{-1}$ IBA (figure 3-4).

**Figure 2.** Impact of carbohydrate and its concentration on the micropropagation of some *L.caerulea* cultivars (LSD$_{05}$=2.72)

**Figure 3.** Impact of auxin type on rooting of *L.caerulea* microshoots, % (P<0.05)

**Figure 4.** In vitro rhizogenes *L.caerulea* Yugana on the medium supplemented with: A – IAA (1.0 mg L$^{-1}$); B – IBA (1.0 mg L$^{-1}$)
Similar results were found in the study of Y.S. Zapolsky [23] and Z.K. Kadhim [24] where the highest rooting percentage was obtained using IBA in concentration of 1.0 and 1.5 mg L\(^{-1}\). However, most cultivars of our study showed no significant dependence of auxin type on the rooting. Callus formation was observed at the base of basal shoots when using IBA as an auxin which negatively affected on the further adaptation process. That is why IAA was chosen for further researches in \textit{L.caerulea} rooting.

The impact of auxin concentration on the honeysuckle rhizogenesis was studied. The results indicated that when IAA concentration increased (from 0.5 mg L\(^{-1}\) to 3.0 mg L\(^{-1}\)), the number of rooted micro-shoots of Goluboy Desert increased. Diana and Yugana showed better rooting on the medium supplemented with 1.0 mg L\(^{-1}\) IAA while Zolushka was preferable to root on the concentration of 0.5 mg L\(^{-1}\) (figure 5). Mass callus formation on the medium containing 3.0 mg L\(^{-1}\) IAA was observed. Mass callus would negatively affect the further adaptation of regenerants [25-26].

![Figure 5](image)

**Figure 5.** Effect of IAA concentration on rooting of \textit{L.caerulea} microshoots, % (P<0.05)

Adaptation is important and final stage of clonal micropropagation. It determines the efficiency of all the technique because plants get stressed during transferring to unsterile conditions which is accompanied by low viability and sometimes death of the planting material [27].

An optimal substrate is important for the adaptation of \textit{L. caerulea} regenerants. It most often includes peat, sand, perlite and topsoil in various combinations and ratios [29, 28].

The whole period of adaptation was 4-5 weeks. It was found that genetic features of the taxon influenced not only in vitro plant development but also its adaptation to aseptic conditions. All the honeysuckle regenerants were characterized by high viability and cultivar-specific growth dynamics (Table 1).

| Cultivar          | Height of the plant (cm) | Before adaptation | After adaptation |
|-------------------|--------------------------|-------------------|------------------|
| Diana             | 3.0±0.20                 | 13.88±4.37        |
| Moskovskaya 23    | 3.4±0.20                 | 7.95±2.92         |
| Yugana            | 2.5±0.20                 | 15.97±5.75        |

In general, \textit{L. caerulea} regenerants were characterized by high viability and stable growth and development after being adapted to ex vitro conditions.

We found that the plants undergone the whole cycle of in vitro propagation were ahead conventionally propagated plants in development and formed stronger aerial part. This is consistent with the work of Medvedeva T.V., et al [30]. Moreover, 85% of plantlets began to bear fruits in the second year after transferring to field (figure 6).
Figure 6. Plants bearing fruits in the second year after transferring to field: A- Vostorg; B- Diana

4. Conclusions
During the researches we optimized the clonal micropropagation technique of *Lonicera caerulea* L. Positive effect of substituting sucrose for glucose in medium composition at the multiplication stage was revealed for cultivars Diana (30 g L\(^{-1}\) and 40 g L\(^{-1}\)), Moskovskaya 23 (20 g L\(^{-1}\)) and Yugana (40 g L\(^{-1}\)). When studying the influence of different auxin types on honeysuckle rhizogenesis, preferred addition of 1.0 mg L\(^{-1}\) IBA into the medium was observed for Solovey and Yugana. During the research of influence of different auxin concentrations on *Lonicera* rooting the increased number of rooted microshoots of Goluboy Desert was demonstrated with the increasing of IAA concentration (from 0.5 to 3.0 mg L\(^{-1}\)). Meanwhile, Diana and Yugana showed better rooting on the medium containing 1.0 mg L\(^{-1}\) IAA but Zolushka reached its highest rooting percentage on the medium with IAA of 0.5 mg L\(^{-1}\) concentration. The genetic features of cultivars were found to have a great impact on plant development at the adaptation stage. 85% of plants undergone the whole cycle of in vitro propagation began to bear fruits in the second year after transferring to field.

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