

**ADAPTATION OF BLUE HONEYSUCKLE (*LONICERA CAERULEA* L.)
MICROPLANTS AFTER IN VITRO CULTURE UNDER DIFFERENT LIGHTING
CONDITIONS AND MYCORRHIZAL PREPARATIONS**

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Abstract

This study was conducted to identify the optimal lighting source in combination with mycorrhizal preparations for successful adaptation of honeysuckle microplants to non-sterile conditions of the varieties 'Gigant's Heart' and 'Jugana'. We tested two types of lighting: high-pressure sodium lamps (HPS) and light-emitting diodes (LEDs). The mycorrhizal preparations 'Kormilitsa', 'Aegis', and 'Great White Premium Mycorrhizae Plant Success' were added to the substrate for adaptation. Plant measurements were taken on the 42nd day of adaptation. The results showed that the honeysuckle plants studied ex vitro adapted effectively under full-spectrum white LED lamps combined with additional red and blue diodes (peak wavelengths: ~450 nm, ~660 nm), with the addition of the mycorrhizal preparations 'Aegis' or 'Great White Premium Mycorrhizae Plant Success' to the substrate. Moreover, in the honeysuckle plants of the 'Gigant's Heart' and 'Jugana' varieties studied ex vitro, the survival at the adaptation stage increased by 1.2 times, and the total root length by 1.6-2.0 times.

Math. Subject Classification: 62P10, 92C75.

Keywords: blue honeysuckle clonal micropropagation, adaptation stage, mycorrhizal preparations, High-pressure sodium lamps Light-Emitting Diode

1. Introduction

In recent years, there has been great interest in blue honeysuckle (*Lonicera caerulea* L.). The popularity of honeysuckle is due to the very early ripening of berries, which are ready for consumption in mid-June, 7-10 days before the beginning of strawberry ripening. Honeysuckle berries contain dry matter (13.2-16.4%), sugars (1.48-12.5%), organic acids (0.98-5.3%), pectins (1.1-1.6%), tannins and coloring agents (0.08-0.30%), as well as a large number of P-active compounds (1000-1856 mg%), represented by anthocyanins (410-1500 mg%), catechins (250-500 mg%), leucoanthocyanins (106-770 mg%) [2][17].

There is currently a need to expand the area of honeysuckle industrial plantations. Therefore, it is essential to improve methods for accelerated honeysuckle propagation to obtain sufficient quantities of high-quality planting material [4].

Research into effective methods for accelerated propagation using clonal micropropagation technology is particularly widespread. Clonal micropropagation is a method for mass and accelerated vegetative propagation of plants in vitro culture, which is widely used in the production of garden planting material. Currently, this technology requires the development of techniques to improve the survival rate of microplants during the adaptation phase and ensure the production of high-quality planting material with a closed root system [9].

Lighting plays a crucial role in the adaptation of microplants to non-sterile conditions, as it influences the morphological and physiological parameters of plant development. The spectral composition of light, as well as different parts of the spectral range, have a specific effect on the regulation of various morphogenetic and physiological processes [3][11].

High-pressure sodium lamps (HPS), which are characterized by high efficiency of emission in the red spectrum, are typically used for the adaptation and further growth of ex vitro plants. However, modern technologies offer the use of more energy-efficient Light-Emitting Diode (LED) lamps, which have the ability to adjust the spectral composition of the light, allowing for optimization of the conditions for the adaptation and further growth of ex vitro plants [6].

Currently, full-spectrum white LEDs combined with additional red and blue Light-Emitting Diodes are used in most cases. It is known that the emission spectra of red and blue LEDs coincide with the absorption spectra of plant photosynthetic pigments [13][15][20].

One area for improving clonal micropropagation technology is the expanded use of mycorrhizal fungi, which stimulate plant growth and development. Blue honeysuckle is known to be a facultative symbiont with mycorrhizal fungi, meaning it can form associations with mycorrhizae but does not necessarily require such interaction [21].

Arbuscular mycorrhizae are one of the most widespread plant-microbe symbioses in nature. This symbiosis involves the roots of 85-90% of all higher

plants and fungi belonging to the Glomeromycota family. The most common arbuscular mycorrhizae belong to the *Glomus* genus. These fungi supply plants with growth factors and hormone-like substances, promoting their mineral nutrition [1][10][19].

Arbuscular mycorrhizae play a vital role in helping plants to adapt to conditions where there are low levels of phosphorus, nitrogen and micronutrients available to them in the soil. This improves plant growth and increases resistance to stress and pathogens [8][22][23]

Therefore, the aim of our research was to identify the optimal light source in combination with mycorrhizal preparations for the successful adaptation of blue honeysuckle microplants to non-sterile conditions.

2. Materials and methods

2.1. Plant Material

The studies were conducted on the honeysuckle varieties 'Gigant's Heart' and 'Jugana'. 'Gigant's Heart' is a late-ripening, dessert variety with a tall, dense bush and barrel-shaped berries with an average weight of 2.5-3.5 g. 'Jugana' is a mid-season, all-purpose variety with a medium-sized, dense bush and pitcher-shaped, pleated berries with an even ridge at the top, blue in color with a strong waxy coating and medium-thick skin.

The experiments were carried out threefold in 2024-2025 at the Russian State Agrarian University, Moscow Timiryazev Agricultural Academy, in the department of biotechnology and berry crops of the Edelstein Educational Scientific and Production Center for Horticulture and Vegetable Growing.

2.2. In vitro Culture Conditions

At the stage of multiplication explants of blue honeysuckle ‘Gigant's Heart’ и ‘Jugana’ were planted on a nutrient medium with mineral salts according to Quoirin and Lepoivre (Quoirin, 1977) enriched with the following substances: thiamine hydrochloride, pyridoxine hydrochloride, nicotinamide - 0.5 mgL⁻¹; glycine - 1 mgL⁻¹, mesoinositol - 100 mgL⁻¹, 6-Benzylaminopurine - 0.2 mgL⁻¹, sucrose - 30000 mgL⁻¹, plant micropropagation agar-agar – 6000 mgL⁻¹.

At the stage of rooting explants were placed on a nutrient medium with mineral salts according to the Quoirin and Lepoivre [18] for the stage of rooting, which contained ½ part of macroelements and microelements enriched with the following substances: thiamine hydrochloride, pyridoxine hydrochloride, nicotinamide - 0,5 mgL⁻¹; indole-3-butyric acid - 0,5 mgL⁻¹, sucrose - 15000 mgL⁻¹, agar-agar - 6000 mgL⁻¹.

The pH of the medium was adjusted to 5.8 before autoclaving and the medium was poured into glass culture vessels with a volume of 200 mL, while each vessel was filled with 30 mL of the medium. Further vessels with the nutrient medium were sterilized using pressurized saturated steam (p = 101 kPa) at 120 °C for 20 min. The process of planting the explants on a medium was carried out in a laminar box according to the rules for working with sterile materials.

At the multiplication and rooting stage, the plants were maintained for 60 days in a growth room under fluorescent lamps (46.25 μMm⁻²s⁻¹ PPFD) and a 16/8 h (light/dark) photoperiod, at 20 ± 2°C.

2.3. Experimental Treatments

At the end of the rooting stage, the transplant-ready microplants were thoroughly washed off the nutrient medium.

And planted for acclimation to nonsterile conditions in plastic cassettes with a cell diameter of 4 cm, which had been preliminarily filled with a mixture of enriched peat brand Agrobalt-C (Pindstrup, Russia) which includes low-decomposition sphagnum peat, dolomite flour and complex mineral fertilizer (N – 150 mgL⁻¹, Ca – 120 mgL⁻¹, Mg – 30 mgL⁻¹, P₂O₅ – 150 mgL⁻¹, K₂O - 250 mgL⁻¹, pH 5.5-6.6) with perlite in the ratio of 3:1.

Before planting microplants, the following was added to the substrate: the mycorrhizal preparations ‘Kormilitsa’ (BashInkom, Ufa, Russia), Aegis (BioTekhnologii, Moscow, Russia) and Great White Premium Mycorrhizae Plant Success (Plant Success - USA) were used (Table 1).

Table 1. Composition, method of application and concentration of mycorrhizal preparations

Mycorrhizal preparations	Composition	Application method	Concentration (g/L)
Mycorrhiza forming additives			

Kormilitsa	Granular mycorrhizae containing mycelium and spores of fungi of the genus <i>Glomus</i> , colonised root fragments, natural organic fillers, humic substances	Substrate application	6
Aegis	Aegis Microgranules combination of two highly effective strains of mycorrhizal fungi <i>Glomus intraradices</i> , <i>Glomus mosseae</i> , growth promoting Rhizobacteria	Substrate application	5
Great White Premium Mycorrhizae Plant Success	Powdered preparation containing fungi (endomycorrhizal fungi of the genus <i>Glomus</i> , <i>Paraglomus brazilianum</i> , <i>Gigaspora margarita</i> ; ectomycorrhizal fungi: <i>Pisolithus tinctorious</i> , <i>Rhizopogon luteolus</i> , <i>Rhizopogon fulvigleba</i> , <i>Rhizopogon villosullus</i> , <i>Rhizopogon amylopogon</i> , <i>Trichoderma koningii</i> , <i>Trichoderma harzianum</i> , <i>Saccharomyces cerevisiae</i>). The preparation contains various species of bacteria (Bacteria <i>Azotobacter chroococcum</i> and bacteria of the genus <i>Bacillus</i> , <i>Paenibacillus durum</i> , <i>Paenibacillus polymyxa</i> , <i>Pseudomonas aureofaciens</i> , <i>Pseudomonas fluorescens</i>)	Substrate application	1

The honeysuckle microplant cassettes were placed in a mini-greenhouse, where the air humidity was maintained for 2 weeks at a level of 80-90%, temperature at $20 \pm 5^\circ\text{C}$, under photoperiod and irradiance with day/night 16/8.

The plants were exposed to different types of illumination: High-pressure sodium lamps (HPS) - Osram Plantastar 400 W E40 Data Sheet (power 400 Watts, PPFD $725 \mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$, voltage: 100 Volt, Color Temperature 2000 Kelvin, Luminous Flux 56500 Lumens) (Figure 1). And Light-Emitting

Diode (LED) Lamp Rootster 225W (Board Lite) (power 225 Watts, PPFD $1610 \mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$, Spectrum range: Full-spectrum white LEDs combined with additional red and blue diodes (peak wavelengths: $\sim 450 \text{ nm}$, $\sim 660 \text{ nm}$)) (Figure 2).

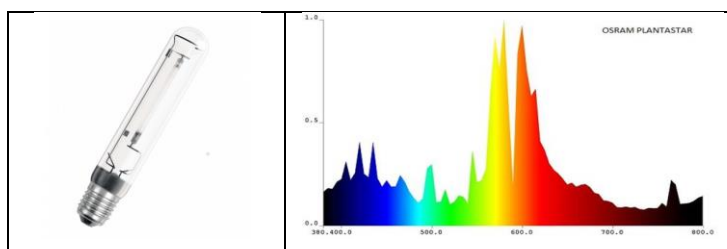


Figure 1. Spectrum Osram Plantastar 400 W E40 Data Sheet

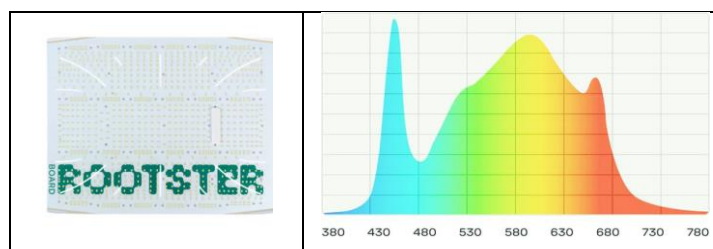


Figure 2. Spectrum LED Lamp Rootster 225W

Table 2. Experimental treatment variants

Treatment variant	types of illumination		Mycorrhizal preparations		
	High-pressure sodium lamps (HPS)	Light-Emitting Diode (LED)	Kormilitsa	Aegis	Great White Premium Mycorrhizae Plant Success
blue honeysuckle cv. 'Gigant's Heart' (GH)					
V1(Control 1)	+				
V 2	+		+		
V 3	+			+	
V 4	+				+
V 5(Control 2)		+			
V 6		+	+		
V 7		+		+	
V 8		+			+

During the acclimation period, beginning from the 14th day after the transfer to ex vitro conditions, the humidity was reduced step by step by daily ventilation of the greenhouse. On the 42th day of the acclimation stage, the parameters of the microplants' growth and development were recorded.

2.4. Data Analysis

The experiments were repeated three times, with fifteen plants used for each repetition. The statistical data processing was first performed using two-way analysis of variance (ANOVA). When determining the significant difference between the experimental variants, the least significant difference (LSD) at a level of $p < 0.05$ was used.

3. Results

The results of the study obtained on the 42nd day of adaptation of experimental plants of the honeysuckle variety "Gigant's Heart" showed a statistically significant advantage of adding mycorrhiza preparations (Factor A) to the substrate. Ex vitro plants under Light-Emitting Diode showed the best survival and development indicators. At the same time, survival in variants

with mycorrhiza preparations was 94.4-97.8% compared to 75.0-80.5% in plants under High-Pressure Sodium lamps.

At the same time, only types of illumination (Factor B) had a significant effect on shoot length and average number of roots, and the advantage of Light-Emitting Diode lighting was revealed.

The best morphometric indicators of the total length of the roots were observed in ex vitro plants in variants with mycorrhizae “Aegis” and “Great White Premium Mycorrhizae Plant Success”. In the variants with High-Pressure Sodium lamps illumination, the total length of the roots was 39.9-4.6 cm compared to 24.8 cm in the control without mycorrhiza, and in the variants with Light-Emitting Diode illumination, it was 56.1-63.7 cm compared to 31.6 cm without mycorrhiza (Table 3, Figure 5, 6).

Table 3. effect of adding different types of illumination and mycorrhizal preparations on the morphometric indicators of the development of experimental blue honeysuckle plants cv. ‘Gigant's Heart’

Mycorrhizal preparations (Factor A)	Survival, %	Average shoots length, cm	Average number of roots, pcs.	The total length of the roots, cm
illumination High-Pressure Sodium lamps (HPS) (Factor B)				
V1 (Control 1)	66.6	5.6±0.92*	6.4±1.01	24.8±4.17
V 2	75.0 ^{a**}	5.8±0.74	6.6±1.12	31.7±4.43 ^a
V 3	80.5 ^a	5.7±0.87	7.1±0.94 ^a	39.9±3.40 ^a
V 4	77.7 ^a	5.7±.52	7.2±0.96 ^a	45.6±5.35 ^a
illumination Light-Emitting Diode (LED) (Factor B)				
V 5 (Control 2)	80.5 ^b	6.1±0.77 ^b	8.2±1.34 ^b	31.6±5.77 ^b
V 6	94.4 ^{a,b}	6.0±0.83 ^b	7.8±1.17 ^b	40.2±3.74 ^{a,b,ab}
V 7	97.2 ^{a,b}	6.2±0.87 ^b	8.7±1.41 ^b	56.1±4.25 ^{a,b,ab}
V 8	97.8 ^{a,b}	6.3±0.96 ^b	9.6±1.86 ^{a,b}	63.7±5.35 ^{a,b,ab}
LSD ₀₅ a	4.82	Fe < Ft	0.60	2.33
LSD ₀₅ b	3.41	0.27	0.42	1.64
LSD ₀₅ ab	Fe < Ft***	Fe < Ft	Fe < Ft	3.29

The least significant difference (LSD) $p < 0.05$ was calculated by two-way variance analysis; * the results were expressed as mean _ standard deviation (SD); ** “a, b, ab”- the difference between the average with the control was significant based on the comparison of the differences between the average with LSD at a 5% significance level: “a”- by factor “a” (Mycorrhizal preparations), “b”- by factor “b” (types of illumination), “ab” -in the combination of factors; *** “Fe < Ft”- F empirical < F theoretical, not proven difference between averages with LSD at 5% significance level.

Statistical analysis of the data revealed that the factors studied have different effects on the indicators considered. Thus, the survival rate of honeysuckle microplants of the “Gigant's Heart” variety is influenced to a greater extent (60.9%) by types of illumination (Factor B). And the total length of the roots is influenced to a greater extent (60.6%) by mycorrhizal preparations (Factor A).

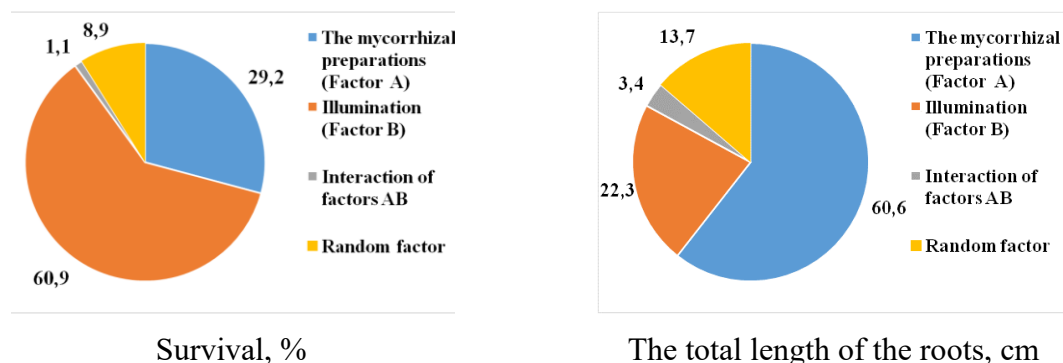


Figure 3. Share of studied factors (factor A- mycorrhizal preparations, factor B – types of illumination) influence on survival rate and total root length of honeysuckle plants blue honeysuckle plants cv. ‘Gigant's Heart’

The 'Jugana' variety exhibited lower survival and morphometric parameters for ex vitro adapted plants. Results obtained after 42 days of adaptation also showed a statistically significant advantage for the factors studied. The best survival was demonstrated by ex vitro plants under LED, while the survival in variants with mycorrhiza preparations was 80.5-83.3% compared to 66.6-72.2% for plants under high-pressure sodium lamps.

However, only the type of LED illumination (Factor B) significantly affected shoot length and average root number. The best morphometric indicators of the total length of the roots were demonstrated by ex vitro plants in the variants with mycorrhiza ‘Aegis’ and ‘Great White Premium Mycorrhizae Plant Success’. In the variants with LED illumination, the total length of the roots was 42.2-52.9 cm compared to 25.5 cm in the control without mycorrhiza (Table 4, Figures 5, 6).

Table 4. The effect of adding different types of illumination and mycorrhizal preparations on the morphometric indicators of the development of experimental blue honeysuckle plants cv. ‘Jugana’ on the 42th day of cultivation

Mycorrhizal preparations (Factor A)	Survival, %	Average shoots length, cm	Average number of roots, pcs.	The total length of the roots, cm
Illumination High-pressure sodium lamps (HPS) (Factor B)				
V1(Control 1)	58.3	4.9±3.69*	4.4±0.95	20.3±3.96
V2	66.6a**	4.7±7.60	4.8±0.97	25.4±7.60a
V3	69.4a	5.0±4.40	5.2±0.85a	31.7±4.40a
V4	72.2a	4.9±7.70	5.4±0.90a	36.6±7.70a
Illumination Light-Emitting Diode (LED) (Factor B)				
V5 (Control 2)	69.4b	5.2±4.91b	6.2±1.34b	25.5±4.91b
V6	80.5a,b	5.1±4.59 b	5.5±1.12b	33.2±4.59a,b,ab
V7	80.5a,b	5.4±7.70b	6.7±1.33b	42.2±7.70a,b,ab
V8	83.3 a,b	5.5±5.90b	7.1±1.03a,b	52.9±5.90a,b,ab

LSD05 a	4.41	Fe < Ft	0.51	2.87
LSD05 b	3.12	0.34	0.36	2.03
LSD0505 ab	Fe < Ft***	Fe < Ft	Fe < Ft	4.06

The least significant difference $p < 0.05$ was calculated by two-way variance analysis; * the results were expressed as mean _ standard deviation (SD); ** “a, b, ab”- the difference between the average with the control was significant based on the comparison of the differences between the average with LSD at a 5% significance level: “a”- by factor “a” (Mycorrhizal preparations), “b”- by factor “b” (types of illumination), “ab” -in the combination of factors; *** “Fe < Ft”- F empirical < F theoretical, not proven difference between averages with LSD at 5% significance level.

Statistical analysis of the data also revealed that the studied factors had different effects on the measured parameters for the honeysuckle cultivar 'Jugana'. For example, illumination types (Factor B) had a greater impact on microplant survival (48.6%), while mycorrhizal preparations (Factor A) had a greater impact on the total root length (50.7%).

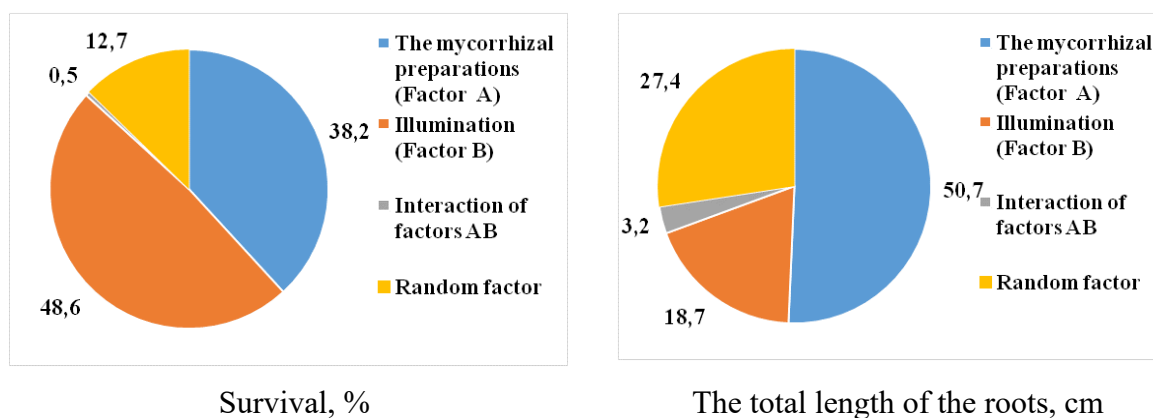


Figure 4. Share of studied factors (factor A- mycorrhizal preparations, factor D – types of illumination) influence on survival rate and total root length of honeysuckle plants blue honeysuckle plants cv. 'Jugana'

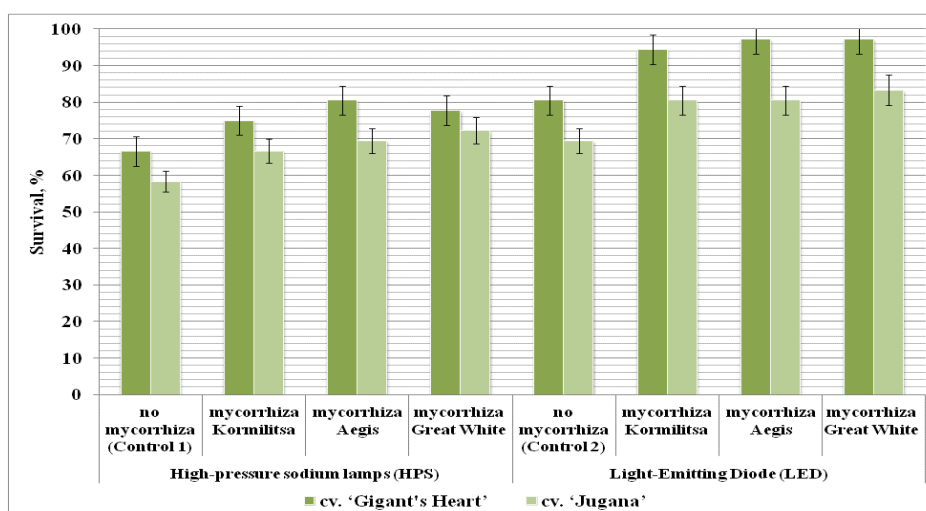


Figure 5. Survival (%) of the development of experimental blue honeysuckle plants depending on types of illumination and mycorrhizal preparations

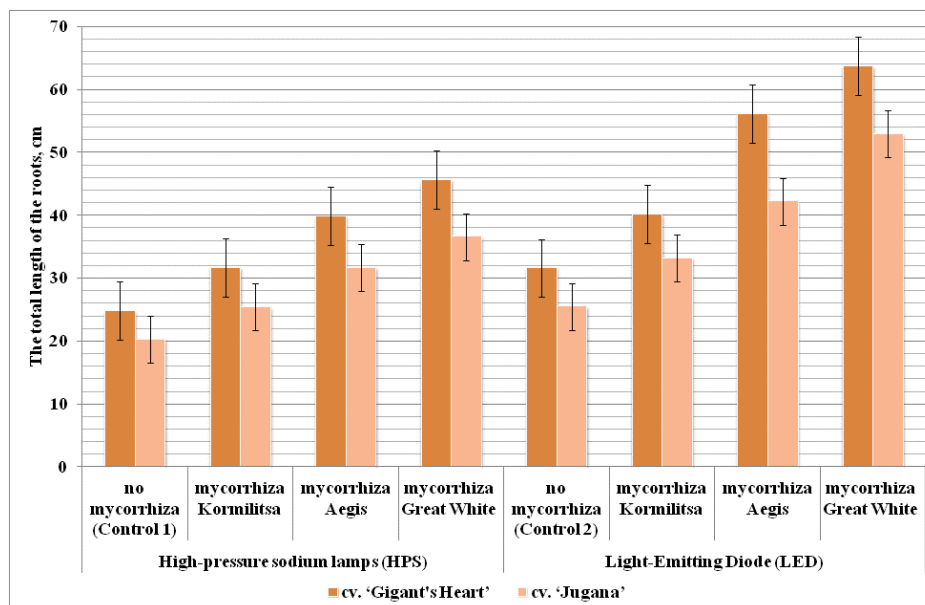


Figure 6. The total length of the roots (cm) of the development of experimental blue honeysuckle plants depending on types of illumination and mycorrhizal preparations

4. Discussion

The adaptation stage of microplants to non-sterile conditions is key in clonal micropropagation technology. When regenerated plants are transferred from sterile *in vitro* conditions to non-sterile *ex vitro* conditions, they experience severe stress, and plant losses can often reach 80-90%. Therefore, during the adaptation stage, it is important to create all the conditions conducive to effective microplant survival (Kolbanova et al., 2020).

High-pressure sodium (HPS) lamps are known to emit highly efficient red light, which contributes to improved photosynthetic activity in *ex vitro* plants. However, this type of lamp lacks blue light (Matsuda et al., 2004; Hogewoning et al., 2012; Olle et al., 2013; Guo et al., 2016).

However, as plants primarily absorb blue and red light, it is possible that they could be successfully adapted using full-spectrum white LEDs combined with additional red and blue light-emitting diodes (LEDs) (Tang et al., 2009; Semenova et al., 2022).

The blue spectrum of LED lamps stimulates root formation and activates phototropins, which regulate stomatal function and increase a plant's resistance to water stress (Dorokhov et al., 2022).

This is probably why our research has shown that the *ex vitro* honeysuckle plants of the 'Gigant's Heart' and 'Jugana' varieties are more effectively adapted to full-spectrum white LEDs combined with additional red and blue diodes (peak wavelengths: ~450 nm, ~660 nm).

One promising method of increasing the survival of microplants during the adaptation phase is to enrich the substrate with arbuscular mycorrhizal fungi. Although blue honeysuckle can grow successfully without mycorrhizae, mycorrhizal fungi increase total root length and enhance water and dissolved mineral nutrient absorption (Renker et al., 2003; Al-Karaki et al., 2004; Li et al., 2006; Shi et al., 2013).

This is probably related to the proven effectiveness of options with mycorrhizal preparations such as 'Aegis' or 'Great White Premium Mycorrhizae Plant Success'.

The 'Aegis' preparation is a combination of two highly effective strains of mycorrhizal fungi (*G. intraradices* and *G. mosseae*) and growth-promoting rhizobacteria. As a result, the 'Gigant's Heart' and 'Jugana' honeysuckle varieties tested *ex vitro* demonstrated a 1.2-times increase in survival during the adaptation phase, and a 1.6-1.8 times increase in total root length.

The best results of the experiment were obtained when adding 'Great White Premium Mycorrhizae Plant Success' to the substrate, which includes: endomycorrhizal fungi of the genus *Glomus*, *Paraglomus brazilianum*, *Gigaspora margarita*; ectomycorrhizal fungi: *Pisolithus tinctorius*, fungi of the genus *Rhizopogon*, *Trichoderma koningii*, *Trichoderma harzianum*, *Saccharomyces cerevisiae*, and various species of bacteria (*Azotobacter chroococcum* and bacteria of the genus *Bacillus*, *Paenibacillus durum*, *Paenibacillus polymyxa*, *Pseudomonas aureofaciens*, *Pseudomonas fluorescens*). As a result, the 'Gigant's Heart' and 'Jugana' honeysuckle plants studied *ex vitro* showed a 1.2-times increase in survival during the adaptation phase and a 1.6-2.0-times increase in total root length.

These results will be useful for adapting blue honeysuckle microplants to non-sterile conditions.

5. CONCLUSIONS

The obtained results allowed us to improve the technology for adapting blue honeysuckle microplants to non-sterile conditions using various types of illumination and mycorrhizal preparations.

The results obtained on the 42nd day of the adaptation stage showed that the *ex vitro* honeysuckle plants of the 'Gigant's Heart' and 'Jugana' varieties adapted more effectively under LED lamps with the addition of the mycorrhiza preparation 'Aegis' or 'Great White Premium Mycorrhizae Plant Success' to the substrate. The survival of 'Gigant's Heart' microplants was 92.7-97.8% compared to 80.5% in the control without mycorrhiza, and 80.5-83.3% for 'Jugana' compared to 69.4% in the control.

The results of the study highlight the importance of adding mycorrhiza preparations to the substrate before adaptation of honeysuckle microplants and subsequent adaptation under LED illumination, which allows obtaining better-quality *ex vitro* adapted plants for further propagation in nurseries.

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