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THE EFFECT OF LOW-INTENSITY COHERENT RADIATION ON THE EFFICIENCY OF RHIZOGENESIS OF PLANTS OF THE GENUS *Rubus* L.

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ABSTRACT

The article presents the results of research on the effect of laser irradiation on rooting microcuttings of plants of the genus *Rubus*. The effect of helium-neon (λ = 632.8 nm) and red semiconductor laser (λ = 655 nm) on the frequency of rooting, the intensity of root formation, the growth of roots and shoots were studied. It has been established, that the use of LCR in 1,5-2,2 times increased the efficiency of rhizogenesis, accelerated the formation of roots, and growth of shoots.

Keywords: *Rubus*; clonal micropropagation; laser irradiation; rhizogenesis process; microcuttings.

INTRODUCTION

The use of coherent laser radiation offers wide possibilities for stimulating morphogenetic processes *in vitro*. Despite the fact that coherent radiation sources of almost the entire visible part of the spectrum can stimulate the growth and development of plants, the optimal wavelengths are those that correspond to the maximum absorption of phytochromes. The form that stimulates the physiological activity of plants is $ΦX_{730}$. Its high concentration is supported by radiation in the spectral range of 600 to 690 nm [1]. Thus, it is promising to use helium-neon (λ = 632.8 nm) and red semiconductor lasers (λ = 655 nm) to irradiate plants to stimulate their growth, reproduction, and root formation [2].

The response of plant organisms to laser radiation depends on a number of factors that must be considered in a biotechnological experiment. The most important is the genotypic characteristics of the plants, the physiological state of the explants, cultivation conditions, and exposure parameters.

Numerous experiments have found that the response of plants to irradiation of various durations is nonlinear multimodal in nature with maxima and minima of the stimulation effect. Different researchers report that laser irradiation of tissues *in vitro* increases the regenerative activity of meristems and improves the rhizogenesis of berry crops [3,4,5,6].

In order to increase the efficiency of the rhizogenesis of plants of the genus *Rubus*, a series of experiments was carried out on the irradiation of explants at the rooting stage using different types of lasers.

MATERIALS AND METHODS

The experiments were carried out on microcuttings of cultivated *in vitro* blackberry plants of the Chester Thorless and Black Satin varieties, Loganberry and Boysenberry raspberry hybrids, everbearing raspberries of Oranzhevoe chudo, Rubinovoe ozherelie, and Brilliantovaia varieties.

At the stage of microcutting rooting, the mineral base of the nutrient medium MS [7] or QL [8,9] was used with a 2-fold decrease in the concentration of macrosalts and with the addition of 20 g/l of sucrose, 50 mg/l of mesoinositol, a complex of vitamins according to Murashige-Skoog, 8 g/l agar. The rooting medium for MS_{rm} or QL_{rm} contained 0.5 or 1.0 mg/L of IBA. All growth regulators and vitamins were sterilized by ultrafiltration through Millipore filters (0.22 µm pore diameter) and added to the media after autoclaving.

For rooting, shoots were used that reached 1.5-2.0 cm in length on the propagation medium.

Sterile microcuttings were treated with GN-40 helium-neon laser (632.8 nm) and semiconductor HLDPM12-655-10HJ (655 nm) radiation with a light flux density of 2 W/m^2 and a light spot diameter of 14 cm at various exposures (30, 60, 120, 240, 480, 960 s) 3-4 days after planting on the rooting medium directly in the culture vessels. The calculation of a longer period of irradiation was performed by doubling the previous time. For uniform irradiation, the flask with explants was placed on a stand rotating at a constant speed.

Shoots were subcultured in 250 ml wide-necked conical flasks and 400 ml glass jars. The culture vessels were covered with thin aluminum foil and sealed with Parafilm tape. After irradiation, the vessels with plants were placed in the culture room and for the testing integrity, were separated by partitions. Thus, the control and experimental plants were in the same cultivation conditions but were also optically isolated from each other.

The plants were cultured in a specially equipped culture room at 16-hour daylight with the illumination of 1600-1800 lux (Osram L36W Cool Daylight fluorescent lamps), air temperature 24±2°C and air humidity 50-60%.

The number of rooted cuttings, the number of roots formed and their length was considered. Statistical data processing was performed using Microsoft Excel.

RESULTS AND DISCUSSION

The results of our studies showed that laser irradiation of microcuttings can significantly affect the efficiency of rhizogenesis and the growth of shoots of cultivated plants. The effect of laser radiation was most pronounced on crops with lower morphogenetic potential. There are certain difficulties at the stage of the rooting of microcuttings of everbearing raspberry *in vitro*. In general, the process of rooting of microcuttings of everbearing raspberry is quite long (up to two months). As a result, 2–4 long roots with second-order roots are formed on the rooted micro-cutting. Therefore, various methods of stimulating rhizogenesis are used, both with auxin active drugs, biological products, and by regulating the spectral composition of light at the rooting stage [10,11,12,13].

In our studies, the use of LCR 1.5–2.2 times enhanced the efficiency of rhizogenesis of everbearing raspberries of the Oranzhevoe chudo variety (Fig. 1). Under optimal exposure, rooting was significantly accelerated. After six weeks of cultivation, the rooting rate of control was 33.3%, with a helium-neon laser irradiation of 72.7% (exposure 60 s), 61.3% (exposure 240 s), with a semiconductor laser irradiation 63.6% (exposure 60 s), 66.6% (exposure 240 s). The best versions of the experiment showed an increase in the number of roots per rooted microcuttings and faster growth of roots. So, the average root length under helium-neon laser irradiation at an exposure of 60 s was 4.7 cm, at an exposure of 120 s - 4.5 cm, under semiconductor laser irradiation - 4.7 cm, at exposures of 60 s and 480 s compared to 3.5 cm in intact control.

A more powerful development of the root system led to faster growth of shoots of irradiated microcuttings already at the rooting stage (Fig. 2).

Similar results were obtained on other varieties of everbearing raspberries. Thus, we managed to increase the rooting rate of microcuttings of everbearing raspberries of the Rubinovoe ozherelie variety on MS_{rm} medium with 1.0 mg/L IBA from 55.6% in the control to 84.4% in subjects exposed to a helium-neon laser (exposure time 120 s) and 80.4% (exposure 960 s). The number of roots per rooted microcuttings increased respectively from 2.0±0.2 pcs. up to 2.9±0.3 and 3.0±0.3 pcs. respectively. In the best versions of the experiments, well-developed microcuttings of everbearing raspberries were obtained, which could be planted in the soil for adaptation.

Laser stimulation also showed a positive effect on crops with a good rooting ability *in vitro*.

Thus, the rooting rate of Black Satin blackberries on QL_{rm} medium with 0.5 mg/L IBA after a month of cultivation with a 2-minute exposure to helium-neon laser irradiation was 75.9% compared to 46.1% in the control. The stimulation effect was achieved both using a helium-neon laser and a semiconductor laser, however, in each case, the different exposure was the most optimal (Fig. 3). The rooting rate of the Loganberry hybrid of raspberry and blackberry with the 0.5 mg/L IBA with a 16-minute exposure to helium-neon laser radiation was 46.7%, with a 4-minute exposure to a semiconductor laser radiation – 42.1%, compared to 27.8% in control. Laser radiation significantly affected the quantitative and qualitative indicators of the root system. Optimal irradiation ensured a powerful root system in plants without changing the composition of the nutrient medium only due to the activation of the phytochrome mechanism of plants and, as a consequence, increased synthesis of endogenous auxin.

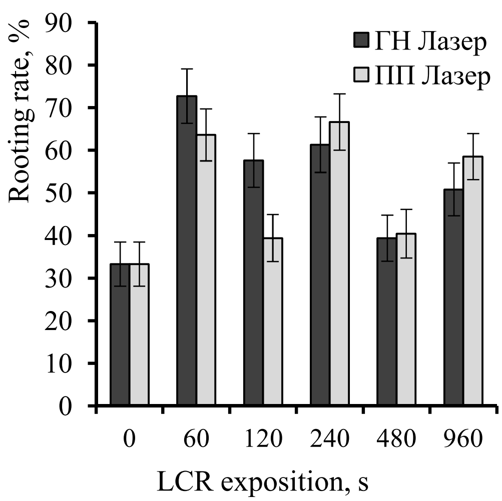

Fig. 1. The effect of laser irradiation on the rooting of everbearing raspberry, Oranzhevoe chudo variety

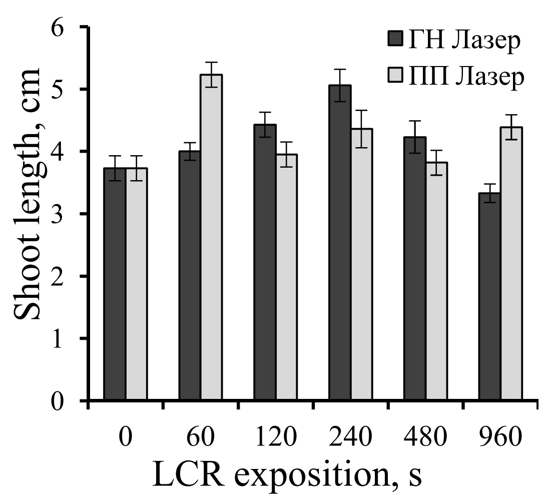

Fig. 2. The effect of laser irradiation on the growth of shoots of everbearing raspberry, Oranzhevoe chudo variety

ГТ лазер	HN laser
ПП лазер	SC laser

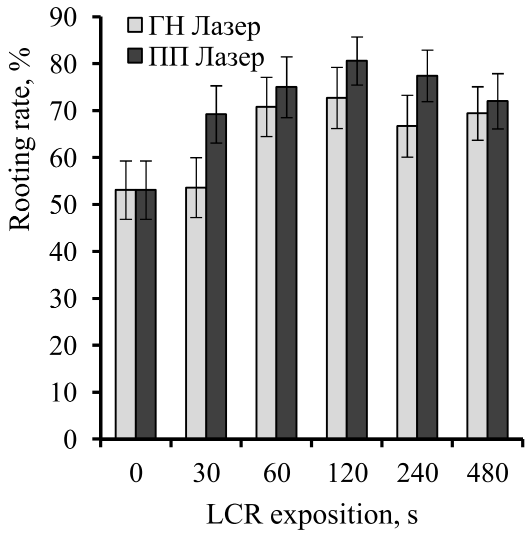

Fig. 3. The effect of laser irradiation on the rooting of Black Satin blackberry

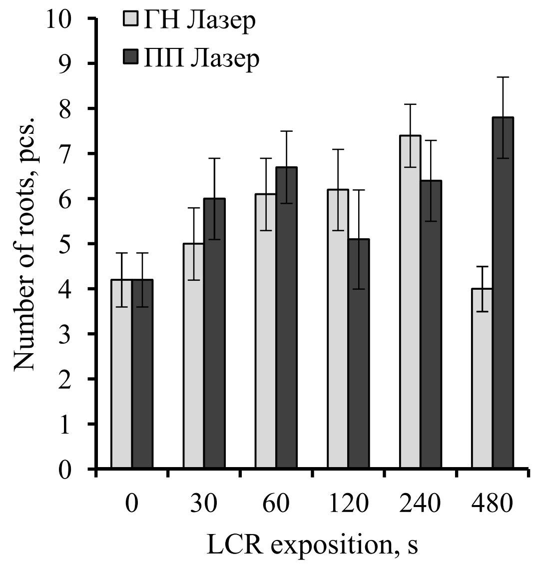

Fig. 4. The effect of laser irradiation on the root formation of Black Satin blackberry

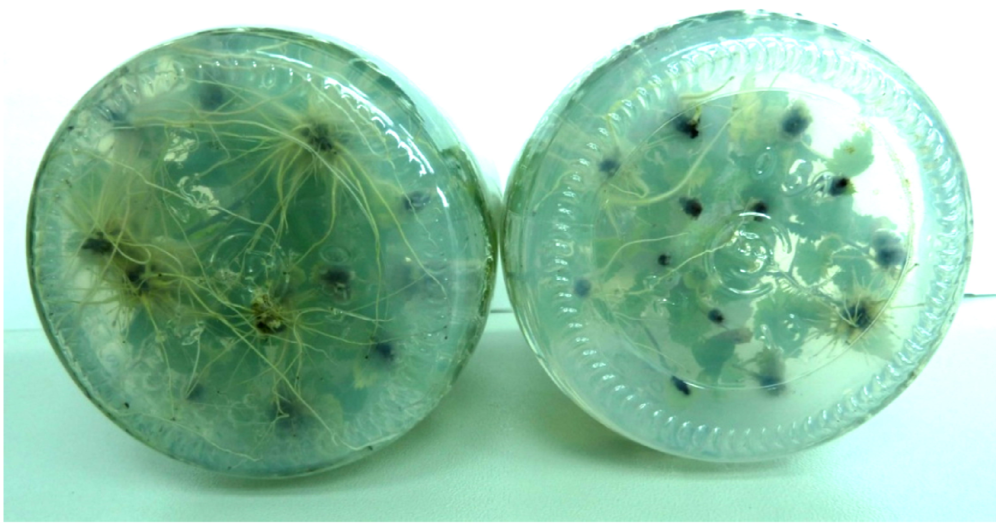

Fig. 5. Chester Thornless blackberry rhizogenesis after irradiation of microcuttings (right - control, left - helium-neon laser, exposure 240 s.):

ГТ лазер	HN laser
ПП лазер	SC laser

For example, the average number of roots in Black Satin blackberry microcuttings on QL_{rm} medium with 0.5 mg/L IBA after a 2-minute exposure to a helium-neon laser at a power density of 2 W/m^2 increased to 5.9 ± 0.6 pcs. and with a 4-minute exposure – up to 6.0 ± 0.6 pcs. compared with 3.9 ± 0.4 pcs. in control. The average number of roots in Black Satin blackberry microcuttings irradiated with a helium-neon laser on a QL_{rm} medium with 1 mg/L IBA after a 4-minute exposure and a power density of 2 W/m^2 increased to 7.4 ± 0.7 pcs. compared with 4.2 ± 0.6 pcs. in control (Fig.

4). Irradiation with a semiconductor laser for 60 and 480 seconds ensured the number of roots equal to 6.7 ± 0.8 pcs. and 7.8 ± 0.9 pcs. respectively. Differences with control are statistically significant (P> 0.98).

Under optimal irradiation, the root system of irradiated plants was characterized by significantly better development compared to control plants. A similar result was obtained on different varieties of blackberries (Fig. 5).

The laser-induced stimulation of the root system both improved rhizogenesis and led to a more intensive growth of berry crop shoots already at the rooting stage. Moreover, depending on the type of laser used, the same exposure provided a different degree of effect.

CONCLUSION

Thus, laser coherent radiation has a positive effect on the growth and development of explants *in vitro*. The use of IBA at the stage of rhizogenesis significantly stimulates the process of rooting microcuttings *in vitro* and contributes to an increase in the number and length of roots and shoot growth. The stimulating effect was obtained both using a helium-neon laser and a semiconductor. The degree of the achieved effect non-linearly depends on the duration of exposure. Optimization of the laser treatment regimens requires using different exposure durations: 4-6 exposures.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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