ORIGINAL ARTICLE

The research of clonal micropropagation efficiency of *Schisandra chinensis* under the influence of low-intensity coherent radiation

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Abstract

Aim: The present study reveals the results of an investigation on the effect of low-intensity coherent radiation (LCR) on the efficacy of clonal micropropagation of medicinal plant - *Schisandra chinensis* (Turcz.) Baill. **Experimental:** Cultivation of plants *in vitro*, a mineral base of the nutrient medium was used with the addition of 30 g/l sucrose, 100 mg/l mesoinositol, 250 mg/l casein hydrolyzate, 8 g/l agar and vitamin complex of Murashige-Skoog. During microcuttings, rooting macrosalts and sucrose concentration reduced to half; indole butyric acid at a concentration kept at 1.0 mg/l. Sterile microcuttings were treated with radiation from a helium-neon laser, GN-40 (wavelength 632.8 nm, power density 2 W/m^2), and semiconductor HLDPM12-655-10HJ (wavelength 655 nm, power density 2 W/m^2) at various exposures (30, 60, 120, 240, 480, 960 s) on 3-4 days after their planting into the propagation medium or rooting directly in the culture bottles. **Result and Discussion:** It has been established, that the use of LCR at the stage of rhizogenesis significantly stimulates the process of microcuttings rooting *in vitro* and promotes an increase in the number and length of roots and growth of shoots. The selection of *in vitro* conditions and irradiation treatment of the obtained *S. chinensis* microshoots by helium-neon laser, during the propagation stage, allow to increase the number of viable explants of this culture from 60.0% ± 6.3% in the control sample, up to 90.9% ± 3.5% in the test sample with 120 s exposure of irradiation. With this exposure, the share of microshoots with a net reproduction more than 1, increased from 44.4% to 75.5%. **Conclusion:** Due to low rates of reproduction and rooting, a slow growth rate of shoots and roots, latent bacterial infections, significantly reduce the method clonal micropropagation for *S. chinensis*. Laser coherent radiation stimulates the process of microcuttings rooting *in vitro* at the stage of rhysogenesis of *S. chinensis* (Turcz.) Baill leads to increase in number and length of root and shoot system.

Key words: Clonal micropropagation, laser irradiation, microcuttings, rhysogenesis process, *Schisandra chinensis*

INTRODUCTION

Schisandra chinensis (Turcz.) Baill belongs to promising medicinal plants, and it is used by the pharmaceutical industry. Preparations, obtained from this plant, are able to activate the reflex activity of the central nervous system, stimulate the cardiovascular system, and stimulate the function of respiration. Pharmaceutical industry is interested in obtaining of standard raw materials from medicinal species and plant varieties,[1,2] especially those, that have a broad spectrum of action, such as *S. chinensis*.

One of the most promising ways of propagation of appreciable varieties of *S. chinensis* is the method of clonal micropropagation. However, the low rates of reproduction and rooting, a slow growth rate of shoots and roots, latent bacterial infections, significantly reduce the possibility of its use.[3]

One of the methods that can significantly increase the efficiency of plant propagation *in vitro* is the use of laser irradiation. Low-intensity coherent radiation (LCR) is an effective photoregulatory factor, capable of increasing the functional activity of plants (Budagovsky, 2008).[4] Studies, conducted

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by different authors on representatives of the genus *Rubus*, actinidia, and other cultures, show that laser irradiation of tissues *in vitro* promotes an increase of the meristems regenerative activity and an improvement of plant rhizogenesis.[5,6]

The purpose of the investigation is to study the influence of laser irradiation of *S. chinensis* explants on the efficacy of their clonal micropropagation.

MATERIALS AND METHODS

For the cultivation of plants *in vitro*, a mineral base of the nutrient medium QL (Quorin, Lepoivre, 1977)[7] was used with the addition of 30 g/l sucrose, 100 mg/l mesoinositol, 250 mg/l casein hydrolyzate, and 8 g/l agar and vitamin complex of Murashige-Skoog.[8] At the stage of microcuttings rooting, the concentration of macrosalts and sucrose was reduced by a half; indole butyric acid at a concentration of 1.0 mg/l was added to the medium. Using other method of rooting, the cuttings were soaked for 20 h in a solution of auxin (50 mg/l), and then, they were planted into hormone-free medium.

Sterile microcuttings were treated with radiation from a helium-neon laser, GN-40 (wavelength 632.8 nm, power density 2 W/m^2), and semiconductor HLDPM12-655-10HJ (wavelength 655 nm, power density 2 W/m^2) at various exposures (30, 60, 120, 240, 480, 960 s) on 3-4 days after their planting into the propagation medium or rooting directly in the culture bottles.

RESULTS AND DISCUSSION

A wide range of possibilities for stimulating morphogenetic processes *in vitro* detected the use of coherent laser radiation.[4,9] In our experiments, irradiation treatment of *S. chinensis* microshoots by helium-neon laser at the propagation stage, made it possible to increase the number of viable explants of this culture from 60.0% ± 6.3% in the control sample, up to 90.9% ± 3.5% in the test sample with 120 s exposure of irradiation [Figure 1].

With this exposure, the share of microshoots with net reproduction more than 1, increased from 44.4% to 75.5%. At the same time, the coefficient of shoots propagation, upon the influence of irradiation, increased insignificantly; but the length of shoots increased substantially. Shoots of the best experiment samples were stronger, with large leaves, practically without necrosis.

S. chinensis is characterized by a low ability to rooting of microcuttings *in vitro* and without using of rhysogenesis inducers on nutrient media, they do not take root. In the control sample without irradiation on QL_{YK} medium, containing 1 mg/l of indole butyric acid, the frequency of rhysogenesis was 26.9% ± 6.1%. The optimum exposures of irradiation, when maximum percentage of microshoots rooting of *S. chinensis* has been obtained in the medium with indole butyric acid, were exposures of 60 and 120 s [Figure 2].

The frequency of microshoots rooting was 60.0 ± 6.9 and 48.0 ± 7.1, respectively, that is, 1.8-2.2 times higher than control. Differences are statistically significant ($P > 0.99$).

DEDUCTIONS

1. It was defined, that one of the methods, which can significantly increase the efficiency of propagation of *S. chinensis* (Turcz.) Baill in conditions *in vitro*, is the use of laser irradiation. LCR is an effective photoregulatory factor, which is capable to increase the functional activity of plants.
2. Irradiation treatment of *S. chinensis* microshoots by the helium-neon laser at the stage of propagation, allowed

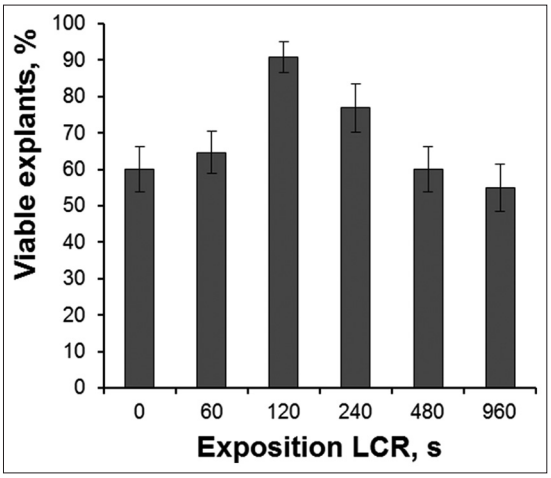

Figure 1: The influence of low-intensity coherent radiation (λ = 632.8 nm) on development of *Schisandra chinensis* explants

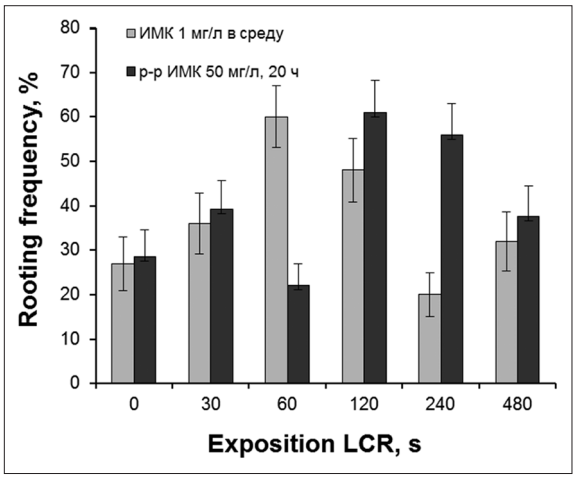

Figure 2: The influence of low-intensity coherent radiation (λ = 632.8 nm) on the effectiveness of *Schisandra chinensis* rooting

to increase the number of viable explants of this culture from 60.0 ± 6.3% in the control sample, up to 90.9% ± 3.5% in the test sample with 120 s exposure of irradiation. With this exposure, the share of microshoots with a net reproduction more than 1, increased from 44.4% to 75.5%.

3. In the control sample without irradiation on $QL_{УК}$ medium, containing 1 mg/l of indole butyric acid, the frequency of rhysogenesis was 26.9% ± 6.1%. The optimum exposures of irradiation, when maximum percentage of microshoots rooting of *S. chinensis* has been obtained in the medium with indole butyric acid, were exposures of 60 and 120 s.

CONCLUSION

One of the most promising ways of propagation of appreciable varieties of *S. chinensis* is the method of clonal micropropagation. However, the low rates of reproduction and rooting, a slow growth rate of shoots and roots, latent bacterial infections, significantly reduce the possibility of its use.

The efficiency of plant propagation *in vitro* can be significantly increased using laser irradiation. During the research, it was established, that laser coherent radiation positively influences the growth and development of *S. chinensis* explants. It is use at the stage of rhysogenesis of *S. chinensis* (Turcz.) Baill significantly stimulates the process of microcuttings rooting *in vitro,* and contributes the increase of number, length of roots and shoot growth. Thus, the integrated use of plant tissues culture methods and biophotonics methods allows to increase the yield and quality of plants *in vitro,* practically without additional expenditures.

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