Effect of irradiation on the growth and rooting of a climbing rose in vitro

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Abstract. The article presents the influence of pulsed and continuous irradiation (400...780 nm) on in vitro growth of the climbing rose variety "Camelot" at the illumination of 80±5 mmol/(m²·s), temperature - 24±1°C, and the relative air humidity - 73±2%. It was found that the experimental led light (LED) pulsed phytoirradiator contributed to a significant increase in the leaf surface area during cultivation of climbing rose microstems in Gamborg's nutrient medium, the average growth was 2.94 mm² compared to 2.80 mm² in the control. Pulse irradiation increases the reproducibility of climbing roses by 1.7 times, and also increases the rooting rate up to 96% compared to 82% in the control. Experimental LED phytoirradiator of continuous irradiation promoted an increase in the leaf surface area growth at the level of the control luminescent phytoirradiator, but also provided a significant increase in the reproduction factor and rooting rate of rose microstems. In the pulsed mode, phytoinstallations consume only 50% of the electricity compared to the continuous irradiation mode.

1. Introduction
It is known that in natural conditions plants can use photosynthesis during the daytime only. Depending on seasons and on the weather, some days have much less light than another. Plants need to adapt to these conditions in a particular region. Many scientists attached great importance to this. Their analysis and work on the influence of the change of light and dark phases on plants are now an important topic for further study. It has been proved that it is necessary to use installations with an adaptive type of irradiation for different plant species [1]. Thus, for efficient impact on the growth and development of a certain culture it is necessary to create a phytoinstallation with a unique spectral composition depending on the growth period of a particular plant.

Previously UV-C light and pulsed light was used as alternative to chemical and biological elicitors for stimulating plant natural defenses against fungal diseases [2]. It was found that UV-C and pulsed light had high potential as plant defense activators, especially in horticulture. Pulsed light treatment increased temperature and mass loss of freshly cut red bell pepper during storage. The results obtained
showed that treatment with pulsed light with a power of more than 4 J/cm² can be used to disinfect red bell peppers without any negative changes in their chemical properties [3].

It has been shown that, therefore, the use of low frequency flashing light as the final stage of industrial production can increase the content of protein, polyunsaturated fatty acids, or pigments in biomass, which will lead to the production of valuable products from algae [4]. In [5], data on strengthening flashlight effect with a pond-tubular hybrid photobioreactor to improve microalgal biomass yield. A pond-tubular hybrid photobioreactor was developed for microalgal growth to increase areal microalgal biomass yield by 54.7%. Flashing light was shown to improve photosynthetic performance and growth of green microalgae [6], while low frequencies \((f < 50 \text{ Hz})\) might improve light harvesting-associated biomolecules production.

The use of LED phytoirradiators for various crops was recently justified. For example, in the article [7] the results of the rooting of garden strawberries using a phytoirradiation installation, operated in a pulse mode, are presented. It has been found that under the influence of such a setup rooting is 5% better in comparison with continuous lighting.

Since the process of photosynthesis is due to two phases - dark and light, the use of a phytoinstallation operating in a pulsed mode does not harm the plants. As known, chlorophyll absorbs the energy of photons and transfers this energy for further development. When the plant is constantly illuminated, some of the photon energy is converted into thermal energy. When illuminated with the use of the dark phases of the phytoinstallation, chlorophyll, absorbing the energy of photons, manages to use it, and take the next "portion". Thus, the energy of photons is used almost in full to build up the green mass of the plant [7].

Growing meristemic plants requires the use of electricity. Monitoring of electricity prices shows their constant growth. Therefore, it is necessary to use electrical energy when growing plants in vitro rationally and efficiently. Research of scientists has confirmed that the use of pulsed irradiation of plants leads to a reduction in electricity consumption by 40 ... 50%. Therefore, the use of the pulsed mode is an actual task.

The purpose of this work is to study the effect of pulsed and continuous irradiation on the growth and rooting of the climbing rose in vitro.

2. Materials and methods
Studies on the meristems of a climbing rose were carried out in the laboratory of the Udmurt Research Agrarian Institute of the Udmurt Federal Research Center of the Ural Branch of the Russian Academy of Sciences. The illumination was within \(80\pm5\ \text{mmol/(m}^2\cdot\text{s)}\), the temperature was \(24\pm1\^{\circ}\C\), and the relative humidity was \(73\pm2\%\). Phytoinstallations worked 16 h a day, from 6 am to 10 pm. Each group contained 33 plants. The object of the research was the meristem plants of the climbing rose variety "Camelot".

Three modes of irradiation of meristem plants were compared: control – using a luminescent phytoirradiator, LED – with continuous irradiation with a cold spectrum, and LED operating in a pulsed irradiation mode. The experimental LED pulsed phytoirradiator has 32 LEDs. The pulsed irradiation mode is based on the K561 microcircuit. The electrical circuit includes an adjustable frequency generator for more accurate selection of parameters, a counter for dividing input pulses, an inverter that allows to start working with heated LEDs, a power transistor, an integrated voltage regulator, a transformer, and a rectifier (figure 1).
Figure 1. Electrical diagram of the phytoirradiator.

Figure 2 shows a diagram of a pulsed irradiation regime for group 3 of plants. Three modes of irradiation of meristem plants were compared. The first group of plants (control) was grown under fluorescent lamps, while the second group – under LEDs, and they shined continuously. The third group of plants was grown under LEDs working in a pulsed mode: the LEDs worked for 0.5 sec ($T_a$) and did not light for a second ($T_b$). The light pulses lasted for 30 sec ($T_p$), then 15 sec of continuous light ($T_N$), then again 30 sec of pulses, then 15 sec of continuous light, and so on [7]. The operating time of the LEDs in 1 min included the following mode: for the period of $T_p = 30$ sec – the LEDs worked for 10 sec, for a period of $T_N = 15$ sec – the LEDs worked for 15 sec. For the remaining 15 sec – the LEDs worked for 5 sec. Thus, in a minute, the LEDs worked for 30 sec and consumed only 50% of the electricity compared to continuous irradiation.

Figure 2. Scheme of the pulsed irradiation regime for the 3rd group of plants: $T_C$ – cycle time; $T_a$ – duration of the light pulse; $T_b$ – duration of the dark phase, and $T_N$ – duration of continuous light.

The irradiation phyto-installation had 32 LEDs. The pulsed irradiation mode was based on the K561 microchip (Microcircuit, Russia). The circuit included variable frequency generator, counter, inverter, fuses, built-in voltage regulator, capacitors, diode bridge. We also used a device for sterilizing the air
space "Sampo" UOS-99-01, model VL-12 (Sampor, Russia, table 1) and Water distiller DE-4-02-"EMO" (EMO, Russia).

Statistical processing of the results was carried out according to the Dospekhov method [8].

**Table 1.** Characteristics of the “Sampo” UOS-99-01 sterilization device, model VL-12.

| Parameter                                           | Meaning  |
|-----------------------------------------------------|----------|
| Filter efficiency for particles 0.3 μm               | 99.995%  |
| Noise level at the workplace                         | ≤ 62 dB  |
| Illumination of the working area                     | ≥ 1000 lux|
| Supply voltage / Mains frequency                     | 220 V / 50 Hz |
| Power consumption                                    | ≤ 400 W  |
| Electrical protection class                          | 2        |

3. Results and discussion

The growth of the leaf surface area of climbing rose microplants was positively influenced by both experimental LED phytoirradiators (table 2, figure 3). In comparison with the traditional luminescent phytoirradiator, the increase in the leaf surface area of the climbing rose microstems at the stage of proliferation increased more intensively under the LED irradiators. When illuminated by a phytoirradiator of continuous luminescence, the increase in the leaf surface area averaged 2.88 mm², with pulsed illumination – 2.94 mm², which is significantly higher than 2.80 mm² (control) with the smallest significant difference with an error of 5% (SSD05) equal to 0.11 mm².

**Table 2.** Leaf surface area of climbing rose microstems at the stage of proliferation, depending on the phytoirradiator.

| Phytoirradiator                | Average leaf surface area, mm² |
|--------------------------------|--------------------------------|
| Luminescent (control)          | 2.80                           |
| LED, continuous irradiation    | 2.88                           |
| LED pulsed                     | 2.94                           |
| SSD05                          | 0.11                           |

![Figure 3](image_url)  
**Figure 3.** Dynamics of growth of the leaf surface area of climbing rose microstems under various phytoirradiators in 2018, mm².
Figure 3 shows that the difference in the growth of the leaf surface in the control was $3.13 - 2.48 = 0.65$ mm$^2$ or 100%. For the second group $3.25 - 2.64 = 0.61$ mm$^2$ or $(0.61/0.65)100\% = 93.8\%$. For group 3 the difference in height is $3.32 - 2.51 = 0.81$ mm$^2$ or $(0.81/0.65)100\% = 124\%$.

At this stage of proliferation, the reproduction factor of climbing rose microstems under illumination with an experimental phytoirradiator of continuous luminescence was 5.5 pcs/explant, and with pulsed light – 7.0 pcs/explant (table 3). In comparison with the control (4.2 pcs/explant), illumination with both phytoirradiators significantly increased the reproduction factor of climbing rose microstems at SSD$_{05}$ 1.5 pcs/explant. Illumination of microstems with a pulsed phytoirradiator provided the maximum reproduction factor, which is significantly higher than both the control indicator and the indicator when using a phytoirradiator of continuous luminescence.

| Phytoirradiator          | Reproduction factor | Rooting rate, % |
|--------------------------|--------------------|-----------------|
| Luminescent (control)    | 4.2                | 82.1            |
| LED, continuous irradiation | 5.5               | 91.8            |
| LED, pulsed irradiation  | 7.0                | 96.4            |
| SSD$_{05}$               | 1.5                | 8.6             |

It can be seen from table 3 that, in comparison with the luminescent phytoirradiators, the experimental LED ones also contributed to an increase in the rooting rate of climbing rose microstems. The use of a phytoirradiator of continuous luminescence increased the rooting rate of climbing rose microstems by 9.7% compared to the control indicator. Under illumination with an experimental pulsed phytoirradiator, the rooting rate of climbing rose microstems was 14.3% higher than for the control experiment. By the end of the phase, all rooted microplants of the climbing rose had a well-developed root system and aerial part, and were successfully adapted to the ground.

Thus, illumination with an experimental LED pulsed phytoirradiator, in comparison with a luminescent and phytoirradiator of continuous luminescence, significantly increased the leaf surface area, the reproduction factor and the rooting rate of climbing rose microstems.

We obtained a positive effect of the LED phyto-irradiator with a flashing light earlier [7]: the rooting rate of micro-cuttings of strawberries increased by 5-10%, depending on the variety; the number of fully formed leaves increased from 6.0 pcs up to 7.2 pcs and, as a consequence, an increase in the surface area of the shoot from 262.2 mm$^2$ to 348.0 mm$^2$; by the end of the stage, all rooted strawberry microplants in terms of the rosette height, the number of fully developed leaves and the length of the root corresponded to the normative requirements.

**4. Conclusion**

In comparison with the luminescent one, the experimental LED pulsed phytoirradiator contributed to a significant increase of the leaf surface area of the climbing rose (2.94 mm$^2$ compared to 2.80 mm$^2$ in the control), reproduction factor (by 1.7 times in relation to control), and rooting rate (up to 96.4% compared to 82.1% in the control experiment).

Experimental LED phytoirradiator of continuous irradiation contributed to an increase in the leaf surface area growth at the level of the control luminescent phytoirradiator, but also provided a significant increase in the reproduction factor and rooting rate of climbing rose microstems.

**References**

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