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Influence of light-emitting diodes on the efficiency of valuable woody plants micropropagation

Abstract. The article presents the results of studying the effect of light-emitting diodes (LEDs) on the efficiency of micropropagation of valuable woody plants. Commercial use of micropropagation requires optimization of various parameters, including light quality and lighting regime. Nowadays, LED have become an alternative source of illumination for plant tissue culture, including woody plants. Recently, along with medicinal herbaceous plants, tree crops have also become widely used as a source of various pharmaceuticals. Therefore, it is promising to optimize the technology of micropropagation for the rapid and massive production of valuable woody plants. The current research focused on analysing the effects of different LEDs on *Paulownia* sp. micropropagation: white (W), red (R), blue (B), mixed red and blue (R:B), and the Fitotron climate chamber. The fluorescent light was used as a control. Micropropagation efficiency was evaluated using the following parameters: height, number of axillary buds, length of internodes, number and length of roots, and photosynthetic pigment content. *P. tomentosa* produced the greatest number of roots under R LEDs, while hybrid *Paulownia Sieb.Zucc.* × *Elongata* – under W and R LEDs. In hybrid *Paulownia Sieb.Zucc.* × *Elongata*, the most intense leaf and internode formation was observed under R:B and R LEDs. The content of chlorophyll *a* and *b* in *P. tomentosa* leaves was significantly higher under R LED, while in HP leaves – under B LED. The obtained results are an important contribution to the commercial use of Paulownia and other valuable woody plants for mass production of plant material with biologically active properties.
Key words: woody plant, micropropagation, LEDs, shoot induction, root induction, efficiency.

Introduction

Paulownia is one of the most useful and fastest growing trees in China and Southeast Asia, and it has been introduced to many countries, such as Europe, America, and Australia due to its multipurpose use [1,2]. Although it is a non-traditional medicinal plant, various parts of the plant (leaves, flowers, fruits, wood, bark, roots and seeds) of paulownia are used to treat various diseases [3,4].

Nowadays, micropropagation technology is widely used to obtain planting material and to accelerate the propagation of valuable tree species and cultivars. Compared to seed propagation, this method offers numerous important advantages for Paulownia sp. propagation. Because of its ability to regenerate plants in vitro, micropropagation considerably expands the scope and possibilities of mass reproduction of garden and ornamental woody plants. To date, systems for Paulownia sp. propagation through direct and indirect embryogenesis and organogenesis have been developed to address issues of hormonal regulation of microclonal propagation, the influence of explant type and genotype on shoot multiplication in vitro, long-term storage under in vitro conditions, as well as rooting and adaptation of microclones to ex situ conditions [5-7].

Although the benefits of using micropropagation for diverse Paulownia species are obvious, commercial implementation of the technology must be tailored to each species.

Microclonal propagation depends on the internal (endogenous hormones, physiological age of explant) and external (nutrients, exogenous hormones, light, temperature, and humidity) factors. Among factors affecting micropropagation, the most crucial is a light regime that includes light quality, intensity, and photoperiod and consequently determines the

efficiency of micropropagation. Plant tissue cultures depend entirely upon artificial light sources for illumination. The illumination should provide light in the appropriate regions of the electromagnetic spectrum for photomorphogenic responses and photosynthetic metabolism. Fluorescent lamps are the most common source of illumination while the cultivation of plant cells and tissues under *in vitro* conditions. However, in the last two decades, LEDs have been used as alternative light sources. LEDs' spectral properties enable regulation of various morphological, anatomical, and physiological characteristics such as shoot elongation, axillary bud formation, somatic embryo induction, rhizogenesis, leaf anatomy, and photosynthetic potential of plants cultivated under *in vitro* conditions [8]. LED lighting affects not only plant regeneration but also induces changes in the generation of reactive oxygen species (ROS) and the subsequent involvement of antioxidant metabolic activity [9]. Moreover, LEDs reduce the cost of micropropagation due to low power consumption and heat generation as well as radiation at a certain wavelength [10,11].

Numerous literature data reported the influence of light quality on the *in vitro* cultivation of herbaceous plants, whereas the effects on woody plants cultivation have been studied much less. Similarly, literature on evaluating the impact of LEDs systems on the growth and development of forest culture species are limited compared to garden plants [12]. Most studies on the light influence on woody plants have been conducted with fluorescent lamps, specifically with cold white light. Nevertheless, a number of research papers evidenced the positive effects of red, blue, and mixed red and blue lights on the micropropagation of fruit and ornamental crops [13, 14]. Thus, LEDs can promote shoot multiplication, zygotic embryos growth and development under *in vitro* conditions, microclones rooting, and plant adaptation to *ex situ* conditions [15-17].

The effects of light quality on *Paulownia* sp. micropropagation are poorly investigated except few studies on the effects of different photoperiods and light intensities on *Paulownia* sp. *in vitro* regeneration and LEDs with mixed red and blue spectra on the growth of micropropagated plants[18- 20]. Therefore, the investigation of the LED influence on the efficiency of *Paulownia sp.* micropropagation is great importance. The development of biotechnological approaches to *Paulownia* sp. *in vitro* propagation will accelerate its introduction in Kazakhstan.

The current research aimed to investigate LEDs' influence on the efficiency of *P. tomentosa* and hybrid *Paulownia Sieb. Zucc.* × *Elongata* microclonal propagation for the rapid and massive production of valuable woody plants.

Materials and methods

Plant material. Paulownia tomentosa (Thunb.) Steud (P. tomentosa) and hybrid Paulownia Sieb. Zucc. × *Elongata* (HP) explants were used to investigate the influence of LEDs on the efficiency of their micropropagation and photosynthetic potential. The parent plants of *P. tomentosa* and the hybrid trees were provided by IE Hayet AE EDIS (Almaty, Kazakhstan), which in turn purchased plants from Paulownia PlantBiotech (Moscow, Russian Federation). The study was performed in the Laboratory of Genetics and Reproduction of Forest Culture of the Institute of Genetics and Physiology, Almaty, Kazakhstan. All the experiments were performed in accordance with relevant guidelines and regulations.

Experiment design. This study consists of two stages: the first stage includes shoots induction and multiplication; the second stage – investigation of the LEDs influence on the efficiency of *P. tomentosa* and HP micropropagation and photosynthetic potential.

Shoots induction and multiplication. The 2-3 cm stem with an axillary bud was used as an explant for *in vitro* cultivation. The explants were sterilized according to the following protocol: washing in soap solution for 7 min, rinsing with sterile distilled water for 3 times, and then treatment with 10% hydrogen peroxide (H_2O_2) for 4 min. In order to initiate shoot induction and multiplication, explants were cultured for 4 weeks on WPM hormone-free nutrient media (25 mL per container) in a heat-resistant 350 mL plastic container. The media contained mineral salts, WPM-based vitamins, 3% sucrose, 6.5 g L^{-1} Sigma agar. Media after adjusting the pH to 5.6 were sterilized by autoclaving at 121°C for 20 min. Plant cultures were grown at 25±2°C, relative humidity of 60%, and a photoperiod of 16/8 (light/dark) hours. At this stage, a cold white fluorescent light with an intensity of 6000 lk was used as the illumination source. The nutrient medium consisted of 5 containers with 4 explants per container. The experiment was performed in triplicate so that a total of 600 explants were observed during the experiment. To evaluate the influence of media content on shoot induction and multiplication, parameters such as the shoots quantity (SQ), shoots length (SL), internodes quantity (NI), and callusogenesis (CG) were measured weekly.

LEDs influence on P. tomentosa and hybrid P. Sieb. Zucc. × Elongata micropropagation. The study of the light quality influence on micropropagation of the research plants was carried out on *hormone-free WPM* at 23±2°C with a relative humidity of 70±5%, and a photoperiod of 16/8 (light/dark) hours using the following six sources of illumination: 1) Fluorescent lamps emitting light with a wide range of wavelengths (400-700 nm) – FL (as a control); 2) LEDs of cold white light (TM Zarya 36W, Russia) – W; 3) LEDs of 100% red spectrum (China, 620 nm) – R; 4) LEDs of 100% blue spectrum (Epileds 12W, China, 460 nm, PPFD: 150 μmol m^{-2} s^{-1} at H = 0.1 m) – B; 5) LEDs of combined 70% red and 30% blue spectra (50W, China) – R:B; 6) Climatic chamber Fitotron containing LEDs of red (620 nm), deep red (660 nm), green (525 nm), blue (460 nm), and deep blue (440 nm) spectra (Fitotron LLC, Russia) – F. The 0.5-1.0 cm long shoot tips of *P. tomentosa and* HP (one tip per 15 mL test tube) were cultured separately under the above illumination sources. LEDs were installed at a distance of 15-20 cm from the test tubes. The experiment was repeated 3 times, one replication consists of 20 shoot tips. The light spectrum was measured using an OHSP-350P spectrometer (Hangzhou Hopoo Light & Color Technology Co). PPFD was measured in the central area of an empty shelf with pre-installation for each experimental tube at 62–65 μmol m^{-2} s^{-1} and a 16-hour photoperiod. After 4 weeks of cultivation under different illumination sources, the *P. tomentosa* and the HP microclones were carefully retrieved from the test tubes. The micropropagation efficiency was evaluated by physiological parameters such as height, length of roots and internodes as well as their quantity.

Photosynthetic pigments content. The content of chlorophyll *a* (*Chl a*), chlorophyll *b* (*Chl b*), and carotenoids (*Car*) was determined in fresh leaves of microclones after 4 weeks of cultivation. In order to prepare a plant extract, 0.2 g of fresh leaves were ground in a mortar with the gradual addition of 20 mL of 96% ethanol, then homogenized and filtered twice. Optical density was measured at 665 nm (*Chl a*), 649 nm (*Chl b*), and 470 nm (*Car*) using a PE-5400UF spectrophotometer (Russia). The content of *Chl a*, *Chl b*, and *Car* in μg g^{-1} fresh weight (FW) was calculated using the equations described in Lichtenthaler et al. [21]:

$$Chl\ a = 13.95A_{665} - 6.88A_{649} \quad (1)$$

$$Chl\ b = 24.96A_{646} - 7.32A_{665} \quad (2)$$

$$Car = \frac{1000A_{470} - 2.86 \times Chl\ a - 129.2 \times Chl\ b}{245} \quad (2)$$

where A is a measured absorbance value at different wavelengths.

Statistical analysis. Data analysis was processed using the RStudio software (version 1.3.959, R Studio PBC, 2020). Two-Way Repeated Measures ANOVA was performed to compare the changes of physiological parameters (length of shoots, callusogenesis, and quantity of shoots and internodes) of explants grown on media with different ratios and concentrations of growth regulators during the experiment (4 weeks). Two-Way ANOVA was carried out to declare a significant difference between physiological parameters (plants height, length of roots and internodes, quantity of leaves, roots, and internodes) and photosynthetic pigments concentrations of two research plants under different sources of illumination. Tukey HSD tests were performed for the pairwise comparison of means when ANOVA showed a significant effect of tested factors. Then treatments were categorized by letters in descending gradation. Significance was declared at $p < 0.05$.

Results and discussion

Shoot induction and multiplication. At this stage, *P. tomentosa* shoot induction and multiplication was examined. In order to initiate shoot induction and multiplication, explants were cultured for 4 weeks on WPM hormone-free nutrient media. After the first week of cultivation of *P. tomentosa,* microclones had the following parameters: the average number of shoots was 2.00 while their length reached 0.50 cm. After 4 weeks of cultivation, the shoot number did not significantly change and remained equal to 2.00,

whereas the length of shoots increased by 5.76 times being equal to 2.88 cm, the number of internodes reached 2.90 and callusogenesis was not observed at all (presented on Figure 1).

Many studies have found that using MS medium with various combinations of BAP, NAA, and IAA is the optimal choice for *P. tomentosa* and many hybrids shoot formation [22,23]. In Paulownia micropropagation, the frequency of WPM medium use is much lower than that of MS. A comparative analysis of MS, WPM, and GD media supplemented with different concentrations of BA and NAA showed that the longest shoot was formed on WPM nutrient media supplemented with 2 mg L^{-1} of BAP [24]. The use of hormone-free media in experiments reduces the possible appearance of somaclonal variants and contributes to the production of genetically homogeneous planting material.

1st week 4th week

Figure 1 – Shoot multiplication of *P. tomentosa* explants in hormone-free WPM medium

Effect of light quality on in vitro propagation. Photoperiod duration and light spectral quality essentially impact plant morphogenesis and development. LEDs have become increasingly popular in recent years as an alternative artificial light source, not only in agriculture and horticulture but also in tissue culture research in a wide range of plant species.

The investigation of the LEDs influence on the research plants' propagation efficiency was performed using the hormone-free WPM medium. The results showed that LEDs in the red (R) and blue (B) regions of the spectra stimulated the growth dynamic of *P. tomentosa* and HP, respectively. The height of *P. tomentosa* did not differ within light treatments except R being equal to 2.63 cm under this light. Contrary, the height of HP was positively affected by B light (2.79) and inhibited under F light (1.48 cm). The same tendencies were observed for internodes length.

Numerous studies with various plant species have shown that red light has a positive effect on the plant's growth and development under *in vitro* conditions. Red LEDs stimulated shoot elongation and multiplication rate of *Myrtus communis* L.[30], as well as increased the frequency of poplar shoots regeneration [25], chestnut roots development and hypocotyls elongation[26], the germination frequency of pine somatic embryos[27], and the micropropagation efficiency of some ornamental species[28].

In our study, the R and F spectra illumination additionally promoted the formation of *P. tomentosa* leaves (5.13 and 4.86 pcs, respectively) and internodes (5.26 and 4.53 pcs, respectively). In HP, the most intense leaves and internodes formation was observed under R:B illumination (4.46 and 4.66 pcs, respectively) and R (5.13 and 4.8 pcs, respectively). R illumination also caused the doubled elongation of *P. tomentosa* and HP internodes. Under FL illumination, there was formed the smallest number of leaves and internodes (Table 1).

Currently, there is nearly no published research on the effect of light quality on the Paulownia micropropagation efficiency. LEDs with mixed light (20% red and 80% blue) influenced the *Paulownia fortuneii* seedlings' growth parameters such as height, the number of roots, fresh weight and chlorophyll

content. Grubišié et al. [29] revealed that *P. tomentosa* seed germination is induced by red light and inhibited by far-red light. Decreasing light intensity from 3000 to 1200 lk led to a higher *Paulownia fortuneii* shoots elongation in *in vitro* culture and contributed to an increase of nodes number with healthier shoots.

Table 1 – Effect of light quality on *in vitro* propagation of *Paulownia* sp.

Light quality	Plant height,cm		Internode length,cm		Number of leaves (explant^{-1})		Number of internodes	
	P. tomentosa	HP	*P. tomentosa*	HP	*P. tomentosa*	HP	*P. tomentosa*	HP
Fl	1.78±0.11	1.88±0.06	0.47±0.04	0.46±0.03	3.73±0.22	3.93±0.20	3.86±0.13	4.20±0.22
W	1.63±0.07	1.79±0.05	0.41±0.02	0.31±0.02	4.46±0.15	4.43±0.18	4.26±0.18	4.40±0.14
B	1.68±0.06	1.65±0.25	0.49±0.03	0.44±0.47	4.66±0.19	3.06±0.41	4.53±0.21	3.26±0.48
R	2.89±0.17	3.35±0.23	0.86±0.06	0.84±0.08	5.13±0.22	4.46±0.18	4.86±0.26	5.13±0.22
R:B	1.57±0.06	1.73±0.05	0.30±0.03	0.33±0.04	5.00±0.26	4.66±0.95	4.73±0.26	4.80±0.18
F	1.61±0.07	1.46±0.05	0.35±0.03	0.27±0.02	5.26±0.24	4.40±0.81	4.53±0.28	3.73±0.20

Effect of light quality on in vitro rooting. The results of studying the effects of light quality on the formation and development of *P. tomentos*a and hybrid HP microclones root system showed that the process was genotype-dependent, and it was found that there were significant differences in the number of roots per seedling and per stem under different light spectra. The highest quantity of *P. tomentosa* and hybrid HP seedlings were observed under R light (2.66 and 5.66 pcs, respectively). In the case of hybrid HP, W light also promoted its root formation. In addition, the B, R:B, F, and W spectral illumination had no significant effect on the number of *P. tomentosa* roots laying in a range from 1.40 to 1.66 pcs. The lowest roots number were observed under FL illumination for *P. tomentosa* while under B and F lights – for HP.

Measurements of root length under different light conditions resulted in its different dependence on the light quality for *P. tomentosa* and HP (Table 2).

Table 2 – The effect of different light spectra on the growth and development of *P. tomentosa* and HP plants in *in vitro* culture

Light quality	Number of roots (explant^{-1})		Root length, cm	
	P. tomentosa	HP	*P. tomentosa*	HP
FL	1.23 ± 0.17a	3.36 ± 0.35a	2.99 ± 0.36bc	3.48 ± 0.42bc
W	1.40 ± 0.17a	5.10 ± 0.45b	2.96 ± 0.32bc	4.03 ± 0.27c
B	1.66 ± 0.18a	2.86 ± 0.45a	2.22 ± 0.27abc	1.12 ± 0.20a
R	2.66 ± 0.25b	5.66 ± 0.43b	3.10 ± 0.22c	2.52 ± 0.27b
R:B	1.60 ± 0.19a	3.06 ± 0.22a	1.93 ± 0.24ab	2.46 ± 0.18b
F	1.56 ± 0.19a	2.86 ± 0.30a	1.55 ± 0.17a	2.39 ± 0.33b

The longest root length for *P. tomentosa* was noted under R light (3.10 cm), while for HP – under W and FL light (4.03 and 3.48 cm, respectively). Moreover, R:B and F (*P. tomentosa*) and B (HP) illuminations significantly inhibited root growth compared to control (FL light). The effect of different light spectra on the growth and development of *P. tomentosa* and HP plants in *in vitro* culture is shown in Figure 2.

The data obtained are consistent with many existing studies, although the scientific information on the light quality physiological role on the root formation of tree species *in vitro* is less comprehensive.

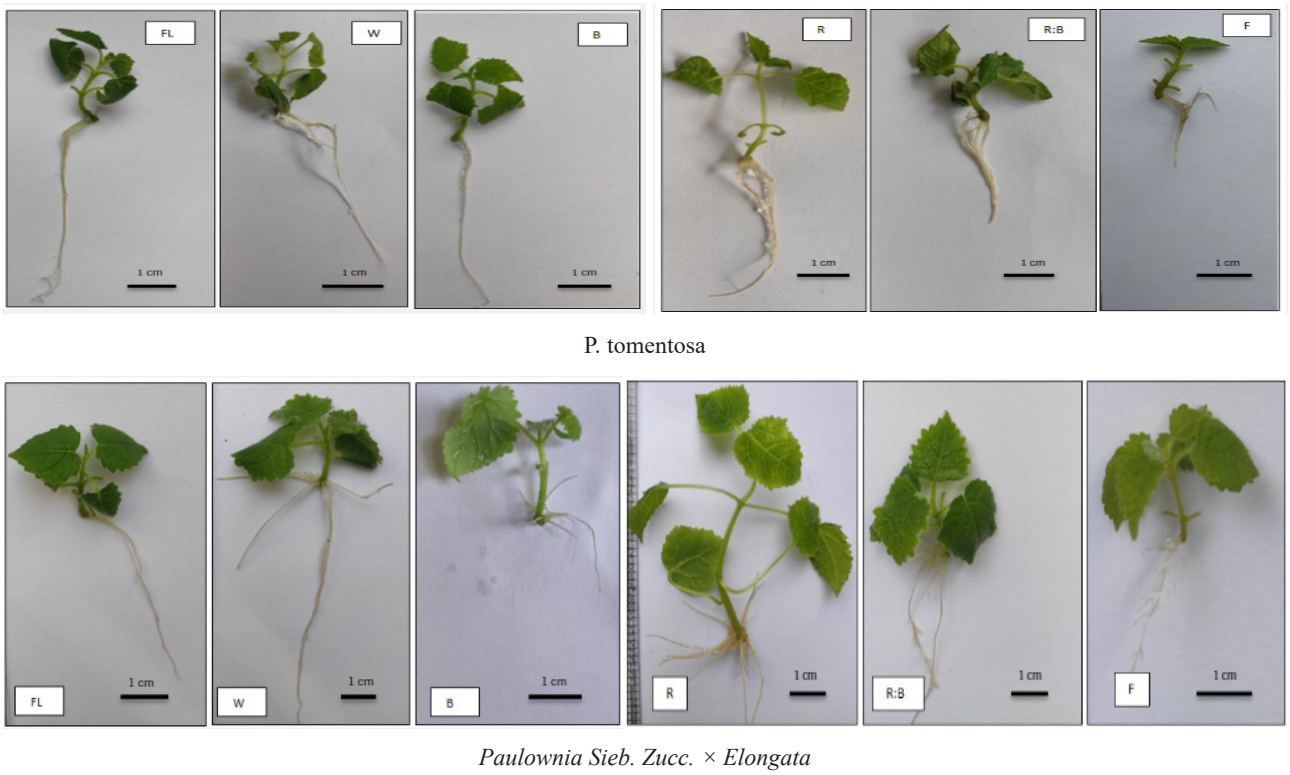

Figure 2 – Influence of different light spectra on the growth and development of *P. tomentosa* and *Paulownia Sieb. Zucc.* × *Elongata* microclones in *in vitro* culture.
Note: FL – fluorescent lamps; LEDs: W – white, R – red, B – blue, B:R – blue + red, F – climate chamber Fitotron

In a series of studies, the genotypic dependence of the tree species rooting process in culture *in vitro* on the light quality of light has been noted. An increase in the formation and growth of the root of regenerated chestnut plants was facilitated by red light [26]. In the case of birch microcuttings, the rooting process and the formation of adventitious roots were stimulated by blue color [30]. Moreover, increased light intensity and duration of application positively influence the rooting speed and roots quantity as well [15,17].

Influence of the light quality on the content of the photosynthetic pigment. The success of commercial micropropagation depends not only on the quantity but also on the quality of the plants produced. The content of photosynthetic pigments (chlorophyll and carotenoids) can be used as a reliable indicator of plant vitality and provide an indirect estimate of photosynthetic ability. During the rooting stage, LEDs of different qualities were found to have a significant effect on the content of the photosynthetic pigment depending on the plant genotype.

The total content of *Chl a* in the *P. tomentosa* leaves was significantly (p <0.05) higher under the R light spectrum (0.99 ± 0.03 μg g^{-1} FW). In the case of hybrid HP leaves, the highest chlorophyll content was noted under B (1.19 μg g^{-1} FW) and FL (0.70 μg g^{-1} FW) lights (p <0.05), respectively. The lowest chlorophyll content was noted under lighting F. Kitajima and Hogan also reported notable differences in chlorophyll *a* and *b* content between four *Bignoniaceae* species [31]. There was established a correlation was between the growth and chlorophyll pigments content in the *Cunninghamia lanceolata* microclones leaves at different photoperiods and light quality. The best microclones were obtained at a 16-hour photoperiod with red, blue and green LEDs [14]. Blue and a combination of red and blue LEDs increased while only red decreased chlorophyll pigments content in flowering plants [30]. Shin at al. reported that blue LEDs and fluorescent light stimulate the formation of carotenoids [32]. In our study, although there was no detected statistically significant difference between the carotenoids content depending on the light quality (p > 0.05), the highest carotenoids content (0.05 μg g^{-1} FW) was observed under R and B lights for *P. tomentosa* and HP, respectively (Table 3).

Table 3 – The influence of light quality on the photosynthetic pigment content in *Paulownia* sp. during *in vitro* rooting

Light quality	Chlorophyll *a* (μg g^{-1} FW)		Chlorophyll *b* (μg g^{-1} FW)		Carotenoids (μg g^{-1} FW)	
	P. tomentosa	HP	*P. tomentosa*	HP	*P. tomentosa*	HP
FL	0.80 ± 0.49a	0.90 ± 0.16 ab	0.62±0.38a	0.70±0.12a	0.04 ± 0.02a	0.03 ± 0.005a
W	0.53 ± 0.25a	0.64 ± 0.10ab	0.40 ± 0.18a	0.49 ± 0.09a	0.01 ± 0.005a	0.02 ± 0.01a
B	0.73 ± 0.50a	1.19 ± 0.31b	0.61 ± 0.32a	0.62 ± 0.25a	0.02 ± 0.001a	0.05 ± 0.02a
R	0.99 ± 0.03a	0.74 ± 0.13ab	0.82 ± 0.01a	0.59 ± 0.09a	0.05 ± 0.001a	0.03 ± 0.003a
R:B	0.44 ± 0.13a	0.76 ± 0.14ab	0.32 ± 0.09a	0.56 ± 0.12a	0.02 ± 0.01a	0.04 ± 0.005a
F	0.26 ± 0.02a	0.35 ± 0.02a	0.20 ± 0.02a	0.28 ± 0.01a	0.04 ± 0.05a	0.03 ± 0.01a

Conclusion

The use of a hormone-free nutrient medium reduces the likelihood of the appearance of somaclonal variants, contributes to the production of genetically homogeneous planting material. LEDs increase the productivity and profitability of paulownia micropropagation. This study shows that red LED lights have a positive effect on the Paulownia micropropagation efficiency on WPM medium without growth regulators. The R LEDs application stimulated the shoots and roots elongation, the leaves and internodes formation, and the chlorophyll *a* and *b* content increase. The use of hormone-free nutrient media can reduce the possibility of somatic cell clonal mutation and contributes to the production of genetically homogeneous planting materials. LEDs improved the productivity and profitability of Paulownia micropropagation. The results are of great importance for optimizing and increasing the efficiency of micropropagation technology and other valuable woody plants.

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