The Effect of Different Wavelengths LED Lighting on the Growth of Spruce (Picea abies L) Plantlets

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Abstract

The present study investigating the reaction of spruce (Picea abies L) germinated and grown for 40 days in a septic or in vitro conditions under ultrabright LEDs lighting (mono-color light) shows a low influence on the plantlets growth (the green light stimulates slightly this process) but reduces almost to half the assimilatory pigments content in the leaf. However, because the use of mono-color LEDs for plant in vitro cultures lighting is very economical, such procedure can be used in units that practice various temporising methods for plantlets growth in aseptic regime increasing their subcultivation interval.

Keywords: spruce (Picea abies L), germination, growth, LED lighting

1. Introduction

Plant biotechnology is a theoretical and practical area of great importance in modern biology with substantial implications in „in vitro” cloning of plantlets and in obtaining and micropropagation of disease free species. On the other hand, the vitroculture is also a way of investigating different phyto-physiological processes (for example using cells and protoplast cultures) and even molecular biology aspects using modern techniques with nanoparticles (CACHITA & al., [1]).

Solar light consist of electromagnetic radiation with a wavelength between 400 and 700 nm (red, orange, yellow, green, blue and violet), in the visible spectrum (TARHON, [2] and also ultraviolet light with a wavelengths below 400 nm and infrared light above 700 nm.

In the plant photosynthetic process only visible light is active. The ultraviolet and infrared light absorbed by the leaves have a harmful effect on them (TARHON, [2]; BERCE, [3]).

Artificial light sources emit differently the spectral wavelengths, their light being different from the natural light which has an equal percentage of all spectral components.

The nature and wavelength of the light has different influence on the different physiological plant processes (BURZO & al., [4], depending on the species and their development stage or studied organ. Thus, green light, in the process of seed germination of Arabidopsis, stimulates the early elongation of the stems, antagonizing the growth inhibition by light whereas the white and red light, in ferns can delay the chlorophyll loss due to senescence.
In *Lolium rigidum* seeds under germination, such light produces an inhibitory effect of the „waking-up” process of the plant embryos from the latent stage (FOLTA, [5]. *Blue light*, in *Arabidopsis thaliana* plays a role in phototropism, in inhibiting the hypocotyl ellogation and influences the opening of the stomata (AHMAD & CASHMORE, [6]; FOLTA & SPALDING, [7]).

In the last century (the 1960s), was launched the illumination of the displays of some instruments with Light Emitting Diodes (LEDs) (TENNESSEN, [8]. The LEDs are small light emitting devices (diodes), 5 -7 cm in length, cheap and have a long lifetime. Also they have very low energy consumption, high luminosity, do not heat the growth chambers and occupy little space (this enables to increase the density of the shelves and more efficient usage of the growth chambers for plant vitrocultures). There are LEDs that emit red, yellow, green, blue, cyan and violet light. The first white LED was created in 1993 by „Nichia Corporation” (https://ro.wikipedia.org/).

The researches on LEDs illumination were expanded for studies on plants. For example *Marigold* and *Salvia* (JEONG & al.[9], *Fragaria* (NHUT & al. [10], *Chrysanthenum* (PETRUS & CACHITA [11], *Pisum sativum* (TOPCHIY & al.,[12], seed germination of *Raphanus* and of *Daucus* (SOMMER & FRANKE [13], cactaeae (VIDICAN & al., [14], *Sequoia sempervirens* (POP & CACHITA, [15 & 16]; *Solanum* (POP & CACHITA, [17 & 18], or *Sorgum* (STANA & CACHITA, [19] and *Pinus nigra*, *Brassica oleracea*, *Beta vulgaris* și *Hordeum vulgare* (MATIOC-PRECUP & CACHITA, [20 & 21].

In most of the cases, positive results were obtained regarding growth parameters. The above list shows that in our country there were research groups that have studied the influence of the colored light emitting LEDs on seed germination and growth of different plant species under a septic or aseptic regime.

From a theoretical and also practical point of view, the question is to what extent the color of LEDs light affects the germination and growth of different plant species and to what extent the nature of LEDs light affects these processes in plantlets which sprouted in transparent plastic containers or the ones from in vitro culture.

It is worth mentioning that in the nordic countries, obtaining seedlings in forestry, their growth, after acclimatization in septic environment, is being done in lighted greenhouses. So practically, the lighting of the seedlings with LEDs is not only efficient but ecological as well.

The purpose of the research presented in this work was to investigate the effects of white or colored LEDs of different wavelengths on spruce (*Picea abies* L.) plantlets in the first 40 days of germination. The final target of the studies is to replace the lighting with fluorescent tubes currently used for in vitro cultures with LEDs panels, a more economic system.

2. Materials and Methods

According to the previously presented it was considered appropriate to follow the effect of the lighting with different color LEDs (different wavelengths) on spruce seeds germination and also the investigation from an ultrastructural point of view of the cotyledon cells as well as the assimilatory pigments content of the plantlets leaflets as a function of LEDs light color.

Different experiments were performed either with plantlets derived from germinated spruce seeds in transparent plastic containers (on filter paper periodically moistened with tap water) or with plantlets germinated and grown „*in vitro***” on agarised MURASHIGE-SKOOG, [22] without growth regulators.

For *in vitro* germination, the seeds were disinfected in 5% sodium hypochloride for 20 minutes followed by rinsing with sterile water. In both situations, lighting of the samples was...
done either with white LEDs (variant $V_{00}$) or red (variant $V_1$), yellow (variant $V_2$), green (variant $V_3$) or blue (variant $V_4$) LEDs. Some of the experiments were also performed under natural light (variant $V_0$) by placing the plastic container in a northward facing window.

In the case of the specimens exposed to LEDs light, seed germination and plantlets growth was done in polystyrene containers, the number and density of LEDs placed in the container’s lid was chosen to give a light intensity of 2000 lux. The adopted photoperiod in all the experiments was 16 hrs light/24 hrs. The LEDs were of ultrabright type, the wavelength was 700 nm for red light, 550 nm for yellow, 500nm for green and 450nm for the blue light.

The type of investigations performed during plantlets growth from spruce seed embryos that were germinated under LEDs lighting depended on the plantlet growth stage. Thus, the percentage of germinated seed per experimental variant was evaluated in the 10th day from placing them for germination on filter paper in plastic containers (Tab. 1).

In the 14th day of germination (Fig. 1) - both in septic or aseptic medium – because the epicotyl and leaflets were not yet present in the plantlets, biometric measurements were not performed, only ultrathin transversal sections through cotyledons were made and were processed according to the specific procedures for ultrastructure studies (CACHITA & CRACIUN, [23]. These studies were made on a Jeol 1010 transmission electron microscope (TEM). The most representative images are presented in figures 1 – 6.

In the 40th day of germination, biometric measurements were done regarding the embryo root, (hypocotyl and epicotyl) lengths. For both the plantlets from the plastic containers and for the in vitro cultivated ones (Fig. 2). A number of 50 plantlets were measured per experimental variant.

To determine the assimilating pigments content, a spectrophotometric method was used. The pigments were extracted with dimethylformamide and the extract was measured at 664 nm to determine the chlorophyll a content, 647 nm to determine the chlorophyll b content and 480 nm to determine the carotenoid pigments content.

In the 40th day, 50 mg of leaflets per sample were harvested from the middle part of the stem and treated with 5 ml of dimethylformamide (DMF) (MORAN & PORATH, [24]. The mixture was kept at 4ºC in a refrigerator for 72 hours. Five measurements were made for each experimental variant.

The data obtained from the spectrofotometer („Speko 11”) was calculated according to the formulas proposed by MORAN & PORATH, [24]:

\[
\text{Chlorophyll} \ a \ (\text{mg/gSP}) = 11,65 \ \text{A}_{664} – 2,69 \ \text{A}_{647} \cdot \frac{v}{SP}; \\
\text{Chlorophyll} \ b \ (\text{mg/gSP}) = 20,8 \ \text{A}_{647} – 3,14 \ \text{A}_{664} \cdot \frac{v}{SP}; \\
\text{Carotenoids} \ (\text{mg/gSP}) = (1000 \ \text{A}_{480} – 1,28 \ \text{clorof.} \ a – 56,7 \ \text{clorof.} \ b) / 245 \cdot \frac{v}{SP};
\]

The numbers after letter „A” represent the wavelength in nanometers (nm) of the filter in the spectrophotometer

$v = \text{solvent volume (ml)}$

$SP = \text{weight of the plant material used for extraction (mg)}$

Both in the case of biometric data and photometric values from the assimilating pigments measurements, average values were calculated and the average numbers obtained from the specimens subjected to white light (LEDs lighting or natural light accordingly) were used as reference values (100 %), all the average results obtained per experimental variant being reported to this.

These percentage values were presented graphically in figures 5 and 6.
3. Results and discussion

The estimations regarding the percentage of germinated seeds depending on the nature of the light the specimens were exposed to, are presented in Tab.1. From the data analysis it can be concluded that the specimens exposed to white LEDs (variant \(V_{00}\)) and green LEDs (variant \(V_3\)) had the highest percentage (95%) regarding the sprouting of spruce seeds.

In opposition to this value, the lowest germination percentage (88%) was noticed in seeds exposed to red light (variant \(V_1\)). The natural light and blue LEDs (variant \(V_4\)) scored only 93% of germinated seeds and under yellow light (variant \(V_2\)), this percentage was further reduced to 92%.

In the 14th day of germination both under septic (Fig. 1A) and aseptic (Fig. 1B) regime, in the specimens exposed to natural light (variant \(V_0\)), the plantlets were the tallest whereas the ones exposed to green LEDs (variant \(V_3\)) had a smaller size but superior compared with the one of the plantlets under red or blue LED (variant \(V_4\)) lighting. It is worth mentioning that at the spruce (as with many other conifers), the embryo and the plantlets have 7 – 8 acicular cotyledons that turn green after light exposure.

<table>
<thead>
<tr>
<th>Experimental variants</th>
<th>Germination percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural light ((V_0))</td>
<td>93%</td>
</tr>
<tr>
<td>White LED ((V_{00}))</td>
<td>95%</td>
</tr>
<tr>
<td>Red LED ((V_1))</td>
<td>88%</td>
</tr>
<tr>
<td>Yellow LED ((V_2))</td>
<td>92%</td>
</tr>
<tr>
<td>Green LED ((V_3))</td>
<td>95%</td>
</tr>
<tr>
<td>Blue LED a ((V_4))</td>
<td>93%</td>
</tr>
</tbody>
</table>

Table 1. Experimental variants and percentage of germinated seeds in the 10th day from placing the seeds to germinate in septic regime, on filter papers in plastic containers

The electron micrographs shown in figures 2 and 3 clearly illustrate that the reduced size of the spruce plantlets from the observations and pictures made in the 14th day on the specimens exposed to red LEDs (variant \(V_1\)) (Fig. 2C and D) and especially blue (variant \(V_4\)) is correlated with the existence in the cotyledon cells of a high starch content in the chloroplasts stroma. Furthermore, in the plantlets exposed to blue light (Variant \(V_4\)), in the vacuolar content of the epidermis cells, spherical-shaped, electrodense, osmiophilic corpuscles can be observed (Fig. 3C–F), that are less frequent and finer in plantlets exposed to other light colors.

These aspects point out that in plantlets, after 14 days of germination, under red or blue lighting, not only the growth is impaired but also some of the metabolic processes are temporized or modified. In turn, according to the ultrastructure aspect of the epidermal cells and of the assimilating mesophyll of the cotyledons exposed to natural light (\(V_0\)) (Fig. 2, poz. A and B) or green light (\(V_3\), Fig. 3, poz. A and B), the plantlets show a normality of the cyto-physiological processes taking place at this level.

In figure 4 it is shown the aspect of spruce plantlets in the 40th day of seeds germination in containers exposed to natural light, placed in a northward facing window and of plantlets from their embryos, age at which the average plantlet size was about 10 -11 cm.

In this developmental stage of the plantlets, the cotyledons for variant \(V_0\) have already fallen whereas at the specimens under green LEDs lighting, the cotyledons were still present.

In the histograms from figure 5A, are presented (as percentage) the average results of the biometric measurements performed on 40 days old plantlets germinated in septic or aseptic
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conditions, in containers exposed to natural light (V₀ - used as reference – control- 100%) and the ones from variants V₁ – V₄ exposed to red, yellow, green or blue LED light.

Thus, both in the case of the experiments performed under septic (Fig. 5 A) and aseptic conditions (Fig. 5 B), it was observed for the spruce plantlets– compared with the control (V₀) – an increase in growth of the embryonary root, for the septic sample under green light LED, the increase being 6% and the hypocotyl size was increased by 12.2%. The yellow light has stimulated up to 3% the growth of the root and hypocotyl, the red light inhibited the growth of the root by 2.9% and of the hypocotyl by 2.4%.

![Figure 1. The aspect of spruce (Picea abies L.) plantlets, 14 days after transfer of seeds to germination medium. A – aseptic conditions: control (V₀), seeds germinated under natural light with northward exposure; B – septic conditions: germination in vitroculture regime. The experimental variants of the plantlets exposed to LED light were: red light - V₁; yellow light - V₂; green light - V₃ and blue light - V₄ (abbreviations: cot – cotyledons; h – hypocotyl; c – coleot; re – embryonary root).]

For the specimens exposed to blue light, the hypocotyl growth was inhibited by 5%. Thus, the green LEDs light has stimulated both the growth of the embryonary root and especially of the hypocotyl (and of course of the whole plant) whereas the blue light has inhibited this process.

The LEDs light (Fig. 5 B) acted in the same manner in the case of the in vitro grown cultivated variants, the percentage values being smaller compared with the same specimens that have been subjected to a varied lighting system. In the aseptic medium, the red light produced a 8.7% inhibition, mainly of the growth of the rootlets size.

In the histograms from figure 6 A and B are shown the results regarding the assimilating pigments content in the spruce leaflets from the plantlets subject to illumination – in septic and aseptic conditions – either with natural light (V₀ – control – 100%) or for the batches exposed to different color LEDs. In the histograms is presented the chlorophyll a and b content in the leaflets, the total green pigments content (obtained by summing the values for chlorophyll a and b) and carotenoids content and by adding all these values, the total content in assimilating pigments is obtained for the samples examined.
The results for both experimental categories performed in septic regime (Fig. 6 A) and aseptic regime (Fig. 6 B), showed a strong inhibition of the assimilatory pigments in all variants compared to the control \((V_0)\) with the exception of the carotenoid pigments extracted from the plantlets exposed to yellow LEDs, mainly in the experiment performed in aseptic regime (Fig. 6 B).

**Figure 2 A-F.** Transmission electron micrographs showing the tissue and cellular structure in the transversal sections through the cotyledons of the 14 days old spruce \((Picea abies L.)\) plantlets. A and B germination under natural light with northward exposure – \(V_0\). Seed germination and plantlet growth under LED lighting: \(V_1\) – red light ( C and D) and \(V_2\) (E and F) – yellow light (abbreviations: cit – cytoplasm; cl – chloroplasts; dv – electrodense vacuolar deposits; ep – epidermis; G – gap, N – nucleus; pa – assimilating parenchyma; cw – cell wall; V – vacuole).
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Figure 3 A-F. Cytological aspects observe dat the transmission electron microscope in the tissue cells identified in the transversal sections through the spruce (Picea abies L.) cotyledons in the 14th day of seed germination. Variant V3 (A and B) – green LED light exposure, variant V4 (C – F) – blue light exposure (abbreviations: cl – chloroplasts; cyt – cytoplasm; dv – electrodense vacuolar deposits; ep – epidermis; G – gap; mz – mezophyll; N – nucleus; pl – plastids; V – vacuole).

Figure 4. The aspect of the Spruce (Picea abies L.) plantlets in the 40th day from placing the seeds for germination in a northward facing window (V0 – control) or exposed to LED green light (V3) (abbreviations: c – colet; cot – cotyledons; epi – epicotyl; If – leaflet; hip – hypocotyl; re – embryonary rootlet).
Figure 5. The growth of the spruce (*Picea abies* L.) plantlets in septic conditions (5 A) and aseptic (5 B) from seeds germinated for 40 days exposed to natural light (V₀) and under different color LED light: V₁ – red light; V₂ – yellow light; V₃ – green light; or blue light - V₄. (the histograms show – in percentages – the data recorded for different variants and organs compared with the parameters of the control – considered 100%).

Figure 6 A and B. The assimilating pigments content in the spruce (*Picea abies* L.) plantlets leaflets taken after 40 days either from seed embryos germinated in plastic container son moistened filter paper, in septic regime (6 A) or „in vitro” on aseptic media (6B) being exposed for 16hrs/day to natural light (V₀ – control, reference values – 100%), or to ultrabright LED with different wavelengths. The experimental variants were as follows: V₁ – red light; V₂ – yellow light; V₃ – green light; or blue light- V₄.

It is interesting that the highest inhibition of all the assimilating pigments was noted when the spruce plantlets were exposed to green LEDs (Fig. 6 A and B) both for the batches maintained in septic or aseptic regime. This light has stimulated the growth in lenght of the organs compared with the control (V₀) and other color LEDs, but at this variant the leaves have undergone a etiolation process, similar to the plants kept in the shade that are more elongated but paler. Thus, from this poit of view, using such monocolor light in the vitroplantlets growth chamber is not recomendated to be used for prolonged lighting of the phyto-innocules. Besides, with the exception of the green light, all the other mono-color lights produced by LEDs not only didn’t have a positive effect on the spruce plantlet growth but also didn’t sustained the assimilating pigments synthesis. Only the lighting of the vitrocultures with yellow light was able to maintain the carotenoid pigments to the same level as the one in the control (V₀).

The blue light from the LEDs has decreased only by 3.7% the size of the plantlets organs and had less inhibition on the assimilating pigments from the spruce leaflets. The yellow light
has reduced the level of chlorophyll \( a \) in the leaflets and had a moderate inhibition on the accumulation of chlorophyll \( b \).

In turn, the red light inhibited to a lesser extent – both in the samples from a septic regime and in the \textit{in vitro} cultivated ones – the accumulation of chlorophyll \( a \) in the spruce plantlets leaves.

Therefore, compared with the spruce plantlets exposed to natural light, in the plantlets exposed to mono-color light from ultrabright LEDs even if such light has produced only a slight decrease in the growth, it severely affected the synthesis and accumulation of assimilating pigments in the leafs. This happened even in the experiments performed with \textit{in vitro} cultures where the plantlets were on a substrate containing all the nutrients necessary to sustain the metabolism and assimilating pigments synthesis processes.

4. Conclusions

The results from the present study demonstrate that the green light stimulates the spruce seed germination and plant growth whereas the blue light inhibits hypocotyl elongation. The results from the chosen experimental model to examine the effect of different color LEDs light on the spruce plantlets were similar with the ones reported for other plantlet types subjected to various lighting conditions.

If in the first 40 days post-germination, the growth of the spruce plantlets was affected to a lesser extent by the color of the LED light, the leaflets content in assimilating pigments was severely affected.

However, for a shorter time period, both the growth of the spruce plantlets under septic or aseptic regime (on appropriate growth media) can be done under lighting by mono-color LEDs as such system is more economical.

This \textit{in vitro} culture procedure can be used in micropropagation units that have phyto-innocules in stock, in a temporised growing regime, increasing this way their subcultivation interval, the procedures being more economical.

References


