A comparative study of various light source influences on the plants regenerative potential using Populus berolinensis root explants as an example

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Abstract. The paper presents the results of a comparative study of the effects of three light source types on micropropagation efficiency of Berlin poplar (Populus berolinensis). As a model explant type for regeneration, root segments were used. Three types of light sources were compared: fluorescent cool white light lamps, LED-based purple (red+blue) light phyto lamps and LED-based neutral white light phyto lamps. The poplar regeneration was more effective under fluorescent lamp illumination treatment then at both types of LED phyto lamps. Among two studied LED light sources, the best result was obtained for white light lamps. The quantity and size of regenerants obtained under LED white light lamps illumination were comparable with those got under fluorescence lamps. The least regeneration efficiency was observed under the purple light LED lamps illumination with a limited spectrum range of emitted light. Nevertheless, in case of discontinuation of fluorescent lamps due to the policy of efficient energy consumption and environment protection, optimized LED phyto lamps illumination has prospects in micropropagation of plants.

1. Introduction

Light plays an essential role in the plants life. Light intensity, its spectral composition, and photoperiod play fundamental importance to photosynthesis and photomorphogenesis processes [1]. The electromagnetic radiation spectrum contributing the photosynthesis is referred to as photosynthetically active radiation (PAR) and is in the waveband between 400 and 700 nm [1, 2, 3]. However, the light of different spectral ranges is absorbed differently, so not all wavelengths are equally efficient in photosynthesis. Despite the reaction centers of the photosystems are activated by the red light only (700 or 680 nm, for I and II photosystems, respectively), light-harvesting complexes are able to absorb in almost the entire region of the visible spectrum. Chlorophylls absorb effectively the red and blue parts of the spectrum, which are the most effective for photosynthesis. However, a significant loss of blue light energy due to radiation capture by non-photosynthetic pigments and inefficient energy transfer by accessory pigments makes it much less effective than red light [1]. The spectral light composition is very important not only for photosynthesis but also for photomorphogenesis which is also referred to as light-mediated development of plants. Regulation of growth, development of leaves and roots, flowering, fruits ripening, seed germination, shade-avoidance, de- etiolation, circadian entrainment, phototropism, chloroplast movement, stomatal opening may be mediated by photoreceptors are sensitive
in red–far-red (600 - 750 nm); ultraviolet-A-green (320 - 520 nm); ultraviolet-A-blue (320 - 500 nm) ranges of the radiation spectrum [1, 4].

The classic light source applying in micropropagation and biotechnology of plants is fluorescent gas-discharge lamps which irradiate white light containing the light waves normally from the wide range of the visible spectrum. Since the beginning of their industrial production, several modifications different in size, shape, and power were released to the wide market. Among them, there are linear lamps with three main types of tube’s diameter: big T12 (38 mm) and T10 (32 mm), medium T8 (25 mm), and small T5 (16 mm).

One of the significant shortage of large and medium gas-discharge fluorescent lamps is increased heat emission emitted by their ballasts, which temperature may reach 60-80 °C. Inhomogeneous and excessive heating of the lamp causes the temperature increasing of the shelf surface located above, which complicates the design of the shelf stands for plant cultivation. To prevent the surfaces overheating, the thick heat-insulating materials or perforated shelves are applied. Both options lead to several inconveniences, consisting either in the loss of usable space or in the instability of plant vessels on the lattice- or ladder-shaped horizontal surfaces. In addition, increased heat emission from the ballasts of fluorescent lamps may lead to an inhomogeneous temperature in the climate room, as well as overheating and even death of plant material in case of the air condition system fail. The T5 luminescent lamp cases have the least heating of surrounding space and are a promising source of lighting for micropropagation of plants and biotechnology. Thus, the selection of the light source for plant growing climate rooms, which has the appropriate radiation spectrum, reduced energy consumption, and heat dissipation, is an important task of plant biotechnology. Moreover, the line spectrum of light from a fluorescent lamp only simulates a continuous spectrum of natural light, and beam intensities in different parts of the spectrum are often unbalanced for plants.

Due to the progress of the electronic industry, the applying of light-emitting diodes (LEDs) for light source development becomes more popular in different life fields and takes place in plant biotechnology as well. In addition to the obvious advantages of LED-based lamps, they have several disadvantages. In the so-called ‘phyto lamps’ with simultaneous emission in blue and red wavelength range, the major portion of the light spectrum which is also important for plants is omitted. Moreover, the purple light irradiated by this type of lamps is very uncomfortable for human eyes. In the case of autonomous agro-industrial installations, this is not a significant problem due to automatization and robotization of the production, but if used for experimental research, this creates certain difficulties associated with the presence of the operator in the climate chamber. Some studies with daily monitoring of plants or plant material require the operator’s presence in the climate room for several hours. In the case of such purple phyto lamps application, the exposition to the deep purple glow could result in the researcher’s eyes overwork and, possibly, may negatively affect his health.

Another popular approach in plant biotechnology is an application of LED-strips. Such strips were originally produced for indoor and outdoor design, but due to relatively cheap cost found application in the plant biotechnology. However, there is one significant drawback that is related to the fact that manufactures of LED-strips were not focused on their application in plant biology where reproducibility of the results and stable equivalent conditions in experiments are required. All LED-strips are often made from heterogeneous LEDs that differ in brightness and spectral composition, which in turn is not acceptable in conditions sensitive experiments with plants. The same problem may be also considered for fluorescent lamps, which often differ from each other depending on the batch and manufacturer. That’s why often different lamps with different characteristics may be found on the one shelf for micropropagation of plants in the laboratories worldwide. This problem can be easily solved by using certified fluorescent or LED-based ‘phyto lamps’ from the same series with known characteristics of brightness and emission spectrum. One of the modern solutions is using LED-based white light phyto lamps due to their full-spectrum and comfort for the human eyes.

Another reason for the increased attention to LED-based light sources among plant biotechnologists is the policy of the transition to environmentally friendly production and the rejection of mercury-containing lamps. The governments of a number of countries have already begun the policy of reducing
the production of mercury-containing lamps and the transition to energy-saving technologies using LEDs. In addition, LED light sources have huge perspectives for their usage in space exploration including plants growing aboard space stations. Therefore, work aimed at studying the application of LED-based light sources in plant biotechnology is a promising task and is important in the transition to an energy-efficient and environmentally friendly lifestyle.

The aim of the present study was a comparative assessment of the influence of three different light sources to the regeneration efficiency of plants using Berlin poplar as an example.

2. Materials and methods

2.1. The plant material

One of the convenient objects for woody plants micropropagation studies is Berlin poplar (Populus berolinensis). It has a high percentage of rooting (99%) after the top part cutting during in vitro cultivation, takes up little space in test tubes due to narrow leaves (in vitro only), and also easily regenerates from different types of explants. Regeneration was induced on the root segments because that type of explants showed the high regenerative potential in the previous study [5].

2.2. Nutrient media preparation and experiment design

Nutrient media for regeneration was prepared on the base of ½ MS5524 [6] manufactured by Sigma-Aldrich with iron chelate and microelements addition to obtaining the full MS medium composition. The base medium was supplemented by thiamine (1 mg/L), pyridoxine (0.5 mg/L), nicotinic acid (0.5 mg/L), and meso-inositol (50 mg/L). Sucrose (2%) was used as a source of carbohydrates. To obtain a solid medium, agar (A7002, Sigma-Aldrich) was used at a concentration of 7 g/L. The acidity of the medium was adjusted to pH 5.7 [5, 7]. As a regeneration induction agent, kinetin in a final concentration of 0.5 mg/L was used. Our previous results [5] showed that the mentioned concentration of kinetin was the most effective for the regenerants formation on the poplar root explants. Freshly prepared media was autoclaved (15 min at 121 °C) and dispensed by 50 ml into sterile polycarbonate GA-7-3 culture vessels (V8380, Sigma-Aldrich).

Nine root explants (1-1.5 cm length) per each vessel were placed on the surface of solid medium precooled to room temperature (24 °C). The explants were incubated at 24 °C for 25 days in an air-conditioned cultivation plant room with 16/8 hours of the day/night photoperiod. Each shelf with one type of light lamps was placed one under another in the rack panel avoiding the reaching light from the other lamp types placed above or below. The surfaces of the shelves were thermo-insulated from the lamps located below, by cellular PVC panels. Open type of shelf constructions assured the proper ventilation and equal stable temperature on each shelf.

2.3. Light sources characteristics

Three types of lamps were chosen for the present study. The classic low-pressure mercury-vapor gas-discharge lamps and two types of LED phyto lamps were used to regenerate plants from poplar root explants. The main characteristics of each lamp are presented in table 1.

| Lamp type | Light source/element types | Brand | Power, W | PPF, µmol s⁻¹ | Light color/color temperature | Light type |
|-----------|-----------------------------|-------|----------|----------------|-----------------------------|------------|
| LED lamp (PPG T8s Agro) | LEDs / 2835 SMD | Jazzway | 15 | 11.2 | purple | bichromatic: 650 and 450 nm spectrum (400 – 700 nm) with peaks at 445 and 660 nm |
| LED lamp (ULI-P11) | BIO-LEDs / FM-P3528WBR-BS | Uniel | 35 | 49 | neutral white / 4000 K | |
| Fluorescent lamp (T5) | electric arc in mercury vapor / TL5 HE 28W/865 lamp | BMC | 28 | 32 | cool white / 6500 K | spectrum (400 – 700 nm) |

Table 1. Characteristics of the light sources (per single lamp).
Each shelf (60×120 cm) for micropropagation was equipped by the five same type lamps orientated parallelly to each other with 12 cm of axel spacing. The distance from the lamp level to the surface of the shelf with experimental vessels was 20 cm. The illuminance directly on the shelf surface under different light sources was measured by CEM DT-1309 digital light meter are presented in table 2.

| Lamp type                      | Illuminance (lx) |
|--------------------------------|------------------|
| LED lamp (PPG T8i Agro)        | 3 700            |
| LED lamp (ULI-P11)             | 9 900            |
| Fluorescent lamp (T5)          | 5 050            |

3. Results and discussion
Obtained results showed that all the studied light sources facilitated Berlin poplar regeneration from root explants on a nutrient medium supplemented with kinetin (0.5 mg/L) (figure 1). The highest poplar regeneration efficiency was observed under T5 fluorescent lamps treatment (figure 1, c). The obtained regenerants were larger compared to those obtained under LED lamps illumination (figure 1, a and b). The regeneration efficiency of poplar under light treatment by T5 lamps was similar to that was shown for larger T8 (25 mm) lamps in our previous study [5]. Taking into account that ballasts for T8 lamps have an increased heat emission, T5 lamps case are more preferable for micropropagation of plants because they simplify the design of the shelves and do not cause additional overheating of the climate room.

Among two studied LED light sources, the best result was obtained for white light lamps. The quantity and size of regenerants obtained under LED white light lamps illumination were comparable with those got under fluorescence lamps. The least efficiency the purple (red+blue) light LED phyto
lamps with a limited spectrum range of emitted light was demonstrated (figure 1, b). It should be also noted that on the contrary to the fluorescent lamps in the case of using purple and white light LED lamps, the red pigmentation leaves and original root explants. Anthocyanins are non-photochemical active flavonoid pigments and their presence is mainly limited to peripheral tissues and structures, such as upper mesophyll, exposed to strong light radiation. Their synthesis and accumulation in tissues may be initiated in response to enhanced light emission for absorbing the excess radiation reaching the plant to minimize the risk of cellular oxidative damage [8]. This fact may be due to unbalanced light spectrum or its intensity for poplar.

In order to reduce the anthocyanins accumulation of regenerants obtained with white light LED phyto lamps, the decreasing of illumination by reducing the lamp numbers per shelf should be tried. It can be assumed that a decrease in illumination to the level of 5000 lx when using LED white light lamps may help to decrease the stress load to plants and avoid the anthocyanins accumulation in tissues. This assumption requires additional experimental studies.

Even though the purple light LED phyto lamps also induced the regenerants formation they can be less recommended for micropropagation of Berlin poplar from the root explants due to low numbers and small sizes of obtained regenerants. The installation of additional sources of white light containing a wider range of the spectrum probably can help to increase the efficiency of regeneration or decrease an anthocyanins accumulation. Moreover, eye discomfort caused by deep purple glow is an important factor reducing the value of this lamp type for the use in the experimental practice as the main light source for plant growth. However, these lamps can undoubtedly be used in the applications related to the studying of the spectral light composition influence on the regeneration of plant tissues.

4. Conclusion
The poplar regeneration from root segments was more effective under fluorescent lamp illumination treatment then at both types of LED phyto lamps (white and purple light). T5 lamps and their cases with low heat emission may be successfully used instead of T8 lamps with high heat-producing ballasts for Berlin poplar micropropagation. Among two studied LED light sources, the best result was obtained for white light lamps. The quantity and size of regenerants obtained under LED white light lamps illumination were comparable with those got under fluorescence lamps. The least efficiency for the purple (red+blue) light LED lamps with a limited spectrum range of emitted light was demonstrated. In case of discontinuation of fluorescent lamps due to the policy of efficient energy consumption and environment protection, optimized LED phyto lamps illumination has prospects in micropropagation of plants. White light LED lamps are more profitable and convenient to use in climate chambers compared to purple light lamps, which make complicated the presence of the operator in the room and require additional upgrades related to switching the light to white.

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