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STUDIES REGARDING THE INFLUENCE OF DIFFERENT WAVELENGTHS OF LEDS LIGHT ON REGENERATIVE AND MORPHOGENETIC PROCESSES IN IN VITRO CULTURES OF *ECHINOPSIS CHAMAECEREUS F. LUTEA*

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ABSTRACT. *Echinopsis chamaecereus f. lutea* is a yellow, ornamental cactus species, which belongs to the group of mutant, chlorophyll-deficient cacti. Their inability to synthesize chlorophyll makes these chlorophyll-deficient cacti survive only if they are grafted onto adequate stock which contains chlorophyll. Chlorophyll-deficient cacti are multiplied through "in vitro" cloning. With regard to the establishment of an "in vitro" culture of *Echinopsis chamaecereus f. lutea*, from the parent plant grown in the greenhouse, we sampled 1-cm explants, which were used as stem segments that were inoculated on an aseptic agarose medium with macroelements and Murashige-Skoog FeEDTA (1962), Heller microelements (1953), vitamins – pyridoxine HCl, thyamine HCl, and nicotinic acid (1 mg/l of each) – m-inositol and sucrose, without growth regulators. In the grow room, the tissue cultures were illuminated with light-emitting diodes (LEDs) of different colors (yellow, red, green, blue or white), with a light intensity of 1000 lx. The evolution (rhizogenesis, callogenesis, and caulogenesis) of the in vitro cultures was monitored for 90 days, tracking the differences in reactivity to the different wavelengths of LEDs illumination. Cultures exposed to white light emitted by fluorescent tubes served as the control sample. After 90 days, compared to the differentiation of the control sample exposed to white light from fluorescent tubes, the samples grown in the presence of green or blue LEDs demonstrated statistically significant increases in the growth of the stems; under red or green LEDs illumination, calusogenesis intensified, while under white or yellow LEDs illumination, the rate of development of the stems was not significantly different from the growth of the control samples. Rhizogenesis was not observed in any of the samples.

Keywords: cacti, in vitro culture, LEDs light source

INTRODUCTION

Echinopsis chamaecereus f. lutea is a yellow, chlorophyll-deficient cactus species. According to Shemorakov (2003), such species have a deficit in the number of chloroplasts present in a cell. Approximately 1/3 of all plastids are chlorophyll-deficient and these types of cacti are unable to carry out photosynthesis. In order to propagate these species of cacti, they must be grafted onto (port graft) stocks of *Cereus, Echinopsis, Eriocereus*, or *Phyllocactus* (Copăcescu, 2001); in this case, *Echinopsis* is grafted onto stock of *Hylocereus triangularis*, cultivated in pots.

Through somatic embryogenesis, the chance that these plants keep their original color is minimal. According to Kornilova (2008), in order to preserve the color of the explant, these plants must undergo vegetative reproduction.

Although chlorophyll-deficient cacti are incapable of photosynthesis, the quality, intensity, wavelength, and incident direction of the light source as well as exposure duration are factors that influence the initiation and development of the principal physiological processes (respiration, transpiration, the absorption of water and nutritional elements, seed germination, growth and development, etc.; Winslow, 2002).

The visible range of the electromagnetic spectrum represents only 40-50% of the total emitted solar radiation. Visible light has a spectrum of colors with wavelengths between 380 and 780 nm: violet (380-400 nm), indigo (400-480 nm), blue (480-500 nm), green (500-580 nm), yellow (580-600 nm), orange (600-650 nm), and red (650-780 nm) (Catrina et al., 1987).

Normally, in vitro cultures are illuminated using white fluorescent tubes. The disadvantage of these tubes is that they produce a large quantity of heat as a byproduct and thus require that the grow rooms are ventilated. Cooling the air in the grow rooms increases the cost of production by increasing the consumption of electricity. The advent of light-emitting diodes (LEDs) technology opened avenues for the replacement of fluorescent tubes with another, more efficient light source.

An LED is a small electronic device that emits light of a specific wavelength without producing thermal energy (infrared). Additional advantages of the use of LEDs in vegetative reproduction include the ability to bring the light source close to the illuminated object and the ability to dissipate, concentrate, or even pulse

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the emitted light. Moreover, LEDs are 30% more energy-efficient than incandescent lamps (Jagers, 2007).

In our experiment, we analyzed the differences in reactivity (morphogenetic activity in the form of rhizogenesis, callogenesis, and caulogenesis) of in vitro cultures of *Echinopsis chamaecereus f. lutea* to the different wavelengths of LEDs illumination (blue, yellow, green, red, or white light).

MATERIALS AND METHODS

The biological material used in our experiments consisted of young, spherical, cauline formations from stems of *Echinopsis chamaecereus f. lutea* (Fig. 1), which were then grafted onto *Hylocereus triangu-laris* stock. The spherical explants where approximately 1 cm in length and 0.5 cm in diameter (Fig. 2).

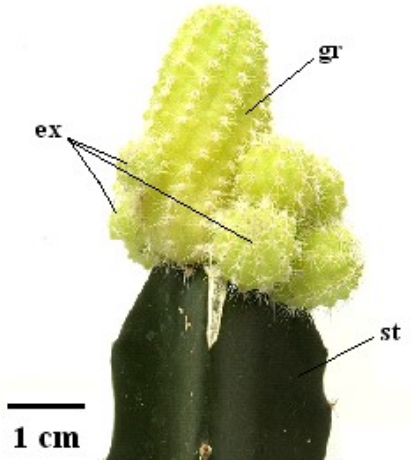

Fig. 1 The parent plant, a young specimen of *Echinopsis chamaecereus f. lutea* grown in the greenhouse, used for the sampling of explants which were inoculated "*in vitro*" (legend: gr-graft; st-stock; ex-explant).

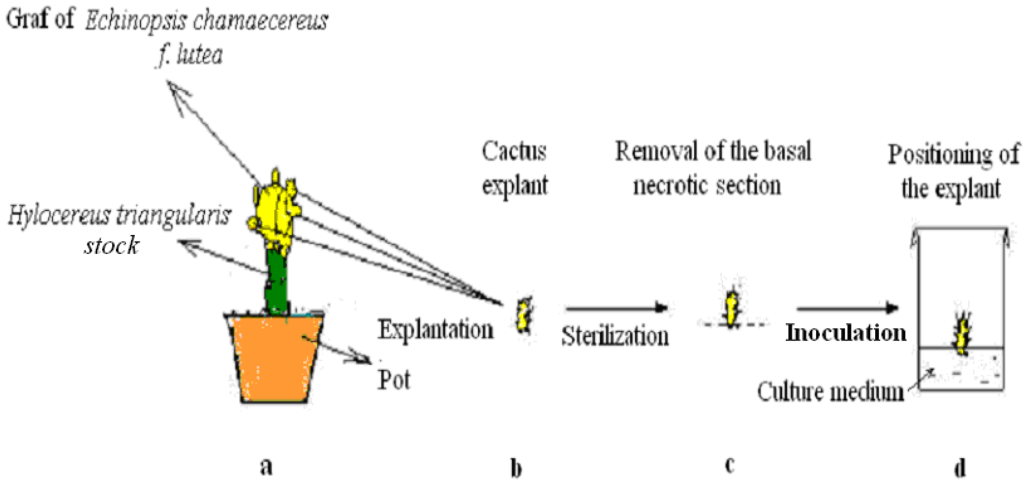

Fig. 2 Schematic representation of the methods used for explanting the fragments of *Echinopsis chamaecereus f. lutea* and the inoculation of the fragments on aseptic culture media (a - *Echinopsis chamaecereus f. lutea* parent plant grown in the greenhouse grafted onto *Hylocereus triangularis* stock; b-explant; c - removal of the basal section of the explant affected by the disinfectant; d–inoculation of the explant)

For the sampling of the explants, the parent plant was sterilized by submersion in 96° ethyl alcohol for one minute followed by submersion in a 0.4% solution of sodium hypochlorite with a few drops of Tween 20 added as a surfactant. During sterilization, the vegetal tissue was agitated continuously (*Cachită et al.,* 2004). After 20 minutes of sterilization, the sodium hypochlorite solution was removed and the vegetal tissue was rinsed – five times – with sterile, distilled water for five minutes each bath. Inside of box with laminar sterile air, flow the vegetal material was deposited onto sterile filter paper (sterilized in a dry-heat oven) on sterile Petri capsules until the excess water evaporated. Also under aseptic conditions, the necrotic portions – those affected by the sodium hypochlorite (Fig. 2c) – were removed and the viable

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portions were inoculated (Fig. 2d) vertically, inside sterile vials, with the basal, sectioned region immersed in the culture medium. The culture medium consisted of macroelements and *Murashige-Skoog* FeEDTA (1962), *Heller* microelements (1953), a mix of minerals with the vitamins pyridoxine HCl, thyamine HCl, and nicotinic acid added (1 mg/l each), m-inositol (100 mg/l), sucrose (20 g/l) and agar-agar (7 g/l). The pH of the growth medium was buffered at 5.8 before autoclaving; the culture medium did not contain any growth regulators. The vials containing culture media were sterilized by autoclaving at 121°C for 30 minutes. The 15-ml vials capacitiy made of clear, translucent glass contained 5-ml of culture medium each. After the inoculation of the explants, the glass recipients (containing one explant per vial) were covered with clear, translucent polyethylene foil affixed using rubber bands. Then, the recipients were inserted into wooden boxes which were then covered with wooden lids whose internal surface was equipped with a monochromatic LEDs installation (**red**, **blue**, **yellow**, **green**, or **white**). The dimensions of the boxes were 17 x 48 x 14 cm. Irrespective of the color of the LEDs installation, the light intensity inside the box was set to 1000 lx; the photoperiod was 16 hours out of a 24-hour cycle; the temperature oscillated between 24 and 27 °C. For the wavelength and quantized potential energy of the different color lights emitted by the LEDs, please see Table 1. Control samples were exposed to **white** light emitted by *fluorescent tubes* with a light intensity of 1700 lx and varying temperatures between 20 and 24 °C.

Table 1

Wavelength and quantized potential energy of the different colors of the visible spectrum (*Tarhon*, 1992). (Note: eV-electron-volt; kJ/mol-kiloJoule/mol)

Color	Wavelength (nm)	Quantified energy		
		kCal/mol	eV	kJ/mol
Blue	450	63.5	2.76	260.6
Green	500	57.2	2.48	230.5
Yellow	550	52.0	2.25	206.6
Red	700	40.8	1.77	176.4

The reaction of the inoculi was followed for *90 days*. On *days 60* and *90*, the growth and development of the explants was evaluated. Representative images of particular aspects of the *in vitro* cultures of *Echinopsis chamaecereus f. lutea* explants, at *90 days*, are presented in figure 3, panels A-F. For the LEDs experimental conditions, we calculated the percentage of surviving inoculi, the diameter of the basal region, and the percentage of inoculi that exhibited callogenesis; all variables for the experimental conditions were operationalized as a function of the values recorded for the **white** light, *fluorescent tube* control samples.

RESULTS AND DISCUSSIONS

After 90 days of explants inoculation, the viability statistic remained steady, with 100% survival of the remaining inoculi even if in the previously sectioned regions showed small brown lesions (Fig. 3A and 3C) suggestive of early-stage necrosis.

At 60 days post-inoculation, observed measurements indicated that only the diameter of the inoculi that were exposed to blue, yellow, or green LEDs illumination surpassed that of the control group (Fig. 4A). Compared to the control sample, the blue LEDs-illuminated sample grew 1.4%, the yellow LEDs-illuminated sample grew 1%, and the green LEDs-illuminated sample grew 16%. Diameter growth in the white LEDs-illuminated sample was 2.8% lower than in the white fluorescent tube control sample; growth in the red LEDs-illuminated sample was 25.7% lower than in the control sample. Our results are compatible with findings by Miler and Zalewska (2006), who observed that red LEDs illumination impeded the in vitro growth of chrysanthemum stems while green LEDs illumination spurred the growth of the same species, as determined by net wet weight of the explants.

Our experimental measurements at 60 days also revealed a 70% increase in callogenesis for explants exposed to green LEDs light as compared to the control explants exposed to fluorescent white light. Samples exposed to red LEDs light exhibited a 100% increase in callogenesis compared to the control explants. Explants exposed to blue, yellow, or white LEDs illumination had no evidence of callogenesis.

Rhizogenesis and *caulogenesis* respectively, were not observed in any of the LEDs samples.

Observations 90 days after the inoculation of the explants of *Echinopsis chamaecereus f. lutea* exposed to green LEDs light revealed that the average diameter of the explants remained at the value observed 60 days post-inoculation. Thus, the total growth of explants exposed to green LEDs light was 16% higher than the growth of control explants exposed to the white light emitted by fluorescent tubes. At a 90 day, the growth of explants exposed to blue LEDs light was 4.2% higher than the growth of the control explants. These results are supported by previous observations obtained by Michalczuk and Michalczuk (2000) with in vitro cultures of Petunia plants. In the case of *Sequoia sempervirens* grown in vitro, under similar laboratory conditions, Pop and Cachiţă (2007) found – compared to the control condition (white light from fluorescent tubes) – that green LEDs exposure resulted in the largest increases in the size of the in vitro stems (18%).

In our experiment, yellow, white, and red LEDs illumination of *Echinopsis chamaecereus f. lutea* inhibited total growth by 2.8% as compared to the control condition (Fig. 4A). Despite this disadvantage with respect to growth, the inoculi exposed to red LEDs light exhibited the largest increases in percentage of callogenesis (100% compared to the control condition), with explants exposed to green LEDs light faring second best (77% total callogenesis). Blue, yellow, and white LEDs light exposure produced no callogenesis.

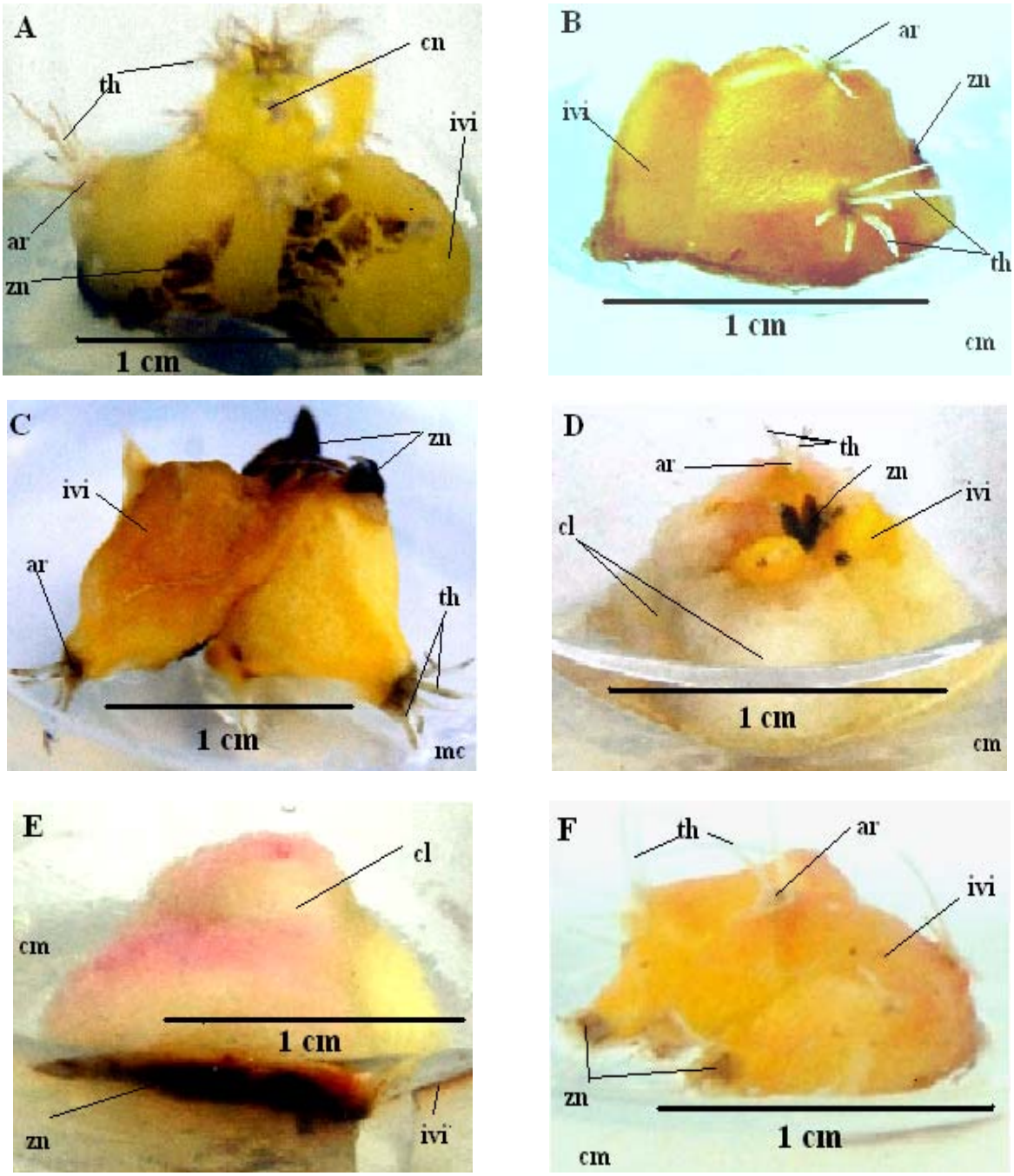

Fig. 3 *In vitro* cultures of *Echinopsis chamaecereus f. lutea* explants on aseptic culture media: **A -** explants grown in the presence of **white** light emitted by *fluorescent tubes* (control sample); panels **B-F** - explants grown in the presence of LEDs illumination; **B** - **white** light, **C** - **blue** light, **D** - **yellow** light, **E** - **green** light, **F** - **red** light (ivi = initially viable inoculi; cn = caulinary neoformations; th = thorns; cl = callus; zn = zone of necrosis; cm = aseptic culture medium).

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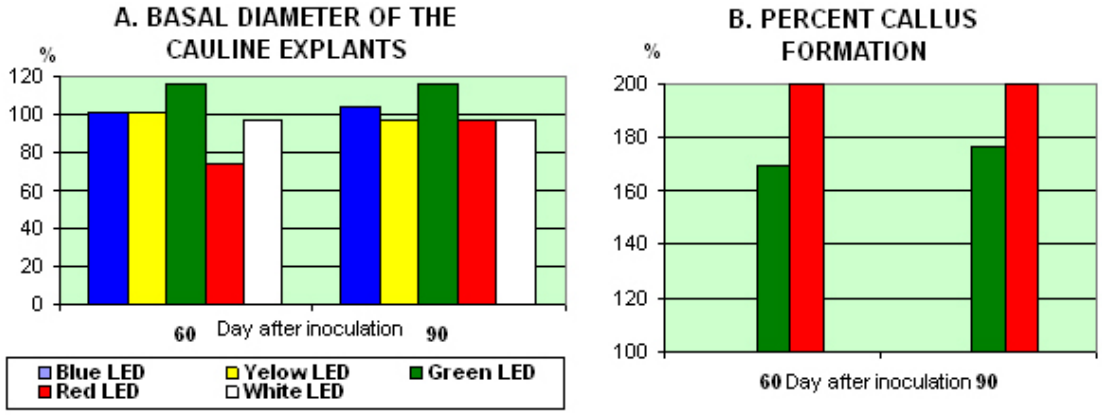

Fig. 4 Growth of the basal diameter of the cauline explants of *Echinopsis chamaecereus f. lutea* in the presence of different colors of LEDs illumination (data expressed in percentages based on values normed to the results observed in the control sample, which was exposed to **white** light emited by *fluorescent tubes*).

CONCLUSIONS

At *90 days* after the initiation of *in vitro* cultures of *Echinopsis chamaecereus f. lutea,* the average diameter of the explants reared under **blue** or **green** LEDs illumination surpassed the growth in the control condition (**white** light from *fluorescent tubes*) by 4.2% and 16%, respectively. Illumination of the *in vitro* cultures with **yellow**, **red**, or **white** LEDs light, resulted in inhibited explants growth (2.8% decrease) compared to the control condition. *Callogenesis* was stimulated by **green** and **red** LEDs-illuminated rearing. *Rhizogenesis* and *caulogenesis* were not present in any of the LEDs samples. Depending on the color of the LEDs illumination to which the explants were exposed, different rates of development were observed: - in **yellow**, **white**, and **red** LEDs-illuminated explants, the growth of the *basal diameter*, *90 days* post-inoculation, presented no statistically significant variation from the growth observed in the control samples reared in **white** light from *fluorescent tubes;* - **green** and **red** LEDs-illuminated rearing both favored *callogenesis*, producing an increase of 77% and 100% with respect to the rates observed in the control condition.

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