DETERMINATION IN ASSIMILATING PIGMENT CONTENT OF THE *Echinocactus* (Pfiff.) *mihanovichii* FROM EXPOSED VITROCULTURES TO LIGHT OF DIFFERENT COLORS AND WAVELENGTHS EMITTED BY FLUORESCENT TUBES OR *LIGHT-EMITTING DIODE* (LED)

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Abstract

Deficient cacti chlorophyll – occurring spontaneously in cultures as a result of mutations, a phenomenon largely influenced by temperature and light, the number of chloroplasts in these species does not normally exceed one third of all plastids, the rest is mutated and are without possibility to synthesize chlorophyll. It is known that in vitrocultures, the light react differently depending on the species and the source of used radiation, an optimal light regime applied to cactuses influencing the nature and the pigments in plastids. In the current experiment of Echinocactus mihanovichii explants inoculated on culture media without growth regulators, were exposed to white or colored fluorescent tubes light, also at the high brightness of LEDs - Ultra bright – of white or colors used in fluorescent lighting of vitrocultures, respectively, blue, yellow, green or red.

After 90 days of culture in vitro, we realized extraction assimilatory pigments - chlorophyll a and b and carotinoid pigments - separated from each experimental variant grown under UV lights of different colors and wavelengths and compared it with similar parameter values to witness group - vitrocultures illuminated by white fluorescent tubes (V_0) - benchmarking data found that, as 100%. The results were different depending on the source of light and color to which they were exposed, but we can say that the amount of accumulated carotinoid pigments in the explants of Echinocactus mihanovichii was in most cases higher than that of green pigments (chlorophyll a and chlorophyll b).

Keywords: cactus, vitrocultures, fluorescent tubes, LEDs, carotinoid pigments.

INTRODUCTION

In carrying out several physiological activities of plants it has been adopted to exploit light energy, their evolution over acquiring a wide range of photoreceptors that perceive and respond to the stimulus of the light spectrum, quantifying quality and light intensity, light direction and light period (Winslow, 2002). Vitroplantlets existence due to the presence of carbohydrates in the culture medium isn't strictly conditioned on the achievement of photosynthesis, which is mixotrophs, but the light affects a series of physiological reactions, phenomena closely related to optical features of chlorophyll (Mustaţă and Mustaţă, 2003).

Visible spectrum of solar radiation, photo energical and photo-chemical effects exercising on plants include the following emission wavelengths (in nm): 380-400 (purple), 400-480 (indigo), 480-500 (blue), 500 - 580 (green),

580-600 (yellow), 600-650 (orange) and 650-780 (red) (Catrina and Popa, 1987). Light does not have the same kind of influence on all plants, it act differently depending on the species and the source of radiation used, such use of *light-emitting diode* as an alternative source of lighting in vitrocultures is becoming increasingly used, particularly after discover the benefits arising from their use. According to (Van Ieperen and Trouwborst, 2008), artificial light sources can be calibrated to emit a specific wavelength so that to deliver plants evolving an optimal light conditions, they also seek through filters can stop UV radiations, and their light can be directed or can choose the most efficient viewing angle for plants. At present there are studies to determine how much photons with a specific wavelength, induce growth and development of plants (Jagers, 2007).

Chlorophyll-deficient cacti, or their various aberrant forms, ensures a completely unusual occurrence but highly appreciated, as it is explained that, their revaluation has become a real industry, highly profitable, in countries like Holland, Japan, Korea, Germany and others where he currently is seeking new technologies for rapid and economically efficient multiplication of these plants (Son, 2000). *Echinocactus mihanovichii* is a chlorophyll-deficient species of cactus, decorative by the trumpet-shaped flowers, yellow - green, white or pink (Fig. 1), depending on variety (Copăcescu, 2001) and the stem port due to flat shape - globular (Fig. 1B), green-gray, reddish brown, red or purple.

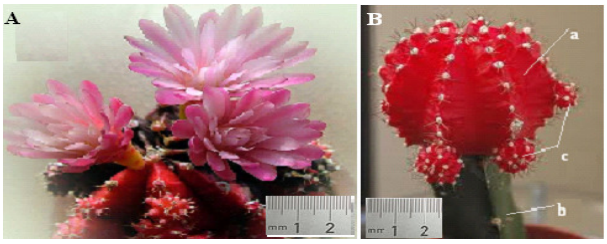

Fig. 1. *Echinocactus* (Pfiff.) *mihano-vichii*, **A**-decorative plant with flowers, **B**-orna-mental plants by stems (where: **a** - slips, **b** - rootstock of *Hylocereus trian-gularis*, **c** - buds).

The present research paper is used chlorophyll cactus as biological material - represents a continuation of experiments performed by us (Vidican and Cachiță, 2010). Stated purpose was to study changes in the assimilatory pigment content of *Echinocactus mihanovichii* vitrostems illuminated - for *90 days* – by fluorescent or LED lights issuing various colors compared to determine the level of these pigments with similar cultures illuminated by white fluorescent light.

MATERIALS AND METHODS

The plant material was sterilized - by submersing – in 96 º ethyl alcohol for one minute, after which it was covered with a solution of 0.8% sodium

hypochlorite, in a ratio of 1:2, mixed with sterile water to which were added - like three drops of surfactant Tween 20 (Cachiță et al., 2004), after continuous stirring for 20 minutes.

Sizing future inocula (Vidican and Cachiță, 2010), so that each explant was made to hold 3-4 areolae. Inoculation of explants was done on a basic medium (MB) (Murashige and Skoog, 1962), microelements (Heller, 1953), to which were added vitamins: pyridoxine HCl, thiamine HCl and nicotinic acid (each 1 mg/l), 100 mg/l myo -inositol, 30 g/l sucrose and 7 g/l agar - agar, pH of the medium - prior autoclaving it - has been set at a value of 5.7. Culture media were without growth regulators.

The vials with inocula were transported and deposited in the growth chamber, where the temperature ranged from 20 to 24ºC, and in this experiment to illuminate the Echinocactus mihanovichii vitrocultures, it was used as a source of light – classical light, ie white or colored fluorescent tubes, also high brightness white or colored LEDs - Ultra Bright - used in fluorescent lighting of the in vitro cultures, respectively in blue, yellow, green or red color.

For this purpose LEDs were placed in growth chambers made from wood, which are placed at a distance of 1 cm from the vials with inocula, the cap and wooden walls were lined with reflective aluminum foil (Fig. 2).

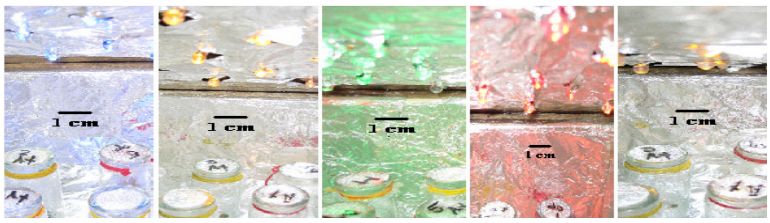

Fig.2.*Echinocactus mihanovichii* vitrocultures, grown in growth chambers (boxes lined with aluminum foil), issuing illuminated LED lights, colored and different wavelengths, respectively, blue - 470 nm, yellow-580 nm, green-540 nm, red-670 nm and 510 nm-white.

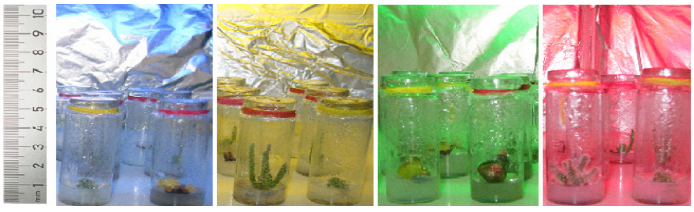

Fig. 3. Vitrocultures of *Echinocactus mihanovichii*, illuminated by *fluorescent tubes* emitted by colour lights and different wavelength, respectively, *blue*-470 nm, *yellow*-580 nm, *green*-540nm, *red*-670 nm.

In order to obtain a DC voltage, for operating the LEDs, power supply facility was provided by a network processor. Adjustment of light intensity was done by using a voltage regulator mounted in each growth chamber, known as the technical data supplied by the company producing LED, such that the different colored light sources, requires different voltages. In the

case of vitrocultures exposed at fluorescent lighting, with different colors (Fig. 3), adjusting the light intensity – 1200 lux - at the base of inocula was achieved with the help of a light meter, and the vials with culture was adjusted at the distance from the lighting source, and their location in the places established for conducting experiments.

Depending on the source of light the inocula were exposed have obtained two experimental series, as follows:

S1- artificially illuminated vitrocultures with fluorescent lamps generating light of different colors;

S2 - vitrocultures illuminated artificially by LED lights generating various colors.

In order to compare the obtained results in the case of extracts from lots of *Echinocactus mihanovichii* vitrocultures were reported to similar values reported in the control group samples - vitrocultures illuminated by white fluorescent tubes (V_0) - data considered as reference values 100%. Depending on the color of the light generated by fluorescent tubes or LEDs, in each experimental series we obtained several variants, as follows:

S_1 Series: V_0 - white fluorescent lighting - control group(λ=400nm); N_1-blue fluorescent light(λ=470nm); N_2-yellow fluorescent light(λ=580nm); N_3-green fluorescent light (λ=540nm); N_4-red fluorescent light(λ= 670nm).

S_2 Series: V_0 - white fluorescent lighting - control group (λ=400nm); L_0-light LED white light issuing(λ=510nm); L_1-LED illumination with blue light issuing(λ=470nm); L_2-LED illumination with yellow light(λ=580nm); L_3-LED illumination with green light(λ=540nm); L_4-LED illumination with red light(λ=670nm).

Extraction of assimilating pigments for each experimental variant, with pure dimethylformamide (DMF 99.9%). The working method consisted of crushing a 50 mg fragment cladodes in 5 ml DMF [after Moran and Porath, 1980]; achieved composition was maintained for 72 hours at 4°C, then supernatant was decanted and the resulting solution was made assimilatory pigment content determination by an photometric extract with SPEKOL 11 type spectrophotometer. In the present operation, the determination of pigments from the extracted photometric liquid samples was done by using selective filters with different wavelengths, as follows: 664 nm for chlorophyll *a*, chlorophyll *b* and 647 nm to 480 nm for carotenoids pigments. For each experimental variant were made five repetitions.

Data obtained were processed by photometric mathematical formulas as proposed by [Moran and Porath (1980)]. The averages of five repetitions were performed per experimental variant figure which was operated in the rest of the calculations. By summing the average obtained data from measurements of chlorophyll *a*, with those from carried out tests to identify

the level of chlorophyll *b* was obtained in total content of green pigment, by adding to these figures the average values resulting a complete content of the result in carotinoid pigments resulting a complete picture of assimilatory pigments level determined in the *Echinocactus mihanovichii* vitrostems, after *90 days* of vitroculture exposed to various lighting regimes.

RESULTS AND DISCUSSION

After *90 days* of culture *"in vitro"*, we realized assimilatory pigment extraction, separately from each experimental variant grown under UV lights of different colors and wavelengths. As already noted belongs to the *Echinocactus mihanovichii* cacti chlorophyll-deficient class monocolor group, and after (Shemorakov, 2001), the number of chloroplasts in these plants normally not exceed 1/3 of all plastids, the rest are mutated and without any possibility to synthesize chlorophyll, the author believes that mutations occur spontaneously in culture and are greatly influenced by temperature and light.

Blue light (variant L_1) LED-s, carotenoid pigments synthesis inhibit (0.4036 mg/g plant material) that were located as a percentage of 50.29% (Fig.4B) as witness V_0 (0.8118 mg/g plant material), while under *blue* light (N_1 variant) fluorescent tubes of *Echinocactus mihanovichii* explants improved over 76.42% (Fig.4B) more assimilatory pigments (1.4793 mg/g plant material), reporting to the control group (0.8385 mg/g plant material), similar situation to that recorded by (Kurilčik et al., 2008) at *Chrysanthemum*, but also (Mercado et al., 2004) on *Nitzschia thermalis*, *Nitzschia laevis* Hust and *Navicula incerta* vitrocultures, also by (Gordillo et al., 2004) to species of *Dictyota dichotoma, Gelidium sesquipedale* and *Ulva rigida.*

Under the *yellow* light emitted by fluorescent tubes or LEDs it was noted that the green pigment content does not change compared with control V_0 (0.0267 mg/g plant material), but substantially increase the amount of carotinoid pigment treated with 50.27% (1.2199 mg/g plant material) in explants exposed to light LED (L_2 variant) (Fig.4B) and 96.39% (1.6468 mg/g plant material) total assimilatory pigments in the case of exposed vitrocultures to light fluorescent tubes (variant N_2) (Fig. 4). While the seaweed vitrocultures where exposed to light yellow LEDs, it was reduced the amount of assimilatory pigments (Mouget et al., 2004).

The *in vitro* culture of *Echinocactus mihanovichii* exposed to *green* light (variant N_3) fluorescent tube boasts an extra 98.74% of the chlorophyll *b* content (0.0305 mg/g plant material) compared with control group V_0 (0.0177 mg/g plant material) (Fig. 4) also were synthesized with 96.8% more assimilatory (green and carotenoids) pigments, and for explants grown on *green* LED light (variant L_3) that parameter has exceeded 98.46%

(Fig.4B) the witness (1.6641 mg/g plant material), these results are in concordance with those published by (Mouget et al., 2004), in the case of exposed seaweed vitrocultures to similar light sources.

The weaker effect in stimulating the synthesis of assimilatory (green and carotenoid) pigments on both the *red* light emitted by fluorescent tubes (variant N_4) and the *red* LED light (L_4 variant), therefore, reported to the witness V_0 (0 , 8385 mg/g plant material), to this parameter registered a loss of 74.35% (0.2151 mg/g plant material) (Fig. 4) in the first case and 40.28% (0.5008 mg/g plant material) in the second case (Fig.4B).

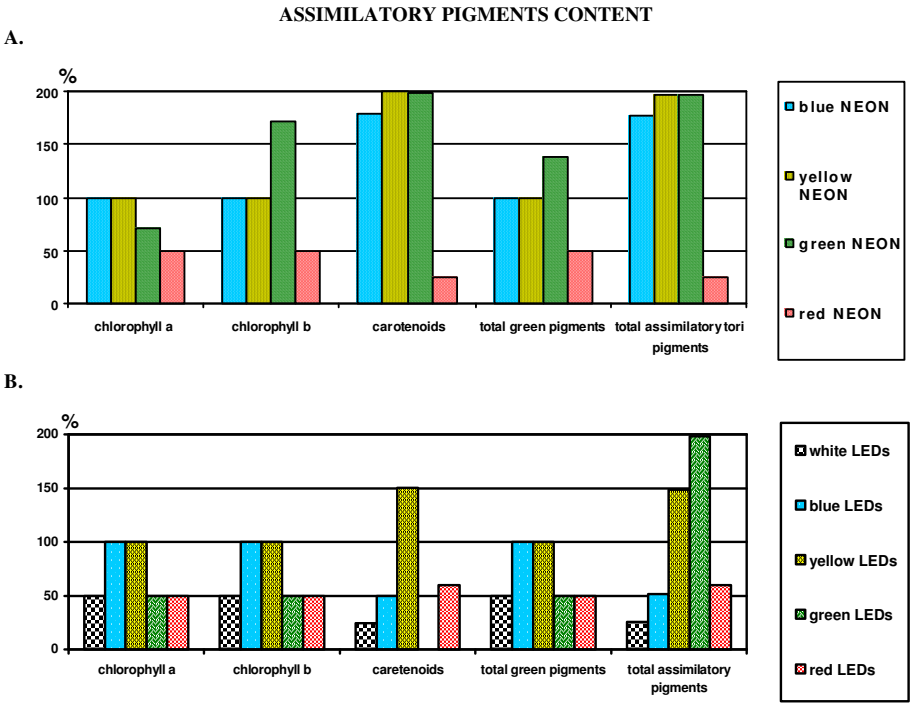

Fig. 4. The assimilatory pigment content of the *Echinocactus* (Pfiff.) *mihanovichii* vitrostems, *90 days* after inoculation, where: **A**-cultures exposed to light emitted by fluorescent tubes of different colors (blue, yellow, green or red), **B**-cultures exposed to light from different *LED* colors (white, blue, yellow, green or red), data expressed in percentage values obtained by comparing the values recorded in those parameters determined in the control group (V_0), values considered 100% respectively to light produced by white fluorescent tubes.

And *white* LED light (variant L_0) inhibit the synthesis of assimilatory (green and carotenoids) pigments, this parameter value was higher with 74.35% (0.6234 mg/g plant material) as that recorded value on the control variant V_0 (0.8385 mg/g plant material) (Fig.4B). Similar results have been communicated by (Segovia and Figueroa, 2007) on *Evernia prunastri*, by

(Pop and Cachiță, 2007) to *Sequoia sempervirens*, and by (Vidican and Cachiță, 2010) on *Opuntia fragilis* var. *fragilis*.

Analyzing the obtained results it is noted that the amount of synthesized carotenoid pigment in caulinar neoformations regenerated from explants of *Echinocactus mihanovichii* the very large limits differ from one variant to another, thus, are highlighted with the lowest values (0.2018 mg/g plant material) for the explants exposed to *white* LED light (variant L_0) and *red* fluorescent tubes light (variant N_4), where recorded a loss of 75.15%, while the opposite, with highest values of this parameter (1.6281 mg/g plant material) is situated on vitrocultures under *yellow* fluorescent tubes light (variant N_2), which marked an increase of 99.56%.

Regarding the influence of light of different colors and wavelengths emitted by fluorescent tubes and LEDs, on the assimilatory pigments synthesis, it appears that the *Echinocactus mihanovichii* vitrocultures have a similar response with *Sequoia sempervirens* (Pop and Cachiță, 2007), *Evernia prunastri* (Segovia and Figueroa, 2007), *Chrysanthemum* (Kurilčik et al., 2008) or with *Opuntia fragilis* var. *fragilis* (Vidican and Cachiță, 2010). We should mention that in the literature have been published results of similar experiments with chlorophyll-deficient cactus.

CONCLUSIONS

At *90 days* after initiation of the *in vitro* culture, it was found that the level of *Echinocactus mihanovichii* vitrostems exposed to blue and yellow light emitted from fluorescent tubes and LEDs, it has been assimilated an amount of chlorophyll *a* and *b* identical to that of samples from the control group, the explants grown on *white* light fluorescent tubes - reference data considered to be 100%.

The *green* light emitted by fluorescent tubes stimulated the synthesis of more chlorophyll *b*, this parameter compared with control, showed a higher value with 72.31%, also found an increase of 100% of carotinoid pigments content in explants of *Echinocactus mihanovichii* exposed to neon and LEDs of the same color.

Under *red* light emitted by fluorescent tubes and LEDs, compared to control variant, and chlorophyll *b* content decreased by 50% and 39.95% of the carotinoid pigments in the case of exposed vitrocultures to LED lights and increased to 75.15% for the same color of neon light.

In the case of vitrostems grown *white* LED light was a 50% decrease in the amount of chlorophyll *a* and *b*, respectively with 75.15% in carotenoids pigments, compared with similar parameter values recorded to the control group.

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