DETERMINATION OF THE CONTENT OF ASSIMILATORY PIGMENTS IN VITRO CULTURES OF *Echinopsis chamaecereus f. lutea*, EXPOSED TO LIGHT EMITTED BY FLUORESCENT TUBES OF DIFFERENT COLORS AND WAVELENGTHS

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RESEARCH ARTICLE

Abstract

As a result of spontaneous mutations in cactus cultures, today we enjoy the unprecedented beauty of chlorophyll-deficient cacti, 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. In the current experiment we used explants of Echinopsis chamaecereus f. lutea, the chlorophyll-deficient cactus, inoculated on culture media without growth regulators and exposed to the light of white or colored fluorescent tubes, respectively, blue, yellow, green and red, the control sample being exposed in natural light.

After 90 days of in vitro culture, we extracted the assimilatory pigments - chlorophyll a, chlorophyll b and carotenoid pigments - separately from each experimental variant grown under fluorescent tube lights of different colors and wavelengths and compared it with similar parameter values from the control variant - vitro culture grown in natural light (N1) - data considered as representing 100%.

The results differed depending on the wavelength and color of the light emitted by the fluorescent tubes. We can conclude that regardless of the color emitted by the fluorescent tubes, in the explants of Echinopsis chamaecereus f. lutea, carotenoid pigments accumulated in a much greater amount than chlorophyll a and b, a phenomenon probably due to the fact that this cactus is a chlorophyll-deficient species. Among the chlorophylls, chlorophyll was synthesized in greater quantity in the case of phytoinoculae grown under the yellow light of fluorescent tubes.

Keywords: vitro culture, fluorescent tubes, chlorophyll a and b, carotenoid pigments
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INTRODUCTION

In its evolution, the plant world has adapted so that plants are able to exploit light energy in the development of physiological processes. For this purpose they have acquired a wide range of photoreceptors that perceive and respond to the stimulus of the light spectrum, quantifying the period, direction, quality and intensity of light (Galvao V.C. and Fankhauser C., 2015; Volta K. and Carvalho S.D., 2015).

The existence of vitroplants is due to the presence of carbohydrates in the culture medium and is not strictly conditioned by the realization of photosynthesis, they are mixotrophs, but light affects a series of physiological reactions, phenomena closely related to the optical characteristics of chlorophyll (Ptushenko et al, 2020, Seyedi et al., 2024).

The visible spectrum of solar radiation, the photoenergetic and photochemical effects exerted on plants include the following emission wavelengths (in nm): 380-400 (violet), 400-480 (indigo), 480-500 (blue), 500 - 580 (green), 580-600 (yellow), 600-650 (orange) and 650-780 (red) (Nassau K. , 2024). Light does not have the same type of influence on all plants, it acts differently depending on the species and the color of the light, so the use of fluorescent tubes of different lighting colors in vitrocultures is becoming more and more used, especially after the discovery of the benefits arising from their use. (Frade et al, 2023; Gonçalves et al, 2023)

According to (Van Ieperen and Trouwborst, 2008, Sontag-González M.,et al, 2022), artificial light sources can be calibrated to emit a specific wavelength, so that in order to provide plants with optimal light conditions, they they look through filters to be able to stop

UV radiation and their light can be directed or can choose the most effective viewing angle for plants. There are currently studies to determine how many photons of a given wavelength induce plant growth and development (Friesen, 2024).

Chlorophyll-deficient cacti, due to their very different shapes and colors, represent a completely unusual but highly appreciated occurrence. This fact has determined the emergence of a true extremely profitable industry, in countries such as the Netherlands, Japan, Korea, Germany and others where new technologies are currently being sought for the rapid and economically efficient multiplication of these plants (Smith et al, 2021).

In the current experiment, as a continuation of the experiments carried out by us (Vidican et al., 2010), we used as biological material chlorophyll-deficient cactus with the stated aim of studying changes in the content of assimilatory pigments (chlorophyll a, chlorophyll b and carotenoid pigments) from the vitroplants of *Echinopsis chamaecereus f. lutea* illuminated - for 90 days - with fluorescent lamps of different colors compared to those illuminated with natural light (N1), considered as the control sample.

MATERIAL AND METHOD

The plant material was sterilized by immersion in ethyl alcohol at 96º for one minute, after which it was covered with a 0.8% sodium hypochlorite solution, mixed 1:2 with sterile water to which was added, as a surfactant, three drops of Tween 20 (Cachiţă et al., 2004), stirring continuously for 20 minutes.

The future inocula are represented by buds of *Echinopsis chamaecereus f. lutea* sized so that each contains 3-4 areoles (Vidican and Cachiţă, 2011) and approximately 1 cm long, 0.5 cm thick and a diameter of 0.5-1, 5 cm, (fig.1).

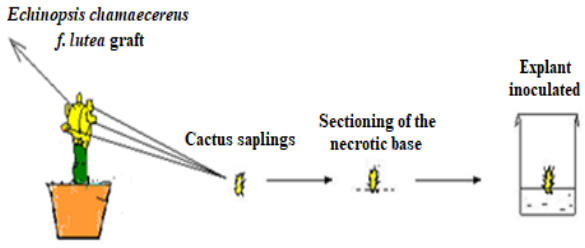

Figure 1. **Schematic representation of how operating fragments *Echinopsis chamaecereus f. lutea* to be inoculated aseptic environments.**

Explants were inoculated on basic medium (MB) (Murashige and Skoog, 1962), microelements (Heller, 1953), to which vitamins were added: 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, the pH of the medium - prior to its autoclaving - was set at a value of 5.7. Culture media do not contain growth regulators.

The inoculum vials were transported and placed in the growth chamber, where the temperature varied between 20 and 24ºC.

For this experiment, when lighting the vitro cultures of *Echinopsis chamaecereus f. lutea*, white or colored fluorescent tubes, respectively blue, yellow, green or red, were used as a light source (fig.2). The control sample (N1) was exposed to natural light. We obtained the following experimental series:

N1 – vitro cultures exposed to natural light - data considered as 100% reference values.

And depending on the color of the light generated by the fluorescent tubes, we obtained several variants, as follows:

N2 - white fluorescent light (λ=400nm);
N3 - blue fluorescent light (λ=470nm);
N4 - yellow fluorescent light (λ=580nm);
N5 - green fluorescent light (λ=540nm);
N6 - red fluorescent light (λ=670nm);

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

The obtained data were processed by photometric mathematical formulas, as proposed by [Moran and Porath (1980)]. Averages of five replicates/experimental variant were performed. By summing the average data obtained from the measurements of chlorophyll a, with those from the tests carried out to identify the level of chlorophyll b, the total content of green pigment was obtained, by adding these figures of the

average values, a complete content of the result in carotenoid pigments was obtained, resulting in a complete picture of of the level of assimilatory pigments determined in vitro in *Echinopsis chamaecereus f. lutea* plants after 90 days of culture in vitro, exposed to different lighting regimes.

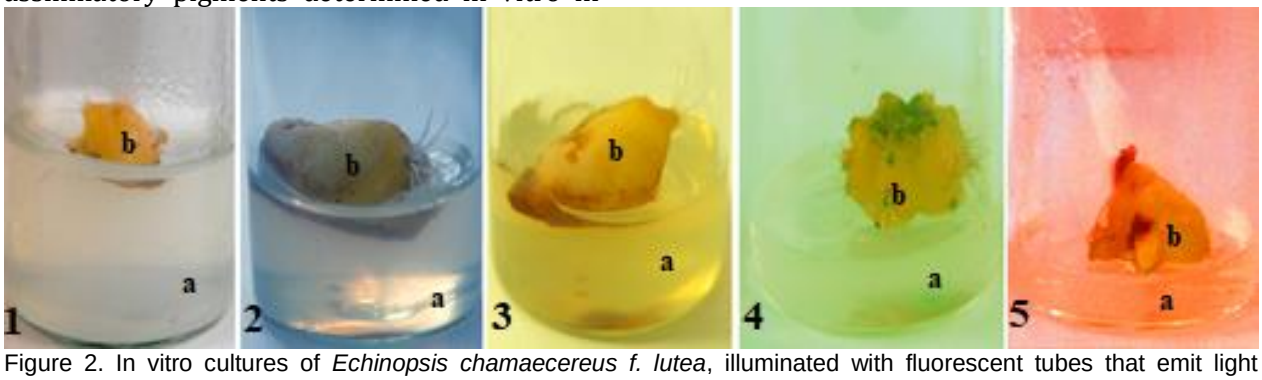

Figure 2. In vitro cultures of *Echinopsis chamaecereus f. lutea*, illuminated with fluorescent tubes that emit light of different colors, respectively, different wavelengths. Where: 1-white light (λ=400nm); 2-blue light (λ=470nm); 3-yellow light (λ=580nm); 4-green light (λ=540nm); 5-red light (λ=670nm); a-culture medium; b-phyto inoculum.

Statistical analysis was performed using analysis of variance or the Anova test for completely randomized multifactorial experiments. By entering the data, I obtained the following general data:
The monitored indicator: the amount of pigments
Unit of measure: mg/g
Number of factors: 2
Number of repetitions: 3
The N factor – the color emitted by the fluorescent tube – 6 graduations
Witness
Factor P – type of pigment – 3 graduations

RESULTS AND DISCUSSIONS

Following the observations made at 90 days in the current experiment, from the data presented in table 1, it is noted that variant N1 (control sample, values considered to be 100%) in vitro seedlings grown in natural light accumulated the largest amount of assimilatory pigments, respectively, 0.28 mg pigments/g plant material while in vitro seedlings grown in the green light of fluorescent tubes (N5) with 0.05 mg pigments/g plant material recorded the lowest value of this parameter which represents a minus of 81.9%. The best result was recorded in vitro with seedlings grown under the blue light of fluorescent tubes (N3) where with 0.15 mg pigments/g plant material a deficit of 46.3% was found, followed by in vitro seedlings grown under the incidence of white light neonates (N2) which, with 0.14 mg pigments/g plant material, shows a minus of 50.9% compared to the control N1. In vitro seedlings grown under yellow (N4) and red (N6) neon lights assimilated 0.07 mg pigments/g plant material, which represents a minus of 74.8%. In the case of this parameter, in all experimental variants the difference compared to the control was statistically very significantly negative (table 1).

Table 1
The influence of the N factor (light type) on the quantity of pigments

Cr. no.	Variant	Absolute production of pigmenți mg/g	Relative production of pigmenți %	± d g/mg	Significance
1	N1	0,28	100,0	0,00	Mt
2	N2	0,14	49,1	-0,14	000
3	N3	0,15	53,7	-0,13	000
4	N4	0,07	25,2	-0,21	000
5	N5	0,05	18,1	-0,23	000
6	N6	0,07	25,2	-0,21	000

$DI_{5\%}$=0,03 $DI_{1\%}$=0,05 $DI_{0,1\%}$=0,07

Table 2 shows the influence of the P factor (pigment type) on the amount of pigments assimilated by in vitro seedlings exposed to different colored light from fluorescent tubes, it can be noted that carotenoid pigments (P3) have the largest contribution to the amount of synthesized pigments expressed in mg pigments/g plant material, thus with 0.36 mg carotenoid pigments/g plant material this parameter was above control P0 by 186%. This result being considered from a statistically significant positive point of view.

Table 2.

The influence of the P factor (pigment type) on the amount of pigments

Cr. no	Variant	Absolute production of pigmenți mg/g	Relative production of pigmenți %	± d g/mg	Significance
1	P0	0,13	100,0	0,00	Mt
2	P1	0,01	7,6	-0,12	000
3	P2	0,01	7,6	-0,12	000
4	P3	0,36	286	0,23	xxx

$DI_{5\%}$=0,02 $DI_{1\%}$=0,03 $DI_{0,1\%}$=0,04

The influence of chlorophyll a and chlorophyll b on the amount of pigments assimilated by in vitro seedlings exposed to different colored light from fluorescent tubes is below the control P0 with 0.12 mg chlorophyll a and b/g plant material which represents a deficit of 92.4%, provided statistically very significant negative compared to the witness.

Table 3.

The interaction of the factor P (type of pigment) to N (type of light)

Cr no	Variant	Absolute production of pigmenți g/mg	Relative production of pigmenți %	± d g/mg	Significance
1	P0 N1	0,28	100,0	0,00	Mt.
2	P1N1	0,01	3,6	-0,27	000
3	P2N1	0,03	10,2	-0,26	000
3	P3N1	0,81	286	0,53	xxx
5	P0N2	0,14	100,0	0,00	Mt.
6	P1N2	0,01	6,3	-0,13	000
7	P2N2	0,01	6,3	-0,13	000
8	P3N2	0,81	286	0,27	xxx
9	P0N3	0,15	100,0	0,00	Mt
10	P1N3	0,04	24,2	-0,12	000
11	P2N3	0,02	11,6	-0,14	000
12	P3N3	0,74	264,3	0,25	xxx
13	P0N4	0,07	100,0	0,00	Mt.
14	P1N4	0,01	6,3	-0,07	0
15	P2N4	0,01	6,3	-0,07	0
16	P3N4	0,79	281,5	0,13	xxx
17	P0N5	0,05	100,0	0,00	Mt.
18	P1N5	0,01	17,1	-0,04	-
19	P2N5	0,01	17,1	-0,04	-
20	P3N5	0,77	274,2	0,09	xxx
21	P0N6	0,07	100,0	0,00	Mt.
22	P1N6	0,01	12,3	-0,06	0
23	P2N6	0,01	12,3	-0,06	0
24	P3N6	0,79	281,5	0,13	xxx

$DI_{5\%}$=0,05 $DI_{1\%}$=0,07 $DI_{0,1\%}$=0,09

The interaction between the two factors P (type of pigment) and N (type of light) is presented in table 3. It should be noted that the carotenoid pigments (P3) showed a significant increase compared to the control (P0) regardless of the color of the light emitted by fluorescent tubes. Thus, the variant P3N1 the amount of carotenoid pigments in vitro seedlings grown under natural light and P3N2 the amount of carotenoid pigments in vitro seedlings grown under the white light of fluorescent tubes exceeded the control (0.28 mg/g plant material) by 0.53 mg carotenoid pigments/g plant material, which represents an increase of 186%. With 0.79 mg carotenoid pigments/g plant material, the varieties P3N4 (in vitro plants grown under yellow light) and P3N6 (in vitro plants grown under red light) had an increase of 181.5% compared to the control, while with 0 .77 mg carotenoid pigments/g plant material, the P3N5 variant (in vitro plants grown under green light) recorded an increase of 174.2% compared to the control. P3N3 (in vitro plants grown under blue light) with 0.74 mg carotenoid pigments/g plant material has an increase of 164.3% compared to the control. For all the variants presented, the differences compared to the control were statistically very significantly positive.

In the case of seedlings grown in vitro under yellow light, the amount of chlorophyll a (P1N5) and chlorophyll b (P2N5) with an absolute value of 0.01 mg pigment/g plant material recorded a deficit of 82.9%, in which case none of the values exceeded the LD5%=0.05 threshold and thus were not statistically assured.

In the variants of Echinopsis chamaecereus f. lutea grown under yellow light, the amount of chlorophyll a (P1N4) and chlorophyll b (P2N4) is 0.27 mg pigment/g plant material lower than in the control, thus in these variants a minus 82.9%, we find the same absolute values recorded in vitro for seedlings grown under red light (P1N6) and (P2N6) where a deficit of 83.7% is noted. Variants where the difference was ensured statistically negative, significant.

From table 3, it can be seen that in vitro seedlings grown both in natural light and in the white or blue light of fluorescent tubes recorded absolute values of chlorophyll a and chlorophyll b below the control value, differences that from a statistical point of view were ensured very negative significant.

Analyzing the data from table 4, namely the influence of the color of the light emitted by the fluorescent tubes on the amount of pigments: chlorophyll a, chlorophyll b and carotenoid pigments, it is observed that; chlorophyll a has the highest absolute value of 0.04 mg/g plant material in the case of phytoinocula grown under the yellow light of

fluorescent tubes which represents an increase of 258.3%, however it did not exceed the threshold of DL5% =0.05 and thus the value is not statistically assured. In the case of the other variants grown under the incidence of white, blue, green or red neon lights, a chlorophyll a deficit of 56.3% is noted, values not statistically guaranteed.

The absolute values of chlorophyll b synthesized in vitro Chamaecereus f. lutea cultures were below the control (0.03 mg/g plant material), thus with 0.02 mg/g plant material in vitro the cultures grown under blue light (N3P2) a minus of 38.8% was found, while in vitro cultures grown under white, green, yellow and red neon lights this parameter was 0.01 mg/g plant material, registering a deficit of 69.6%. Values that did not exceed the threshold of LD5%=0.05 and thus are not statistically assured.

Table 4.
The interaction of the factor N (type of light) to P (type of pigment)

Cr no	Variant	Absolute production of pigmenți g/mg	Relative production of Pigmenți %	± d g/mg	Significance
1	N1P1	0,01	100,0	0,00	Mt
2	N2P1	0,00	43,7	-0,01	-
3	N3P1	0,04	358,3	0,03	-
3	N4P1	0,00	43,7	-0,01	-
5	N5P1	0,00	43,7	-0,01	-
6	N6P1	0,00	43,7	-0,01	-
7	N1P2	0,03	100,0	0,00	Mt
8	N2P2	0,01	30,4	-0,02	-
9	N3P2	0,02	61,2	-0,01	-
10	N4P2	0,01	30,4	-0,02	-
11	N5P2	0,01	30,4	-0,02	-
12	N6P2	0,01	30,4	-0,02	-
13	N1P3	0,81	100,0	0,00	Mt
14	N2P3	0,41	49,9	-0,41	000
15	N3P3	0,40	49,6	-0,41	000
16	N4P3	0,20	24,8	-0,61	000
17	N5P3	0,14	17,4	-0,67	000
18	N6P3	0,20	24,8	-0,61	000

$DI_{5\%}$=0,05 $DI_{1\%}$=0,07 $DI_{0,1\%}$=0,10

As for carotenoid pigments, compared to the control, they showed different values influenced by the color of the light to which the in vitro cultures were exposed. The highest values of this parameter are below 0.81 mg/g plant material, a value recorded in the control, so in vitro cultures grown under the white light of fluorescent tubes (N2P3) showed 0.41 mg carotenoid pigments/g plant material , closely followed by in vitro cultures grown under the blue light of fluorescent tubes (N2P3), with 0.40 mg/g plant material, which represents a deficit of 50.1% and 50.4%, respectively. The variants grown under the yellow (N4P3) and red (N6P3) light of fluorescent tubes recorded an absolute value of this parameter of 0.20 mg carotenoid pigments/g plant material and the in vitro culture grown under green light (N5P3) synthesized 0.14 mg carotenoid pigments/g plant material, a deficit of 75.25 and 82.6% corresponding to the N5P3 variant was recorded. %. In all the variants presented above, the difference was ensured statistically negatively, very significantly.

CONCLUSIONS

1. After 90 days from the initiation of in vitro culture in *Chamaecereus f. lutea*, different values of the assimilative pigment content are noted: chlorophyll a, chlorophyll b and carotenoid pigments depending on the wavelength and color of the light emitted by the fluorescent tubes.

2. The light factor directly influenced the amount of pigments, natural light proving to determine the highest value of this parameter, followed by the blue light of fluorescent tubes, the minimum value was found to be recorded in vitro seedlings grown in green light of neons.

3. It is noted that carotenoid pigments (P3) have the largest contribution to the amount of synthesized pigments, thus with 0.36 mg carotenoid pigments/g plant material this parameter was above the control P0 by 186%.

4. In the interaction between the two factors P (type of pigment) and N (type of light) it is found that carotenoid pigments show a significant increase compared to the control (P0) regardless of the color of the light emitted by the fluorescent tubes, a phenomenon probably due to the fact that *Chamaecereus f. lutea* is a chlorophyll deficient cactus.

5. The yellow color of the light emitted by fluorescent tubes directly influenced the amount of chlorophyll a, in vitro cultures grown under this color showed an increase of 258.3% compared to the control.

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