BIOCHEMICAL DETERMINATIONS MADE ON AFRICAN VIOLETS *(SAINTPAULIA IONANTHA)* EXVITROPLANTLETS, BEING ILLUMINATED DURING THEIR ACCLIMATIZATION TO A SEPTIC MEDIUM, WITH DIFFERENT TYPES OF LIGHT

Adriana PETRUŞ-VANCEA[*1], Dorina CACHIŢĂ-COSMA[2]
[1]Biology Department, Faculty of Science, University of Oradea
[2]"Vasile Goldiş" Western University Arad, Romania, Faculty of Natural Sciences

*** Correspondence:** Adriana Petruş-Vancea, University of Oradea,Faculty of Science, Biology Department Universitatii Str., no 1, 410087, Oradea, Bihor, Romania, fax (Dean Secretary) +40.259.408.461, tel. +40.259.408.278, GSM (Vodafone) +40.723.143.399, E-mail: adrianavan@yahoo.com
Received: march 2008; Published: may 2008

ABSTRACT. In the current experiment we wanted to study the effect of lighting the African violets exvitroplantlets, with fluorescent light of different nature, namely: *white* (400 nm), *red* (660 nm), *yellow* (580 nm), blue (430 nm) and green (544 nm), of the quantity of assimilating pigments from the leaflets, as a meter of the photosynthesis process intensity, and also of the peroxidazic activity at the rootlets level, as a marker of rizogenezis process, both phenomena (photosynthesis and rizogenezis) having a definite role during the acclimatization period of the plantlets cultivated *in vitro*, to the conditions of a septic medium. After 30 days from the acclimatization of the African violets exvitroplantlets, in "Top soil" and perlite substratum, with a 3:1 report, the carried out biochemical determinations have shown that, red light illumination have determined the stimulation of creating assimilating pigments, especially of chlorophyll a, at the still living plantlets (half of the plantlets maintained under this type of light faded), on the other hand the peroxidazic activity was stimulated at all four experimental variants, reaching statistically significant values, expressed in percent with 20% plusses, in the case of the exvitroplantlets rootlets illuminated with blue light, which implies an intense rizogenezis process at this batch.

Keywords: peroxidazic activity, chlorophyll pigments, color light, exvitroplantlets

INTRODUCTION

The presence of assimilating pigments into the chloroplasts, located into the grana lamels, ensures within the thylacoids the developing of the primary anabolic reactions, with the glucose synthesis, compound which is afterwards polymerized in starch particles, formations that lay down into the chloroplasts stroma because of the heterotrophic or mixotrophic regime of the vitroplantlets, blocking their activity, the starch being unable to migrate into the plantlet (Cachiţă, 2000).

The stimulation of chlorophyll forming, at *Coleus* vitrocultures, was tried through maintaining them under fluorescent tubes that give different types of light (Radoveţ – Salinschi and Cachiţă, 2004), noticing that the blue and green light, in comparison with the red and orange one, had good effects in this case.

The illumination of *Cymbidium* protocorms with different colors fluorescent tubes, led to a diminishing of the growth rate of the protocormial mass (Blidar et al., 2007), comparatively with the recorded results at the batch that was kept under the white fluorescent light.

An unconventional system of lighting the *Sequoia sempervirens* vitrocultures, with white light was imagined and applied by Pop and Cachiţă (2007), through using "high brightness" LED (Light Emitted Diodes) light. The authors reported the diminishing of the vitrocultivating costs, but also a stimulating of the growth of *Sequoia* vitroplantlets for the batches kept under the LED, comparatively with the ones illuminated with fluorescent light.

During the *acclimatization* period, the growth parameters at African violets and *Cymbidium* exvitroplantlets were superior at the level of the batches that were kept 16/24 hours, for 30 days, under the *green* and *blue* light (Petruş – Vancea and Cachiţă, 2005). The *red* light proved to be harming for the African violets, *Cymbidium* but especially for *Chrysanthemum* exvitroplantlets, which faded for 100%, after being illuminated with this type of light (at 660 nm).

The peroxides are a good enzymatic marker in identifying the intensity of the root forming processes which increases before the beginning of the rizogenezis phenomena (Gaspar et al., 1992), but they can also be considered as key molecules in the success of the fast rehabilitation, post-cryoconservation, of the normal physiological parameters for plants (Şipoş et al., 2007).

Petruş and the contributors (2004) have proved that the peroxidazic activity at the level of chrysanthemum, African violets and cymbidium orchid rootlets was

50% - 150% higher than at vitroplantlets and exvitroplantlets (30 days after the "ex vitro" transfer), towards the one registered at the roots of the greenhouse (control) plants, that were in a advanced stage of growth, which demonstrates their involvement in the active rizogenezis process.

MATERIALS AND METHODS

For accomplishing this experiment we have chosen African violets propaguls taken off from vitrobush, lying after *90 days* of vitroculture, in a basis culture medium (MB) Murashige – Skoog (MS)(1962), without growth regulators, having a number of 4 - 5 rootlets, with a height of 1 cm, and approximately 6 leaflets/propagul – which we have planted, individually, into a mixture of "Top soil" soil and perlite, in a 3 : 1 report (Vancea et al., 2000), prepared in incubators (Vancea and Cachiţă, 2002), substratum previously humidified with 300 ml of tap water each time, brought at the laboratory's temperature. The incubators were placed on shelves protected with aluminum foil, away from the incidence of natural light, the illumination of the cultures being made through fluorescent tubes which gave *white* (400 nm), *red* (660 nm), *yellow* (580 nm), *blue* (430 nm) and *green* light (544 nm), with the intensity of 2 500 lx (*white*), 300 lx (*red*), 670 lx (*yellow*), 350 lx (*blue*) and 410 lx (*green*), and the power from the neon was of 13 V, the intensity was of 103 A and the light flow was of 0.44 lm. The photoperiod was of 16/24 h, and the temperature form the substratum was of 24°C ± 2°C, the one from the atmosphere being two degrees lower.

At *30 days* from the acclimatization, the post-acclimatization survival percent was calculated for the exvitroplantlets and it was determined the quantity of chlorophyll at the level of the foliar limb and the peroxidazic activity at the exvitroplantlets rootlets level.

Establishing the *assimilating pigments* content form the leaflets, respectively the a, b chlorophylls and carotenoid pigments was made through their extraction in N, N - dimethylformamide (DMF) 99.9% Merck, according to Moran and Porath method (1980).

For the pigments extraction 0.5 g of foliar limb was submersed and macerated in 2.5 ml N,N - dimethylformamide (DMF), for 72 hours, at 4 ºC temperature and in the dark for chlorophyll extraction, afterwards the supernatant being clarified and serving for the quantitative determination of the assimilating pigments, through its photometration using a spectrophotometer Spekol 11 type, Carl Zeiss Jena, using filters with 480 nm bands (for the carotenoid pigments), with 647 nm (for chlorophyll b) and with 664 nm (for chlorophyll a). Adjusting the apparatus was made using in the control box a DMF solution, which helped the pointer to go to zero, after reading each test extinction.

The medium values of the photometric units of the assimilating pigments, determined in the extracts made from the exvitroplantlet leaflets kept under colored lights were reported to the control ones (the exvitroplantlet leaflets kept under *white* light, considered of reference, 100%) and graphically represented.

The determination of the peroxidazic activity was made with p-phenylendiamine (Şipoş et al., 2003 a and b, adapted after Lück method, 1974). The method principle consists in the fact that, in a vegetal extract that contains peroxidases, p-phenylendiamine is oxidized by them, phenomena that leads to changing the color of the obtained mixture into violet, and between the peroxidazic intensity (measured spectophotometric, with a Spekol 11 Carl Zeiss Jena type spectrophotometer with a 483 nm filter) and activity, there is a proportionality direct report (Lück, 1974).

Obtaining the peroxidazic extract was made trough triturating of the radicular tissues in a buffer solution made of phosphate 0.067 M, with pH 7. For this, 0.5 g rootlets (freshly ingathered), were crushed together with quartz sand in a grinding mortar (previously washed and sterilized through dry heat, in the stove at 120ºC), in the presence of 4 ml of ultra diluted phosphate buffer 1 : 9 (1 ml from the 0.067 M solution, with pH 7 plus 9 ml distillated water). The obtained extract was separated from the vegetal wastes through whizzing with a MSE centrifuge, at 6 000 rpm, for 25 minutes. Each specimen's supernatant was collected and was kept in the refrigerator for 2 hours. The determination of the specimen's extinction was made as a report with the distillated water, with a filter adjusted for a wave length of 483 nm. For reading the extinction in the box we introduced the mixture made of: 0.5 ml vegetal extract (supernatant), 0.05 ml oxygenated ultra diluted water, 1 ml phosphate buffer pH 7, 0.067 M, and after that it as added a 0.05 ml p-phenylendiamine 1% solution. For each option (at 30 s, 60 s and 90 s from the obtaining of the final mixture) there were made three checking/readings for each. The oxygenated water used in our experiments was ultra diluted and was obtained from 33% hydrogen peroxide. In this way, for 100 ml distillated water there were added 0.3 ml of hydrogen peroxide; from this it was made a 1 : 9 distillated water dilution. Because the solutions are not stable in time, they are prepared only when the spectrophotometration begins. The working temperature was of 4 ºC.

In order to compare the existing peroxidazic activity in the exvitroplantlet rootlets cultivated under different types of light, the average data of the extinctions read at 120 s, the moment when they got constant, came from the samples belonging to extracts that were obtained from the exvitroplantlet rootlets placed under different types of light, were reported to the similar photometric readings registered at the control batch (the exvitroplantlet rootlets set under white light), values considered as a reference, as being 100%.

RESULTS AND DISCUSSIONS

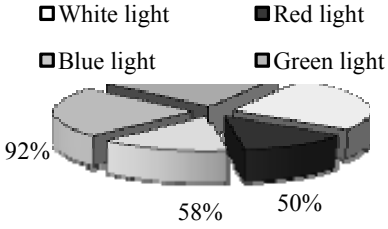
a. The surviving percent

At *30 days* after the „ex vitro" transfer of the African violet plantlets, created in an aseptic medium and maintained during the acclimatization period under different types of light, their *surviving percent*, to the

Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii (Life Sciences Series), vol. 18, 2008

http://www.studiauniversitatis.ro

conditions of the septic medium of life, was maximum, of 100% (Graphic 1), only to the control batch, whose exvitroplantlets were placed under fluorescent *white* light. The lowest percents of survival, of only 50%, respectively 58%, were registered at the exvitroplantlets illuminated with fluorescent *red* and *yellow* light. A good acclimatization to the septic medium, expressed through a high survival percent, of 92%, was found at the exvitroplantlets kept under light produced by *blue* fluorescent tubes, but also *green*, in this last case the success percent for the survival being of 83% (Graphic 1).



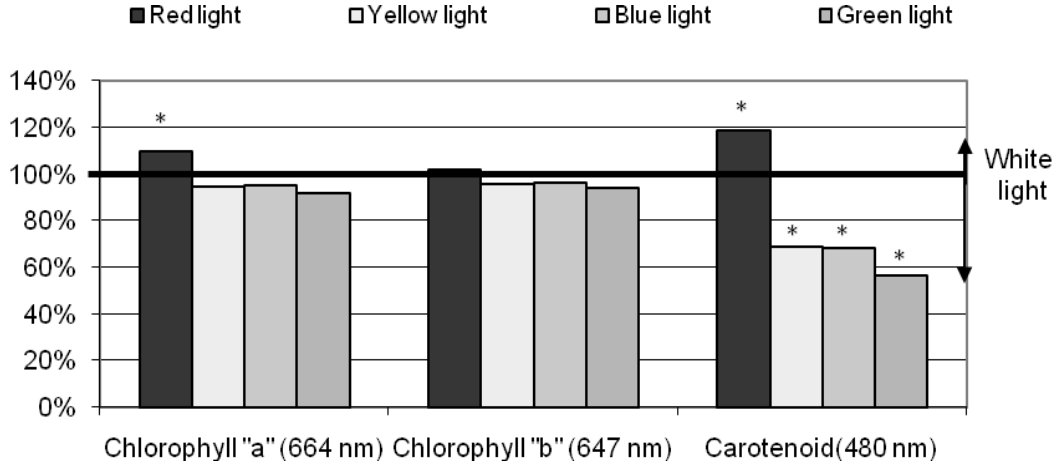
Graphic 1. The survival percent of the African violets (*Saintpaulia ionantha*) exvitroplantlets, *30 days from their transfer into the septic medium*, being illuminated with different types of fluorescent light: *white* (control - M), *red* (R), *yellow* (Y), *blue* (B) or *green* (G), reported to the situation from the moment of their passing „ex vitro", reference values, considered 100%

b. Chlorophyll pigments

At the end of the acclimatization period, *30 days* after its beginning, after the photometry of the samples obtained from the exvitroplantlets leaflets kept under different types of light, about *chlorophyll a,* the average value of the extinctions was the highest, scoring an increase of 10% (Graphic 2), towards the control (extract from the exvitroplantlet leaflets illuminated with *white* light during their acclimatization to the septic life medium). The batches exvitrocultivated under *yellow, blue* and *green* light showed minuses of 5% - 8%, at this parameter (Graphic 2), these being insignificant statistically.

About chlorophyll *b*, the 2% plus, noticed at the batch kept under *red* light, but also the 4 - 6% minuses (Graphic 2), present at the other experimental variants whose exvitroplantlets were kept under *yellow, blue* or *white* light were insignificant statistically.

On the other hand, *carotenoids* differentiated the four experimental variants, grown under colored lights, towards the control (leaflets of the exvitroplantlets kept under *white* light). In this way, in the case of the exvitroplantlets illuminated with *red* light, the carotenoids level was 19% higher than that of the control. If in this situation a plus was noticed, significant statistically, at all other experimental batches, kept under *yellow, blue* or *green* light, were noticed negative differences also significant statistically, expressed in percents with values of 31%, 32% and respectively 43% (Graphic 2). One can certainly declare that, as far as the assimilating pigments are concerned, the most adequate light for intensifying their formation, especially of carotenoids, was the *red* one, and the weakest assimilating pigments genesis was marked under *green* light.



Graphic 2. Expressing the chlorophyll pigments from the African violets (*Saintpaulia ionantha*) exvitroplantlet leaflets in percentage values, *30 days* after their transfer in a septic medium under different types of light: *red, yellow, blue, green,* as reference values there were taken homologous values registered at the exvitroplantlets kept under *white* (control) light, these ones being considered 100% (* significant statistically differences towards the control, $p<0.05$)

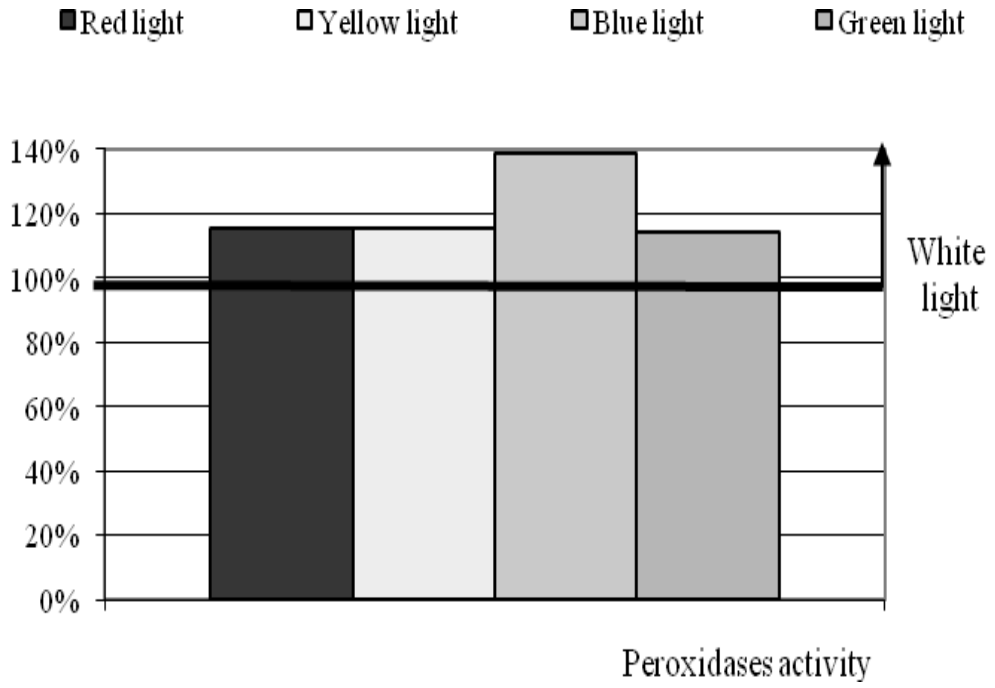
Analyzing the results of this experiment one can notice that, in the acclimatization period of the African violets to a septic medium, the *blue* and *green* light were the optimum ones, after the values registered at the control (*white* light). A possible explanation of these results could be that the *blue* light has a wave length close to the *white* one, 430 nm, and the same for the *green* one; more than that, the *green* light has a wave length of 544 nm, but it is the only one that can illuminate also with 400, 450, 480, 590, 620 nm.

Also *green* light was the most appropriate in the case of maintaining throughout the acclimatization of the *Cymbidium hybridum* cultures under the same types of neon, this conclusion being elaborated by us (Petruş – Vancea and Cachiţă, 2004), after analyzing the results of an identical experiment with the one

described before for the African violets. As for the *assimilating pigments* present in the orchid exvitroleaflets, there were not registered major differences, toward the control, between the batches kept under different colors, the only exception was the 17% inhibition, registered in the case of the 480 nm filter reading (representing the carotenoids), at the samples taken from the exvitroplantlets kept under the *yellow* light and the 86% plus, in the case of reading the same filter, at the samples taken form the exvitroplantlets acclimatized under *green* light. The *Cymbidium* exvitroplantlets kept under *green* light presented only increases toward the control, between 7% and 86%, for the assimilating pigments, at all three categories of filters where the extinctions were read.

c. The peroxidazic activity

The highest peroxidazic activity (Graphic 3), with a significant increase toward the control (exvitroplantlet rootlets kept under *white* light), expressed in percentage with values of over 39%, was registered at the exvitroplantlet rootlets that were kept under *blue* light, then, at the ones kept under *yellow* light, the increase of the activity was 16%, in this last case being insignificant statistically, toward the control; the peroxidazic activity of the exvitroplantlets illuminated with *green* or *red* light during their acclimatization, presented the smallest increases, also insignificant statistically, toward the control, 14%, respectively 15% (Graphic 3).



Graphic 3. Expressing the peroxidazic activity from the African violets (*Saintpaulia ionantha*) exvitroplantlet rootlets in percent values, at *30 days from their transfer into the septic medium* under different types of light: *red, yellow, blue, green*, as reference values there were considered the similar ones registered at the exvitroplantlets kept under *white* light (control), these ones being considered 100% (*significant statistically differences towards the control, $p<0.05$)

CONCLUSIONS

After analyzing the experiment's results where we tried to stimulate the formation of chlorophyll pigments through illuminating the African violet exvitroplantlets with *fluorescent tubes of different colors (red, yellow, blue* or *green*), in their acclimatization period to a septic medium, we could observe that the *blue* light, but especially the *green* one were the most appropriate, after the control (*white* light). Generally, the post-acclimatization survival percent was diminished with approximately 50% at the fluorescent *red* light, even if, the exvitroplantlets illuminated with this type of light presented a *peroxidazic activity* similar to the control, they also showed the only significant value of chlorophyll pigments superior to the control.

REFERENCES

Blidar C.F., Cachiţă C.D., Bandici G.E., Radoveţ-Salinschi D., Pop L., The reactivity of *Cymbidium hybridum* protocorms vitrocultivated under white fluorescent or natural light illumination. Annals of the University of Craiova, vol. XII (XLVIII), Craiova, pp. 287-294, 2007

Cachiţă C.D., Culturile de ţesuturi şi celule la plante – modele experimentale în biologia vegetală. In: Lucrările celui de al IX-lea Simpozion Naţional de Culturi de Ţesuturi şi Celule Vegetale, "Actualităţi şi perspective în biotehnologia vegetală". Cachiţă C.D., Bavaru A., Brezeanu A. (eds.), Constanţa, "Ovidius" University Press, pp. 31-40, 2000

Gaspar T., Keveres C., Hausman J.F., Berthon J.Y., Ripetti V., Uses of peroxidase activity as a predictive merker of rooting performance of micropropagated shoots, Agronomie, 12, pp. 757– 65, 1992

Lück H., Peroxidaza, In: Methods of Enzimatic Analysis, Bergmeyer HU (ed.), Ed. Academic Press, New-York, pp. 895-897, 1974

Moran R., Porath D., Chlorophyll determination in intact tissue using N,N-dimetylformamide, Plant Physiol., 65, pp. 487-479, 1980

Murashige T., Skoog F., A revised medium for rapid growth bioassays with tobacco tissue cultures, Physiol. Plant., 15, pp. 473–497, 1962

Petruş – Vancea A, Cercetări privind procesele morfofiziologice şi biochimice care au loc în decursul aclimatizării plantulelor generate „in vitro", la viaţa în mediul septic. Teză de doctorat, 2007.

Petruş-Vancea A., Cachiţă C.D., Studierea influenţei exercitate de natura luminii în aclimatizarea exvitroplantulelor. In: Lucrările celui de al XIII -lea Simpozion Naţional de Culturi de Ţesuturi şi Celule Vegetale, „Vitroculturile la cormofite, modele experimentale în cercetările de biologie", Cachiţă C.D., Ardelean A. (eds.), Satu-Mare, Editura Bion, pp. 138–151, 2005

Petruş-Vancea A., Cachiţă C.D., Şipoş M., Activitatea peroxidazică în rădăciniţele vitro- şi exvitroplantulelor de crizanteme, violete africane şi de cymbidium, Analele SNBC Vol. IX, Nr. 1., CAP. III – Biologie celulară vegetală, pp. 392–395, 2004

Pop L., Cachiţă C.D., Preliminary research concerning the reactions of *Sequoia sempervirens* vitrocultures to „high brightness" LED illumination, Annals of the University of Craiova, vol. XII (XLVIII), Craiova, pp. 215–219, 2007

Radoveţ-Salinschi D., Cachiţă C.D., Conţinutul în pigmenţi asimilatori în frunzuliţele vitroplantulelor de *Coleus blumei Benth.,* culturi iluminate cu lumină de culori variate. Analale Societăţii Naţionale de Biologie Celulară. Vol. IX nr. 1, Crăciun C, Ardelean A (eds.), Cluj – Napoca, Editura Risoprint, pp. 387–391, 2004

Şipoş M., Chiriac C., Floriş C., Activitatea peroxidazică în embrionii şi în plantulele de grâu (*Triticum aestivum* L. soiul *Turda*) rezultate prin germinaţia cariopselor submersate, în prealabil, în azot lichid (-196ºC). Analele Univ. Oradea, Fasc. Biologie, Tom X, pp. 315-320, 2003 a.

Şipoş M., Chiriiac C., Floriş C., Activitatea peroxidazică în cariopsele de grâu (*Triticum aestivum* L. soiul *Turda*) după submersarea acestora în azot lichid (-196ºC), Analele Univ. Oradea, Fasc. Biologie, Tom X, pp. 333-342, 2003 b

Şipos M., Samuel A.D., Bandici G.E., Peroxidases activity in the barley caryopses after cryopreservation, Analele Universitatii din Craiova, Vol. XII (XLVIII) Seria Biologie, pp. 241-245, 2007

Vancea A, Cachiţă CD, Aclimatizarea vitroplantulelor de *Saintpaulia ionantha*, prin plantarea acestora pe substraturi neconvenţionale. In: Lucrările celui de al X-lea Simpozion Naţional de Culturi de Ţesuturi şi Celule Vegetale, „25 de ani de culturi de ţesuturi vegetale în România", Cachiţă CD, Rakosy TL, Ardelean A (eds.), Editura Risoprint, Cluj-Napoca, pp. 310–315, 2002

Vancea A., Cachiţă C.D., Floriş C., Blidar C.F., Studii preliminare privind aclimatizarea vitroplantulelor de crizanteme, la mediul septic de viaţă, Analele Univ. Oradea, Fasc. Biologie, Tom. VII, pp. 283–294, 2000

Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii (Life Sciences Series), vol. 18, 2008

http://www.studiauniversitatis.ro