THE EFFECT OF LIGHT QUALITY ON SOME PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS OF *IN VITRO* PROPAGATED PETUNIA CV. "BLUE STAR" PLANTS

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Abstract

Petunia hybrida plants were propagated *in vitro* by single-node explants on basic MS medium (3.0% sucrose, 0.7% agar), pH adjusted to 5.7 - 5.8. The explants were cultivated in a growth chamber at different combinations of monochromic LED lighting at equal irradiance of 70 µmol.m⁻².s⁻¹ at the tops of the plants, 12-h photoperiod, 25/25 °C day/night temperatures and 60 - 70% relative humidity. Three different types of LEDs, emitting in the red (635 nm, R), green (531 nm, G), and blue (463 nm, B) region of the spectrum are used to compose 66: 33% RB and 33: 33: 33% RGB light. After four weeks of cultivation the following characteristics were observed: stem number and length, rooting, callus formation, fresh/dry weight index and malondialdehyde (MDA) levels. *P. hybrida* cultivated on red/blue LED form higher number and longer shoots and higher fresh/dry weight index they also have slightly larger content of MDA than plants exposed to cool white light.

Key words: Petunia hybrida, light quality, malondialdehyde

In plant in vitro cultures and greenhouse crops, factors such as growth medium content/soil composition, light qualities, temperature etc., strongly affect physiology, anatomy, morphogenesis and proliferation rates of the cultures. By changing some parameters of light such as wavelength (color), quantity and photoperiod, one can influence traits of horticultural interest- plant stature, growth and productivity. Effect of light spectrum quality on morphology and physiology of some plant species has already been analyzed. Time of flowering is diminished in Arabidopsis plants exposed to blue light, petiole length and leaf area are also affected (Eskins, 1992). The dependence of tiller formation on light spectrum quality and particularly the positive effect of red and far-red light in Triticum aestivus and Lolium spp. is also described (Casal, 1988; Deregibus et al., 1983) Increase of stem length as effect of red light exposure in Pelargonium in vitro cultures is reported by Appelgren (1991). The species from genus Petunia are used worldwide as ornamental plants. Petunia spp. are also potential model systems for research topics such as flower development, flavonoid synthesis pathway, male sterility, retroelement activity, etc. (Gerats and Vandenbussche, 2005). We focus our interest on the dependence of some morphological features of this species - stem number and length, rooting, callus formation, fresh/dry weight index and malondialdehyde (MDA) quantity in P. hybrida extracts, on light spectrum quality.

MATERIAL AND METHODS

<u>Plant material</u>: *Petunia hybrida* cv. "*Blue star*" plants were obtained from Department of Plant Physiology, Faculty of Biology, Sofia University "St. Kliment Ohridski". Explants were micropropagated by single-node explants on basic MS medium (3% sucrose, 0.7% agar), pH adjusted to 5.7 – 5.8. All experiments and measurements were conducted following four weeks of cultivation.

<u>Cultivation conditions</u>: Explants were cultivated at equal irradiance of 70 μ mol.m⁻².s⁻¹ at the top of the plants, 12 h photoperiod, 25/25 °C day/night temperature and 60 – 70% relative humidity and the light spectrum conditions: 66: 33% RB and 33: 33: 33% RGB light.

Extraction: Fresh plant material (300 mg) was homogenized at 4 °C with 0.1% trichloracetic acid (TCA) and centrifuged at 12 000 rpm for 20 min. The supernatants were used immediately for MDA determination.

<u>MDA quantification</u>: For quantification of MDA, the methodology of Dhindsa et al. (1981) with some modifications was used. Samples containing 0.5 ml of fresh plant material methanol extract and 20% TCA, containing 0.5% thiobarbituric acid were incubated in boiling water bath for 30 min, cooled on ice and tempered at room temperature after which their absorbance at 532 nm and 600 nm was measured. MDA content is expressed as mM/g fresh weight.

Variant	Stem number	Stem length, cm	Root formation	Callus formation	Fresh/dry weight index
RGB	1.90 ± 0.57	12.95 ± 3.03	+	-	4.42
RB	2.00 ± 0.41	14.90 ± 1.34	+	-	5.44

RESULTS AND DISCUSSION

Morphological characteristics of floricultural species are narrowly related to their decorative qualities. Effect of different combinations of LEDs on another ornamental species - chrysanthemum was researched by Kim et al. (2003). Chrysanthemums grown on RB light had higher fresh/dry weight, leaf area and chlorophyll content and greater size of stomata, these results were comparable to that obtained for plants grown on fluorescent light. RB light exerted the same effect on fresh weight, chlorophyll content and leaf area in Doritaenopsis plants (hybrid between orchid genera Doritis and Phalaenopsis) (Shin et al., 2008). Petunias grown on monochromic RB light were proved to form higher number of longer stems in comparison to RGB light. Both variants exhibited root formation. Fresh/ dry weight index of P. hybrida cultivated on red/blue light was also slightly higher than those of the explants grown on CWF. These results are consistent with the aforementioned and probably are due to coincidence of the wavelengths used and the absorption maxima of chlorophyll. Neither RGB nor RB monochromic light provoked callus formation. Malonedialdehyde content of the monochromic light-grown plants was also slightly higher (Table 1, Figure 1).



Fig. 1. MDA content of explants cultivated on monochromic red/blue light

Malondialdehyde quantity is considered a reliable indication for levels of peroxidation of polyunsatyrated fatty acids, which in turn is a result of ROS accumulation due to different kinds of physiological stress (Yakimova et al., 2002).

CONCLUSIONS

Given the results from morphometric measurements, it can be concluded that the more appropriate light quality condition for cultivation of this ornamental species is red/blue monochromic light. However, as far as MDA content of plants grown under this condition is slightly higher, in order to determine which of the variants is more suitable for cultivation of *P. hybrida* further examination is needed.

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