RESEARCHES REGARDING THE PHOTOPERIODS INFLUENCE ON PHYSIOLOGICAL INDICATORS AT SOME CULTIVARS OF *EUSTOMA GRANDIFLORUM*, IN THE MULTIPLICATION PHASE OF *IN VITRO* CULTURE

CERCETĂRI PRIVIND INFLUENȚA FOTOPERIODISMULUI ASUPRA INDICATORILOR FIZIOLOGICI, ÎN FAZA DE MULTIPLICARE IN VITRO, LA UNELE SOIURI DE *EUSTOMA GRANDIFLORUM*

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Abstract. *This scientifically papers present the experimental results regarding the influence of photoperiod on physiological indicators, in multiplication stage of in vitro culture. Eustoma grandiflorum is flower specie that belongs to Eustoma genus from Gentianaceae botanic family. The experiment studied the main physiological parameters at three cultivars of Eustoma grandiflorum (Asenka, Magic blue, Echo White). We conclude that the influence of photoperiod don't present significant differences from statistic point of view. These aspects justify the decreasing of photoperiod to 12 hours, with positive influence on energetic costs during multiplication phase of in vitro culture.*

Key words: photoperiod, in vitro culture, physiological parameters, *Eustoma grandiflorum*

Rezumat. *Lucrarea prezintă rezultatele experimentale privind influența fotoperiodismului asupra indicatorilor fiziologici, în faza de multiplicare in vitro, la Eustoma grandiflorum. S-au studiat principalii parametri fiziologici la trei soiuri de Eustoma grandiflorum (Asenka, Magic blue, Echo white) în condiții de fotoperioadă de 12 ore şi 16 ore. Rezultatele obținute arată că valorile principalilor indicilor fiziologici analizați, în funcție de cele două niveluri de fotoperiodism, nu prezintă diferențe semnificative din punct de vedere statistic. În acest sens, se justifică reducerea fotoperioadei de la 16 ore la 12 ore, cu implicații pozitive asupra reducerii consumului de energie şi implicit a costurilor energetice din cadrul acestei faze tehnologice a procesului de multiplicare in vitro.*

Cuvinte cheie: fotoperiodism, cultura in vitro, parametrii fiziologici, *Eustoma grandiflorum*

INTRODUCTION

Eustoma grandiflorum is flower specie that belongs to *Eustoma* genus from Gentianaceae botanic family, Gentianales ordin, Dicotyledonates class (*Bailey L.H., 1976; Şelaru Elena, 2002). Eustoma* genus is contenting from 27 herbs and wood species meeting in special at south part of SUA and Mexic (*Roh S.M. şi Lawson R.H., 1988*).

In the origin area, Eustoma *grandiflorum* is called blue bell *(Elena Şelaru, 2002).* In Europa is presenting from 1835.

By utilization of achieved *in vitro* plants the process of flowering is earliest and the period of harvesting is much bigger, due the increasing of flowers on the initial plant (*Farina si Ruffoni, 1993*). *Griesbach R.J. et all (1988)* used in the in vitro culture as a explants top of shoots, fragments of leaves and steam.

Most of researches achieved at the national and international level is referring at the cultivars behaviour on different nutritive media. *Ördögh et all (2004)* has studied the behaviour of four Eustoma cultivars in multiplication and rooting in vitro stages. The highest multiplication rate was registered on nutritive media M&S (1962) improving with 0.1 mg/l BAP.

MATERIAL AND METHODS

The research experiment was carrying out at vegetal biotechnologies laboratory from University of Pitesti.

The research experience have two factors using three cultivars of *Eustoma (Asenka, Magic blue, Echo White)* and two level of photoperiod (16 and 12 hours photoperiod).

Biological material was representing by shoots harvesting in the growing vegetative stage.

The experimental factors were:

- A factor - cultivar with three graduations: Echo White, Magic Blue, Asenka;
- B factor – duration of photoperiod with two graduations: 16 hours light photoperiod, 12 hours light photoperiod.

For studying of *in vitro* multiplication capacity of explants growing in the initial phase of *in vitro* process were used nutritive media based on Murashige and Skoog (1962) (fig. 1). The nutritive media were sterilized by autoclavation at one atmosphere, 20 minutes and 121^{o}C. Work instruments were sterilized by etuve at 120^{o}C and 20 minutes. During multiplication phase, the explants were keeping in the growing room at 22-24^{o}C, 12 and 16 hours light photoperiod, 3000 lucs light intensity.

Physiological observations consist in:

- Determination of assimilatory pigments quantity by spectrophotometer method;
- Determination of dry substance by thermo balance;
- Determination of total water by thermo balance;
- Determination of photosynthesis intensity by Wartburg method;
- Determination of respiration intensity by Wartburg method.
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Fig. 1. Aspects from *in vitro* multiplication phase

RESULTS AND DISCUSSIONS

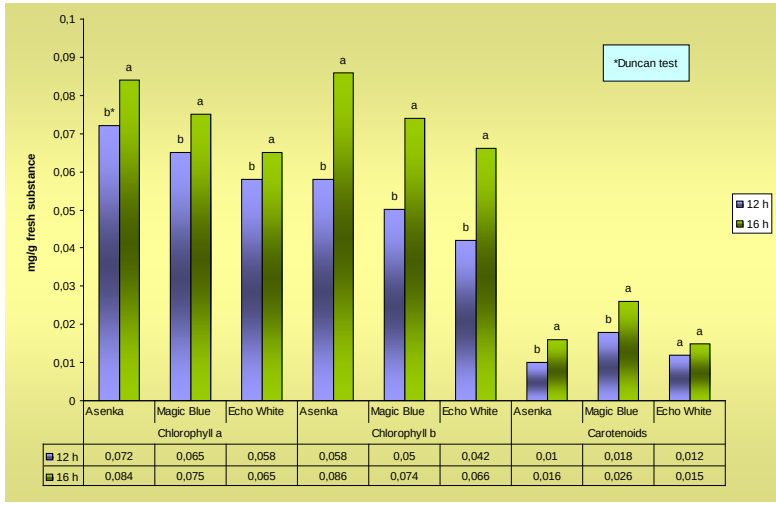
During *in vitro* multiplication phase were studied the main physiological parameters. In this sense was establishing the impact of photoperiod influence on physiological indicators, in the *in vitro* multiplication phase.

Regarding photoperiod influence on assimilatory pigment content at *Eustoma grandiflorum*,(mg/g fresh weight), Asenka cultivar registered the bigger quantity in chlorophyll a (0,084 mg/g fresh weight) and chlorophyll b (0,086 mg/g fresh weight) for both variant of photoperiod with 12 and 16 hours light, the content being much more for 16 hours light photoperiod. The content of carotenoids was the highest at Magic Blue cultivar for both variants of photoperiod (0,026 mg/g fresh weight) (fig. 2).

Echo white cultivar registered a bigger quantity of chlorophyll a than chlorophyll b or carotenoids pigments. The same cultivar achieved at the lowest content of assimilatory pigments in comparison with Asenka and Magic blue cultivars for both level of photoperiod used in the research experiment.

Regarding the photoperiod influence on total water quantity and dry substance at *Eustoma grandiflorum* in the *in vitro* multiplication phase, the cultivars registered significant differences for both variants of photoperiod (fig. 3).

Asenka cultivar achieved the lowest quantity of total water (88,77 %) and the bigger quantity of dry substance (11,3 %). In the same time, Echo white cultivar had a bigger percent of total water (89,9 %) and the smallest percent of dry substance (10,1 %).



	Chlorophyll a			Chlorophyll b			Carotenoids		
	Asenka	Magic Blue	Echo White	Asenka	Magic Blue	Echo White	Asenka	Magic Blue	Echo White
12 h	0,072	0,065	0,058	0,058	0,05	0,042	0,01	0,018	0,012
16 h	0,084	0,075	0,065	0,086	0,074	0,066	0,016	0,026	0,015

Fig. 2. Photoperiod influence on assimilatory pigments content at *Eustoma grandiflorum*

Magic Blue cultivar achieved the accumulation of 91, 3 % total water quantity for 12 hours light photoperiod respective 89,4 % total water quantity for

16 hours light photoperiod. Regarding the content of dry substance Magic blue cultivar registered 10,6 %.

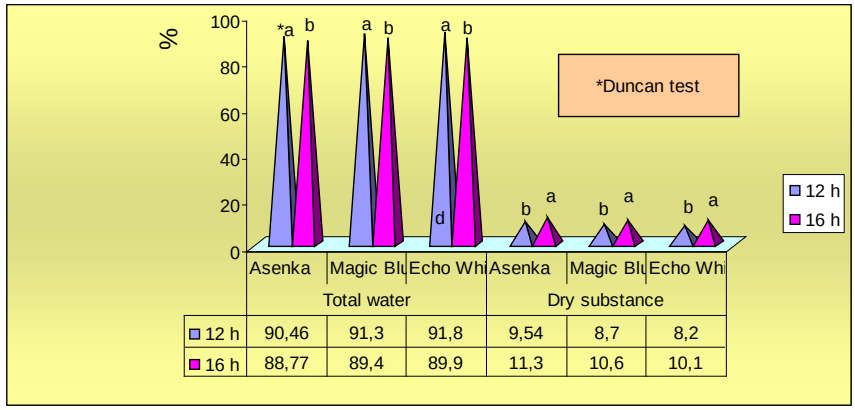


Fig. 3. Photoperiod influence on total water and dry substance quantity

Photoperiod influence on respiration intensity at *Eustoma grandiflorum* in the multiplication of *in vitro* culture expressing by cm^3 O_2/g/h don't was conduct at significant differences, in conforming to statistic interpretation by Duncan test. Thus is confirming the published scientific paper where is emphasize the fact that the vitroplants have a week expression of physiological indicators. Respiration intensity has the bigger value for Asenka cultivar respective Magic blue and Echo white (fig. 4).

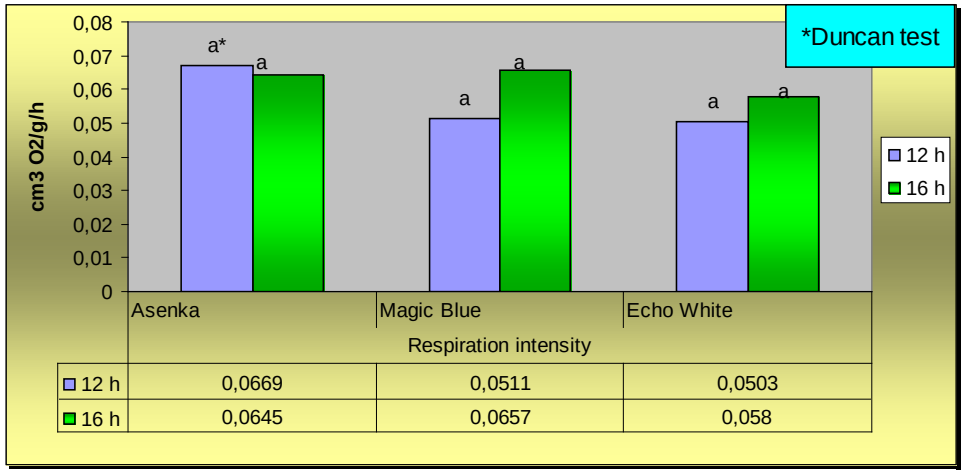


Fig. 4. Photoperiod influence on respiration intensity

The photosynthesis capacity has also expresiing at the small values, without significant differences between values of photosynthesis achieved in conditions of 12 and 16 hours light photoperiod.

The photosynthesis capacity of Asenka cultivar for 16 hours light photoperiod has bigger in comparison with 12 hours light photoperiod (0,012 cm^3 O_2/g/h respective 0,0092 cm^3 O_2/g/h). The same trend is observed at Magic blue and Echo white cultivars. Echo white cultivar has a 0,0097 cm^3 O_2/g/h photosynthesis capacity for 16 hours light photoperiod and 0,0084 cm^3 O_2/g/h for 12 hours light photoperiod (fig. 5). Similar with respiration intensity, the photosynthesis level is going to the follow classification: Asenka, Magic blue, Echo white.

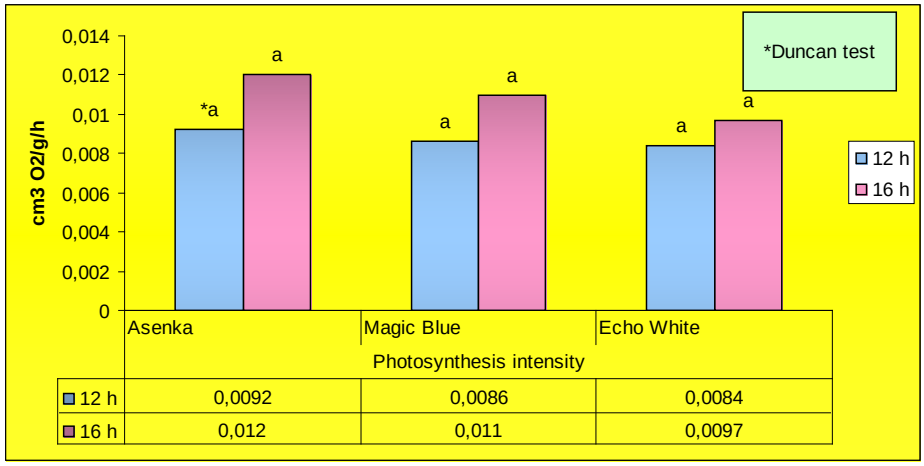


Fig. 5. Photoperiod influence on photosynthesis intensity

CONCLUSIONS

Asenka cultivar registered the bigger quantity of chlorophyll a and b for both variants of photoperiods with more quantity for 16 hour light photoperiod than 12 hours light photoperiod.

Photoperiod influence on respiration intensity at *Eustoma grandiflorum* in the multiplication of *in vitro* culture expressing by cm^3 O_2/g/h don't was conduct at significant differences, in conforming to statistic interpretation by Duncan test.

The photosynthesis capacity has also expresiing at the small values, without significant differences between values of photosynthesis achieved in conditions of 12 and 16 hours light photoperiod. Similar with respiration intensity, the photosynthesis level is going to the follow classification (growing graduation): Asenka, Magic blue, Echo white.

The values of physiological indicators analyzed in function of two level of photoperiod don't present significant differences from statistic point of view. In this sense is justify the reducing of light period from 16 hours to 12 hours with positive implication on reducing of energy consumption respective the energetic costs in the technological phase of *in vitro* multiplication stage.

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