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THE CONTENT IN ASSIMILATING PIGMENTS OF THE COTYLEDONS OF THE RED CABBAGE PLANTLETS ILLUMINATED WITH LEDS

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ABSTRACT. The red cabbage seeds germination and the exposition of the plantlets – for 10 days – to the white or colored light issued by LEDs (Light-Emitting Diodes), compared with the control samples – which were grown in daylight (values considered 100%) – led to a reduction of the content in total assimilating pigments of their cotyledons, as following: with 9.19% - at the samples exposed to white light, with 14.73% - at those illuminated with blue light, with 14.46% - at the seedlings exposed to yellow light, and with 23.61% - at the plantlets lighted with LEDs issuing green light; the seedlings illuminated with red radiations marked an insignificant increase, of 0.39%, as against to the control. Thus, the lowest assimilating pigments content (diminished with 16.34% - chlorophyll a and with 25.48% - chlorophyll b, respectively, with 22.15% - the total amount of green pigments and with 30.72% - the carotenoidic pigments) was registered in the cotyledons of the red cabbage plantlets exposed for 10 days to an illumination with LEDs generating green light.

Keywords: *Brassica oleracea*, seedlings, cotyledons, assimilating pigments, Light-Emitting Diodes

INTRODUCTION

Between the intensity of photosynthesis and the content of plants in assimilating pigments exists a close correlation. Tennessen and his colleagues (1994) showed that the exposure of plants to a narrow-spectrum light, such as that emitted by the electroluminescent diodes (Light-Emitting Diodes or LEDs) can be successfully used in photosynthesis researches. The benefits of LEDs lighting in ecological agriculture are numerous and illumination of cultures with these represents a worthy alternative to be considered for the future. Even today, in some urban areas from Japan there are so-called "plant factories" (Cosmo Plant Co., Ltd., Fukuroi, Japan) where LEDs are used as lighting sources in lettuce production, vegetables which are sold on the local market (Massa et al., 2008). In plants, the effect of spectral quality of light on the accumulation of assimilating pigments in the green organs was studied by Samuoliene and the collaborators (2010), on strawberry plants which were subjected to an illumination, 16 hours/day, with LEDs issuing combined red (640 nm) and blue (455 nm) light (with a photosynthetic photon flux density of 200 µmol·m-2s-1). The authors concluded that this type of light was favourable for the plants growth until the moment of their fructification, promoting the accumulation of carbohydrates. Matioc and Cachiţă (2011a), performing studies on black pine plantlets derived from seeds germinated under light of different wavelengths produced by LEDs (with a luminous intensity of 1200 lx), after 21 days of exposure – 16 hours light / day – at a such regime, in relation with the values of the control samples (maintained in natural light), concluded that the red light emitted by LEDs increased with 39.8% the medium size of the seedlings and with 47.1% their dry biomass; also, at these plantlets, the total amount of assimilating pigments – in cotyledons – was grown by 77.1%, as against to the values recorded to this parameter at the reference lot, samples exposed to natural light, values considered 100%. In the present study we analyzed the content in photosynthetic green pigments – chlorophyll a and chlorophyll b – as well as in carotenoidic pigments, in the cotyledons of the red cabbage seedlings (Brassica oleracea L. var. capitata f. rubra „Red Amager") originated from seeds germinated under the incidence of the white or coloured light, emitted by LEDs, compared to the similar determinations made in the control group, samples exposed to natural light. This work represents, in fact, our further research regarding the investigation of the influence of LEDs light on the germination and growth of the seedlings of various plant species. In a previous paper (Matioc and Cachiţă, 2012b), we had shown that, the exposure of red cabbage plantlets to the green light of LEDs conducted to an increase of 75% in the hypocotyls medium size and of 77% in the dry weight of this organ, compared to the control lot, which was grown in daylight.

MATERIALS AND METHODS

In these experiments, we used as plant material cotyledons of red cabbage seedlings, found in the 10th day of germination, which were resulted from seeds germinated on filter paper substrate wetted regularly with tap water. The germination process took place in plastic boxes, colourless and transparent, allowing free access of light to the seeds and seedlings. The light sources were represented by installations with LEDs (mark Zextar, 5 mm), mounted on panels, having a density of 40 LEDs/600 cm², panels that were placed at a distance of 20 cm from seeds and seedlings. The light intensity was kept constant – at 1200 lx – using a

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potentiometer. Photoperiod was 16 hours of light / day. The natural light used for the seeds germination and seedlings growth of the control group (variant noted with V0) came from a window with a northern orientation. Laboratory humidity ranged from 50-60% and the ambient temperature was 25±1°C, both day and night. The experimental variants were organized in the increasing order of the wavelengths of light (Tab. 1).

Table 1

The experimental variants organized according to the wavelengths of light emitted by LEDs (www.zextar.ro)

Experimental variant	Wavelength	Colour of light
V_1	380-760 nm	white
V_2	465 nm	blue
V_3	520 nm	green
V_4	590 nm	yellow
V_5	650 nm	red

In order to determine the content of the red cabbage cotyledons in assimilating pigments we used the spectrophotometric method described by Moran and Porath (1980). The extraction of the assimilating pigments was made in pure dimethylformamide solution (DMF, 99.9% Merck), the process being carried in dark, at 4°C, for 72 hours. The wavelengths at which the extinctions readings of the samples were made, at a spectrophotometer type Spekol 11, Carl Zeiss Jena, were: 664 nm for chlorophyll a, 647 nm for chlorophyll b and 480 nm for carotenoidic pigments. To convert the values of the read extinctions of the samples in quantitative values we used the coefficients proposed by Wellburn (1994):

Chlorophyll a (mg/g FW) = $(11.65 \cdot A664 - 2.69 \cdot A647) \bullet V/FW$

Chlorophyll b (mg/g FW) = $(20.80 \cdot A647 - 3.14 \cdot A664) \bullet V/FW$

Carotenoidic pigments (mg/g FW) = $(1000 \cdot A480 - 1.28 \cdot clf\ a - 56.70 \cdot clf\ b) /245 \bullet V/FW$

where:

A664, A647 and A480 = pigments absorbance values of these wavelengths;

V = extract volume in ml;

FW = fresh weight of tissue expressed in mg.

The total in chlorophyll pigments was calculated by summing the amount of chlorophyll a with that of chlorophyll b and by adding the carotenoidic pigments values to this sum we obtained the total quantity of assimilating pigments from the extract analyzed.

Three repetitions were made at each experimental variant and the arithmetic mean and the standard deviation from the mean were calculated. The average values of each variant, individually, were reported to the data determined at the control group, values considered as reference, of 100%. The statistical calculus was done by applying Students' t test for independent groups of samples, using GraphPad Software (two-tailed P value), at a confidence level of 95%, thus achieving the statistical significance of the data.

RESULTS AND DISCUSSIONS

In relation to the control group values, cultures illuminated with natural light (V0) – in the 10th day of germination – the content in chlorophyll a, which was determined in the cotyledons of the red cabbage plantlets exposed to the white light emitted by LEDs (V1) (Fig. 1), recorded a minus of only 2.30%, while the amount of chlorophyll b marked a loss of 11.12% as against to the reference values V0, considered 100%; however, at this lot, the content in carotenoidic pigments was reduced by 15.45% in comparison with the control samples. So, compared to the control lot - V0 – the total amount of green pigments was diminished by 7.91% and the content in total assimilating pigments (chlorophylls and carotenoids) registered a 9.19% decrease of their concentration, values statistically significant, at the threshold $p \leq 0.05$.

The illumination of red cabbage seedlings with LEDs issuing blue light (V2) (Fig. 1) induced the reduction with almost 15% of the content in total assimilating pigments, compared with that determined in the cotyledons of the cultures grown in natural light (V0). At these samples, the decrease of the cotyledons content in total assimilating pigments, statistically significant, was found both for the green pigments – chlorophyll a registered a minus of 8.26%, chlorophyll b one of 16.38%, respectively, the total green pigments one of 13.60% -, and for the carotenoidic pigments which marked a 20.22% reduction of their content (relative to the similar values, means determined at the control group V0, considered as 100%). The cotyledons of the red cabbage plantlets which were – on the experimental period – exposed to the green light of LEDs (V3) (Fig. 1) showed the lowest values of chlorophyll a (-16.34%), and of chlorophyll b (-25.48%), respectively of carotenoidic pigments (-30.72%). Consequently, the content in total chlorophyll pigments was lowered by 22.15% as against to the reference values of the control samples (V0), the total quantity of assimilating pigments being diminished with 23.61%, differences statistically validated. These results are in accordance with those obtained by us for the cotyledons of the black pine seedlings exposed to this type of light, at which we found a 20.5% decrease of the content in chlorophyll a, in relation to the samples maintained in natural light (Matioc and Cachiţă, 2012a). After Frencilla and his collaborators (2000), in terms of plant response to the green light, an important role would hold the carotenoids, namely

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zeaxanthin. Moreover, Folta and Maruhnich (2007) had opined that plants possess some “sensors” sensitive to green light, such as phytochromes and cryptochromes, but their efficiency is relatively low compared to the responsiveness of these to the wavelengths corresponding red and blue light.

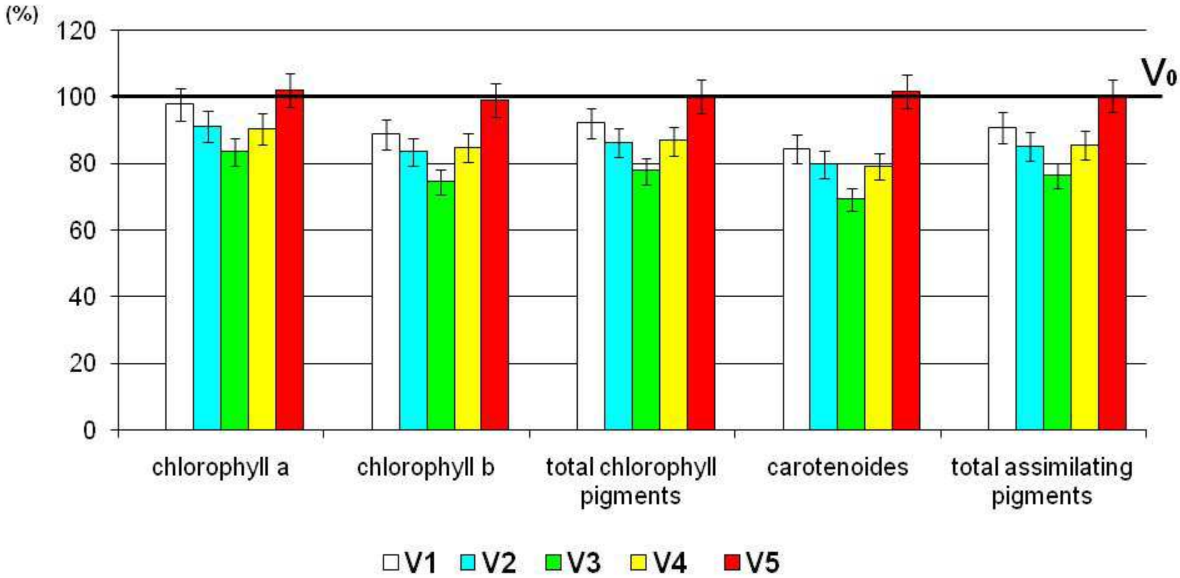

Fig. 1. The assimilating pigments content determined in the cotyledons of the red cabbage plantlets (Brassica oleracea L. var. capitata f. rubra „Red Amager”), in the 10th day of germination, exposed to the LEDs light: V1 – white light (380-760 nm); V2 – blue light (465 nm); V3 – green light (520 nm); V4 – yellow light (590 nm); V5 – red light (650 nm); the control variant - V0, plantlets grown in natural light, reference values, considered as 100%.

In the case of the experiments presented in this paper, we found that the yellow light of LEDs (V4) (Fig. 1) induced a 9.55% reduction of chlorophyll a content in the cotyledons of the red cabbage seedlings illuminated with such a light source, reducing with 15.24% the amount of chlorophyll b and with 13.16% the total quantity of green pigments, respectively lowering with 20.79% the content in carotenoidic pigments, which led to a minus of 14.46% of the assimilating pigments quantity, compared to the reference data considered 100%, of the control samples (V0). All data presented were statistically significant.

Measurements performed on extracts originating from red cabbage seedlings grown in the red light of LEDs (V5) (Fig. 1) revealed that their cotyledons presented a content in total assimilating pigments similar with that dosed in the cotyledons of the plantlets exposed to natural light (V0); therefore, the minuses and pluses noted as against to the control (V0), statistically insignificant, were: +1.98% regarding the chlorophyll a content, minus 0.97% chlorophyll b, +0.11% the total quantity of green pigments, +1.69% the carotenoidic pigments, respectively +0.39% the total in assimilating pigments. So that, from all types of light issued by LEDs, tested by us, at this experimental variant (red light) the highest values of the cotyledons content in total assimilating pigments were registered.

CONCLUSIONS

Red cabbage seeds germination at the LEDs light, and subsequently, plantlets exposure at the same type of illumination, for 10 days, in a 16 hours/day regime, except red light, resulted in a decrease of the total assimilating pigments content in cotyledons, compared with the same parameter determined at the samples kept in daylight, the red light of LEDs causing the maintenance of the total assimilating pigments at levels close to those recorded at the control samples, batches germinated and grown in natural light.

The total assimilating pigments content in cotyledons of red cabbage seedlings was lower than the respective values of the similar parameter determined at the control group plantlets, the decreases noticed being: of 9.19% at the group illuminated with white LEDs, of 14.73% at the plantlets grown under blue light of LEDs and of 14.46% at those exposed to the yellow light issued by LEDs, differences that were statistically significant.

The lowest content in total assimilating pigments in the red cabbage plantlets cotyledons was marked at the group illuminated with green light, issued by LEDs, this being reduced by 23.61%, compared to the values registered at the control samples, considered 100%.

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