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EFFECT OF LED LIGHTING ON THE GROWTH OF RASPBERRY (*RUBUS IDAEUS* L.) PLANTS *IN VITRO*

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Abstract

In recent years, the light emitting diodes (LED) have become an alternative to the fluorescence lamp source of light for plant tissue culture, due to their low energy consumption, low heat emission, specific wavelength irradiation etc. The aim of this study was to investigate the effect of LEDs on the growth of *in vitro* cultivated raspberry (*Rubus idaeus* L. 'Lloyd George'). The plantlets were cultivated *in vitro* under an illumination system based on Philips GreenPower LED research module. Four groups of LEDs emitting in white (W), red (R), blue (B), mixed (W:R:B:far-red=1:1:1:1) lights and fluorescent lamps (control) were used in our studies. Growth parameters, some physiological and biochemical characteristics of the plantlets were measured after three four weeks passages under corresponding light treatment. Our results indicated that different LEDs specifically influence the growth and development of *in vitro* cultivated raspberry plantlets and could be applied as an efficient lighting system for rapid *in vitro* micropropagation of *Rubus idaeus* L. The combination of blue, red, far red and white LEDs (1:1:1:1) stimulated the growth and biomass accumulation, as well as the intensity of net photosynthesis. For optimal results, it would be advisable to shorten the culture period to 3 weeks. This effective and affordable protocol would support the commercial micropropagation of raspberries and other soft fruits.

Keywords: micropropagation, shoot culture, LED, light quality, photosynthetic pigments, chlorophyll fluorescence.

INTRODUCTION

Light is one of the most important factors for growth and development of plants. The intensity, quality, and duration of light affect photosynthesis and photomorphogenesis, of plants. Fluorescence lamps (FL) traditionally have been the most used artificial light source in plant tissue culture, although the different emission spectra of the commercially available lamps do not always match the sensitivity range of plant photoreceptors (Gupta and Jatothu, 2013). In recent years, the light emitting diodes (LED) have become an alternative source of light for plant tissue culture due to their low energy consumption, low heat emission, specific wavelength irradiation etc. (Bourget, 2008; Morrow, 2008). Numerous studies reported the successful applications of LEDs in promoting *in vitro* growth and morphogenesis in various plant species (Gupta and Jatothu, 2013). Better growth and *ex vitro* survival rate and biomass yield have been reported under various LED treatments (Hahn et al., 2000; Nhut et al., 2003; Jao et al., 2005; Shin et al., 2008; Li et al., 2010; Gupta and Sahoo, 2015; Shulgina et al., 2018). In these studies, it is noted that the

requirements of the different genotypes to the spectral composition and photosynthetic photon flux density (PPFD) are specific.

Raspberries, the fruits of the perennial shrub (*Rubus idaeus* L.) of the *Rosaceae* family, are widely recognized as one of the healthiest fruits (Kaume et al., 2012, Stanisavljevic et al., 2019). The importance of raspberries for human health and their recognition as a "superfood" (Hancock et al., 2007, Boone, 2013) have increased their popularity and led to a high demand in recent years.

Micropropagation of raspberries under sterile, controlled conditions has been used for more than 40 years to produce a large number of pathogen-free genetically identical plants from selected genotypes. Availability of many cultivars and the great differences between them in their requirements for regeneration and propagation (Reed, 1990, Zawadzka and Orlikowska, 2006 a,b, Wu et al., 2009) define raspberries as particularly recalcitrant to micropropagation. There are only a few reports in the literature on the effect of different LED lights on raspberries *in vitro*. According to Rocha et al. (2013), red LEDs contribute to increased multiplication and rooting of shoots of 'Batum' and 'Dorman Red' raspberry cultivars in comparison to the fluorescent lamps. In experiments presented by Poncetta et al. (2017), the mixed LED light yielded in a less efficient multiplication of red raspberry in comparison to fluorescent lights, but with higher quality shoots.

The aim of this study was to investigate the effects of LED lighting on the growth of *in vitro* cultivated raspberry (*Rubus idaeus* L.).

MATERIALS AND METHODS

Plant material and experimental conditions

The experiment was carried out on red raspberry (*Rubus idaeus* L. 'Lloyd George'). *In vitro* shoot culture was maintained at 4-week subculture intervals on solid DKW (Driver and Kuniyuki, 1984) medium, supplemented with 2.5 μM 6-benzylaminopurine (BAP), 0.05 μM indol-3-butyric acid (IBA), 30 g L^{-1} sucrose, 6.5 g L^{-1} Phyto agar (Duchefa, The Netherlands). The medium (pH 5.6) was autoclaved at 121°C for 20 min. Plantlets were grown in baby food glass jars with transparent Magenta B-Cap lids with 25 mL nutrient medium per vessel. At each subculture, apical part of shoots (10 – 15 mm) with two-three leaves was transplanted to the fresh medium with five explants per vessel. Cultures were grown at 22±2 °C under a 16-h photoperiod (87 ± 7.5 μmol m^{-2} s^{-1} photosynthetic photon flux density, PPFD), provided by Philips GreenPower LED research module (Philips Lighting, www.philips.com/horti). Four groups of LEDs emitting in white (W), red (R, 650-670 nm), blue (B, 455-485 nm), far red (FR, 725-750 nm) were used. Thus, 4 treatments – W, R, B and mixed (BR, W:R:B:FR=1:1:1:1) were studied. Plantlets grown in the same way under fluorescent lamps (FL) served as a control.

Physiological and biochemical parameters

Growth parameters

In five passages of four weeks, the following parameters of the plants grown under different light were evaluated: fresh (FW) and dry weight (DW), length of main shoot, number and length of lateral shoots, content of photosynthetic pigments, gas exchange rate and chlorophyll a fluorescence. The multiplication index (MI) was calculated as a number of proliferated shoots from the initial one.

Photosynthetic pigments content

The photosynthetic pigments (chlorophyll a, chlorophyll b and total carotenoids) were extracted in cooled 80% acetone, determined spectrophotometrically, and calculated according to the formulae of

Lichtenthaler and Wellburn (1983).

Gas-exchange analysis

Gas-exchange analysis was performed with a portable gas exchange system LCpro+ (ADC, UK) at a light intensity of about 150 µmol m^{-2} s^{-1} PPFD and ambient CO_2 concentration 1100 vpm. Each plantlet was placed in the conifer measurement chamber, and the base of the shoot was wrapped in wet filter paper to prevent it from drying out. Net photosynthesis rate (A, µmol CO_2 $plantlets^{-1}$ s^{-1}), transpiration intensity (E, mmol H_2O $plantlets^{-1}$ s^{-1}) and photosynthetic water use efficiency (A/E) were determined.

Chlorophyll *a* fluorescence

Chlorophyll *a* fluorescence analysis was performed on the youngest native fully developed leaves of 5 representative plants of the respective variant. The basic parameters of the rapid chlorophyll *a* fluorescence were measured by a HandyPEA portable fluorimeter (Hansatech Instruments, UK). The analyzed spots of the leaves were dark adapted for 40 minutes with special clips. The whole plants (together with the clips) were covered with moist filter paper and placed in a plastic bag to prevent them from drying out. Induction curves of the rapid chlorophyll a fluorescence (OJIP test) were recorded for 1 s with 3000 µmol m^{-2} s^{-1} PPFD. The primary data processing was done using the PEA Plus Software (V1.10, Hansatech Instruments Ltd., UK). The parameters measured and calculated using this OJIP test (Table 1.) were interpreted and normalized according to Strasser and Strasser (1995) and Goltsev (2016).

Total polyphenols determination

The total amount of polyphenol compounds in the plant extracts was determined with the Folin-Ciocalteu reagent (Waterman et al., 1994), according to Singleton and Rossi (1965), with slight modifications. The samples (1 g of fresh plant material) were ground with quartz sand and 10 ml 60% acidic methanol, and submerged in an ultrasound bath for 15 min. The homogenized material was left for 15 hours in the dark at room temperature for extraction. Afterwards, the test tubes were centrifuged and the supernatant was used for the measurement of total polyphenols content and antioxidant activity. For the determination of total phenolics, 40 µl of extract were mixed with 3160 µl distilled water, 200 µl Folin-Ciocalteu reagent and in a minute after that 600 µl 20% Na_2CO_3 was added. The test tubes were left for 2 hours at room temperature for the reaction to occur. After that, the extinction at 765 nm wavelength was measured. Total phenolics were calculated as gallic acid equivalents (GAE) using a standard curve and were presented as mg g^{-1}_{FW}. The standard curve was prepared with gallic acid (Sigma-Aldrich, St. Louis, MO) in the range 0–500 mg l^{-1}.

Determination of antiradical activity

For determination of the antioxidant activity the extracts obtained for total phenolics and 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Beta et al., 2007) were used. The incubation mixture contained 100 µl plant extract and 3.9 ml 6×10−5 mol l^{-1} DPPH (0.06 µmol). The extract absorption was determined at 515 nm at min 0 and 30 from the initial mixture of the components. A parallel blank sample was tested, which contained distilled water instead of extract. The antiradical activity is expressed as % discoloration and is equal to:
$(1 - (A_{30min}/A_{0min}) \times 100)$.

Measurement of malondyaldehyde (MDA) content

MDA content was determined by the thiobarbituric acid (TBA) reaction as described by Ali et al. (2005), with slight modifications. Approximately 0.5 g leaves were homogenized with 5 ml of 0.5% trichloroacetic acid (TCA)

and centrifuged at 14 000 rpm for 10 min. After centrifugation, 1 ml of the supernatant was mixed with 4 ml 0.5% TBA in 20% TCA and incubated in hot water (90 °C) for 20 min. Thereafter, it was cooled immediately on ice to stop the reaction and centrifuged at 14 000 rpm for 10 min. Absorbance at 532 and 600 nm was determined, and MDA concentration was estimated by subtracting the non-specific absorption at 600 nm from the absorption at 532 nm, using an absorbance coefficient of extinction (155 mM^{-1} cm^{-1}).

Table 1. Definitions of measured and calculated chlorophyll *a* fluorescence parameters used in the experiment (Based on Strasser and Strasser (1995) and Goltsev (2016)).

Chlorophyll *a* Fluorescence Parameter	**Description**
Measured parameters and basic JIP-test parameters derived from the OJIP transient	
$F_O \sim F20\mu s$	Minimum fluorescence, when all PSII reaction centres (RCs) are open; Fluorescence intensity at 20 μs
F_J	Fluorescence at the J-step (2 ms) of the O-J-I-P transient
F_I	Fluorescence at the I-step (30 ms) of the O-J-I-P transient
$F_M = F_P$	Maximum fluorescence at the P-step when all RCs are closed
$V_J = (F_J - F_O)/(F_M - F_O)$	Relative variable fluorescence at the J-step
$F_V = F_M - F_O$	Variable fluorescence
Quantum yields and probabilities	
F_V/ F_M	Maximum quantum efficiency of PS II photochemistry
$\psi_{EO} = 1 - V_J$	Probability (at t = 0) that a trapped exciton moves an electron into the electron transport chain beyond QA^-
$\varphi_{EO} = (1- F_J/F_M)$	Quantum yield (at t = 0) for electron transport from QA^- to plastoquinone
$\delta_{RO} = (1 - V_I)/(1 - V_J)$	Efficiency/ probability (at t = 0) with which an electron from the intersystem carriers moves to reduce end electron acceptors at the PSI acceptor side
Performance indexes	
PI_{ABS}	Performance index of PSII based on absorption
$PI_{total} = PI_{ABS} \times \delta Ro/(1 - \delta Ro)$	Performance index of electron flux to the final PSI electron acceptors, i.e., of both PSII and PSI

Statistical analysis

For each light treatment six replications, each containing five shoots was tested and the experiment was repeated three times. Statistical analysis of physiological parameters was performed using a one-way ANOVA and the Tukey test to validate the different significance at $P \leq 0.05$.

RESULTS AND DISCUSSION

Different light regimes affected the growth, development and shoot quality of the raspberry plantlets (Figure 1, Table 2). No signs of vitrification, malformations and other disturbances in plant development were observed, which are sometimes associated with in vitro cultivation.

The longest shoots were observed in plantlets exposed to red light (15.52 mm), followed by mixed (BR) light (14.01 mm) and the lowest under blue light, but statistical differences among treatments were found only with blue light. Similarly, red light stimulates stem elongation in other species - chrysanthemum (Kim et al., 2004c), vine (Puspa et al., 2008), stevia (Shulgina, 2018), while Heo et al. (2002) found that the length of tagetes stem was maximal in monochromatic blue LED light. Increased growth of *in vitro* cultured plants provided with red light was also observed in *Vaccinium corymbosum* (Hung et al. 2016), *Scrophularia takesimensis* (Jeong and Sivanesan 2015), *Oncidium* spp. (Chung et al. 2010) and *P. amboinicus* (Silva et al. 2017). According to Manivannan et al. (2015) this growth may be related to the ability of red light to induce the formation of endogenous gibberellins, important growth regulators involved in cell elongation.

Figure 1. Red raspberry (*Rubus idaeus* L. 'Lloyd George') plantlets grown at different light quality treatments. FL - Fluorescence lamps (Control), B – blue LEDs , , R – red LEDs, RB – mixed LEDs, W - white LEDs.

Table 2. Effect of different light sources on the growth parameters and multiplication index (MI) of *in vitro* cultivated raspberry plantlet. FL - Fluorescence lamps (Control), W white LEDs, R – red LEDs, B – blue LEDs, RB – mixed LEDs.

Light treatment	FW	DW	MI	Shoots length	Number of leaves
	mg	mg		mm	
FL	546 a	59.9 b	2.11 a	13.54 a	12.66 b
B	229 b	40.8 c	2.43 a	9.48 b	18.10 a
R	245 b	35.6 c	2.33 a	15.52 a	16.33 ab
BR	552 a	82.7 a	2.50 a	14.01 a	11.31 b
W	284 b	39.4 c	2.67 a	13.67 a	10.66 c

Means in the column, followed by different letters are significantly different at P≤0.05.

Plantlets grown under mixed LED light (BR) had the greatest FW (552 mg), followed by control plantlets (FL) exposed to conventional fluorescent lamps, but no statistical differences were found between them. The lowest FW per plantlet was reported under blue light, but the differences were statistically significant only between control (FL) and BR. The highest DW was found again in plants cultivated under mixed LED light

(BR), and the difference with the control plants was statistically proven. Plantlets grown under red (R) light had the lowest DW value, although statistically the difference with B and W was not proved.

Some other authors emphasize that the combination of blue and red light increased plant growth, fresh and dry mass, compared to monochromatic LED light (Nhut et al., 2000, 2002; Lian et al., 2002; Duong et al., 2003; Kim et al., 2004a, b, c; Poudel et al., 2008; Shin et al., 2008; Li et al., 2010). According to Li et al. (2010) fresh and dry cotton biomass were maximal when combining blue and red LEDs in equal proportions. The results were similar for *Lilium* sp. (Lian et al., 2002), banana (Duong et al., 2003), strawberries (Nhut et al., 2003), chrysanthemum (Kim et al., 2004c), *Lycium barbarum* L. (goji berry) (Oliveira Prudente, 2019). According to Shulgina et al. (2018), mixed red and blue LED light inhibits the growth of *Stevia rebaudiana* Bertoni shoots, but stimulates the development of the root system. Our results are in agreement with those obtained by Heo et al. (2006), who found no increase in fresh and dry biomass of *Vitis* plantlets cultivated under blue light.

The multiplication index varied within narrow limits (2.11 - 2.67) and did not differ significantly between the treatments. This is probably due to the relatively low concentration of cytokinin BAP (2.5 μM). White light was the least effective in inducing new leaves formation. Despite the lowest shoot length, the highest number of leaves was formed in blue light. Our results confirmed the findings of Muleo et al. (2001) that blue light stimulated node formation but reduced internode growth, while red light induced greater internode growth but limited node formation.

The results presented above showed that raspberry plantlets displayed clearly varying growth responses to the different light qualities.

The content of photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) in raspberry plantlets grown under white LEDs was higher than that of plantlets grown under FL, B, R and BR (Table 3).

Table 3. Effect of different light treatments on photosynthetic pigments (mg g^{-1} FW) of *in vitro* cultivated raspberry plantlets. FL - Fluorescence lamps (Control), W white LEDs, R – red LEDs, B – blue LEDs, RB – mixed LEDs.

Light treatment	**Chl a**	**Chl b**	**Chl (a+b)**	**Car**	**Хл(a/b)**	**Chl/Car**
FL	1.57 b	0.59 b	2.16 b	0.59 b	2.64 a	3.69 a
Blue	0.65 c	0.23 c	0.89 c	0.27 c	2.81 a	3.30 ab
Red	0.80 c	0.33 c	1.13 c	0.33 c	2.45 a	3.48 a
BR	0.57 c	0.21 c	0.78 d	0.24 c	2.70 a	3.21 b
White	2.21 a	0.79 a	2.99 a	0.78 a	2.79 a	3.22 b

Means in the column, followed by different letters are significantly different at P≤0.05.

In the plants of the control variant (FL), a lower content of chlorophyll and carotenoids was reported in comparison with W, but higher than the other three variants - B, R, BR. The ratio chlorophyll a/b did not show much difference between treatments (P≤0.05, Table 3). Some variation in total chlorophyll/ carotenoids ratio was observed – the highest in FL, followed by R and B and significantly lower in BR and W. These differences could suggest that light could affect the photosynthetic activity of plants through the light harvesting pigments. Despite the measured lowest values in the content of

pigments (on the FW basis) in plants grown inder mixed LED light (BR), they showed the highest rate of net photosynthetic rate (A) and transpiration (E) (Table 4). For the other treatments, these indicators did not differ significantly.

Table 4. Net photosynthesis rate (A, μmol CO_2 plantlet^{-1} s^{-1}), transpiration intensity (E, mmol H_2O plantlet^{-1} s^{-1}) and photosynthetic water use efficiency (A/E) of *in vitro* cultivated raspberry plantlets. FL - Fluorescence lamps (Control), R – red LEDs, B – blue LEDs, RB – mixed LEDs, W white LEDs.

Light treatment/ parameter	**A μmol CO_2 plantlet^{-1} s^{-1}**	**E mmol H_2O plantlet^{-1} s^{-1}**	**A/E**
FL	67.3 c	26.4 c	2.54
Blue	75.9 b	23.8 c	3.19
Red	69.9 bc	26.1 c	2.68
BR	83.2 a	32.3 a	2.58
White	67.4 c	29.2 b	2.31

Means in the column, followed by different letters are significantly different at P≤0.05.

It should be noted, however, that all plants grown under LED lights had a higher rate of net photosynthesis than the control plants (FL), although the differences were statistically proven only for mixed (BR) and blue light (B). These results were in line with that found by Goins et al. (1997) – the higher rate of net photosynthesis in wheat leaves when combining red and blue LED light. Similar results were reported for chrysanthemum (Kim et al., 2004c). In contrast, Nhut and Nam (2010) noted that net photosynthesis and stomatal conductance were lower in plants grown under red LED light.

In vitro cultivated plants have a heterotrophic type of nutrition due to the presence of carbohydrates in the nutrient medium and under conventional conditions (low light provided by fluorescent lamps) the contribution of photosynthesis to total carbon metabolism has been considered insignificant (Grout and Ashton, 1978; Desjardins, 1995). Our results for more intensive photosynthesis and higher biomass of plants grown in mixed LED light (BR) confirm the hypothesis that under conditions favorable for photosynthesis, in vitro plants could have a positive carbon balance.

Chlorophyll *a* fluorescence determination, along with the intensity of photosynthesis, is another contemporary nondestructive method for the study of the functional activity of the photosynthetic apparatus of plants. The analysis of the induction curves of rapid chlorophyll fluorescence (OJIP test) links the structure and functionality of the photosynthetic apparatus and allows rapid assessment of plant viability, especially in stress conditions (Strasser et al., 2000, 2004). In the five light regimes studied, the rapid chlorophyll a fluorescence curves had a typical OJIP shape from F0 to FM level with distinct J and I phases (Figure 2), indicating that the raspberry plantlets, included in the experiment, were photosynthetically active (Yusuf et al., 2010).

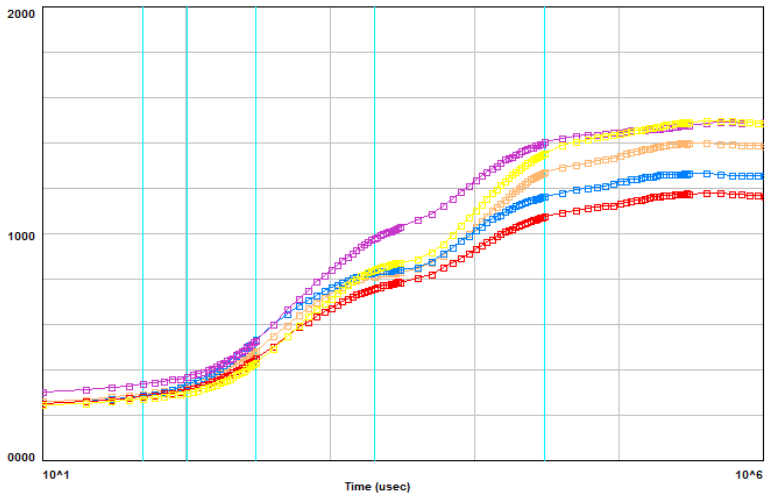

Figure.2. Induction curves of rapid chlorophyll a fluorescence (OJIP test) of raspberry plantlets grown under different light regimes. **FL** - Fluorescence lamps (Control), **W** - white LEDs, **R** – red LEDs, **B** – blue LEDs, **RB** – mixed LEDs.

Table 5. Chlorophyll *a* fluorescence parameters (OJIP test) of the raspberry plantlets grown under different light regimes. FL - Fluorescence lamps (Control), W - white LEDs, R – red LEDs, B – blue LEDs, RB – mixed LEDs.

Light/parameters	FL (Control)	Blue	Red	BR	White
F_0	264 ± 28 ^{ab}	242 ± 17 ^{b}	255 ± 15 ^{b}	306 ± 18 ^{a}	251 ± 10 ^{b}
F_M	1404 ± 179 ^{ab}	1269 ± 22 ^{ab}	1185 ± 143 ^{b}	1493 ± 30 ^{a}	1500 ± 53 ^{a}
Fv	1139 ± 155 ^{ab}	1027 ± 26 ^{ab}	929 ± 154 ^{b}	1187 ± 13 ^{ab}	1249 ± 48 ^{a}
Fv/F_M	0.811 ± 0.01 ^{ab}	0.809 ± 0.01 ^{ab}	0.781 ± 0.04 ^{b}	0.795 ± 0.01 ^{ab}	0.832 ± 0.01 ^{a}
ψ_{E0}	0.513 ± 0.02 ^{a}	0.421 ± 0.09 ^{a}	0.443 ± 0.07 ^{a}	0.427 ± 0.05 ^{a}	0.522 ± 0.03 ^{a}
φ_{E0}	0.417 ± 0.02 ^{a}	0.342 ± 0.08 ^{b}	0.349 ± 0.10 ^{ab}	0.340 ± 0.05 ^{b}	0.434 ± 0.03 ^{a}
δR_0	0.221 ± 0.01 ^{a}	0.212 ± 0.01 ^{a}	0.237 ± 0.04 ^{a}	0.167 ± 0.02 ^{b}	0.214 ± 0.03 ^{a}
PI_{ABS}	2.74 ± 0.41 ^{b}	1.51 ± 0.16 ^{d}	1.77 ± 0.17^{cd}	2.041 ± 0.16 ^{c}	4.43 ± 0.23 ^{a}
PI_{total}	0.80 ± 0.21 ^{b}	0.74 ± 0.23 ^{bc}	0.68 ± 0.18 ^{bc}	0.45 ± 0.12 ^{c}	1.49 ± 0.35 ^{a}

Different letters within column indicated difference at (P<0,05).

The minimal (F_0) and maximal (F_M) fluorescence of the control plants and plants, cultivated under LED light did not differ significantly (Table 5.). Some differences were observed amongst LED lights - F_0 in plantlets, grown under mixed light (BR) was higher as compared to R, B, and W. At the same time, F_M and F_V in R were the lowest, although there were no statistically significant differences with all other treatments.

Despite fluctuations in F_0, F_M and F_V, the quantum yield (Yield = F_V/F_M), reflecting the potential photochemical activity of PS II, ranged from 0.781 - 0.832 and corresponded to normal (0.750-0.830) in healthy, unstressed leaves (Bolhar-Nordenkampf and Oquist, 1993), indicating that a normally developed photosynthetic apparatus was functioning.

However, a more in-depth analysis of the parameters of the OJIP test revealed some characteristic features of the potential of the photosynthetic apparatus in plants, grown under different light regimes (Table 5).

The parameter ψ_{E0}, which reflected the probability of electron transport outside QA, did not differ significantly in the plants studied. But, in the other important parameters of the OJIP test - φ_{E0}, δ_{R0}, the performance index (Pl_{ABS}) and the total performance index (Pl_{total}) some differences were noted.

The parameter φ_{E0} indicated quantum yield for electron transport from Q_{A-} to plastoquinone and the highest φ_{E0} value was calculated for plants exposed to white light, and the lowest for those exposed to blue and mixed light.

In the leaves of plantlets grown under mixed light (BR), a significantly lower value was reported for the parameter δ_{R0}, which provides information on the probability with which an electron from the intersystem carriers moves to reduce end electron acceptors at the PSI acceptor side. In the control and other variants, the values of δ_{R0} did not differ significantly.

The performance index on an absorption basis (PI_{ABS}) in raspberry leaves was the highest under W ($p < 0.05$), followed in order by that FL, BR, R and B, where the minimum value was almost 3 times lower than the maximum (Table 5.).

PI_{total} was also the highest in W light, followed by control plants (FL) and the lowest in variant BR. This indicator for B and R occupied intermediate values.

Keeping in mind the highest values of FW, DW and net photosynthetic rate (A) of plantlets cultivated under mixed LEDs (BR), as well as the normal Fv/F_M ratio (0.795), the higher value of F_0 along with the minimum values of the indicators φ_{E0}, δ_{R0}, Pl_{ABS}, Pl_{total} seemed surprising. Our hypothesis is the following: due to the rapid growth and accumulation of biomass, at the end of the cultivation period the plants have been exhausted and are lacking some important components of the nutrient medium and are already experiencing some nutritional deficiency before it has even affected the growth. One of the possibilities for improving the in vitro cultivation of raspberries is the application of LEDs in a shorter passage - no more than 3 weeks.

The results presented in this study confirm that chlorophyll fluorescence determination is a fast and reliable non-destructive method for early diagnosis of disorders in PS II functionality and growth. Furthermore, the enhanced values of ψ_{EO}, φ_{EO}, PI_{total} and δ_{RO} obtained in plants, cultivated under white LED light as compared to those obtained in plants under conventional fluorescent light (FL) confirmed the better photosynthetic competence of plantlets.

The specific microenvironment (limited gas exchange, poor light and carbohydrates presence in the nutrient medium) under which plants are grown *in vitro* are often associated with stress. They promote production of reactive oxygen species (ROS) and in consequence oxidative stress (Gaspar et al., 2002; Desjardins et al., 2009). The ROS-induced peroxidation of lipid membranes is a reflection of stress-induced damage at the cellular level (Jain et al., 2001). Plants have established through long-term evolution several mechanisms to prevent or alleviate the damage from ROS. The mechanisms of the ROS scavengers include non-enzymatic antioxidants and enzymatic antioxidants (Foyer et al., 1994; Apel and Hirt, 2004).

The antioxidant capacities in raspberry plantlets grown under different LEDs, as measured by DPPH radical scavenging assays, represented by trolox equivalents (mgTE) or percentage of DPPH inhibition, were higher than the control plants, cultivated under fluorescent lamps (Fig.3 B and D).

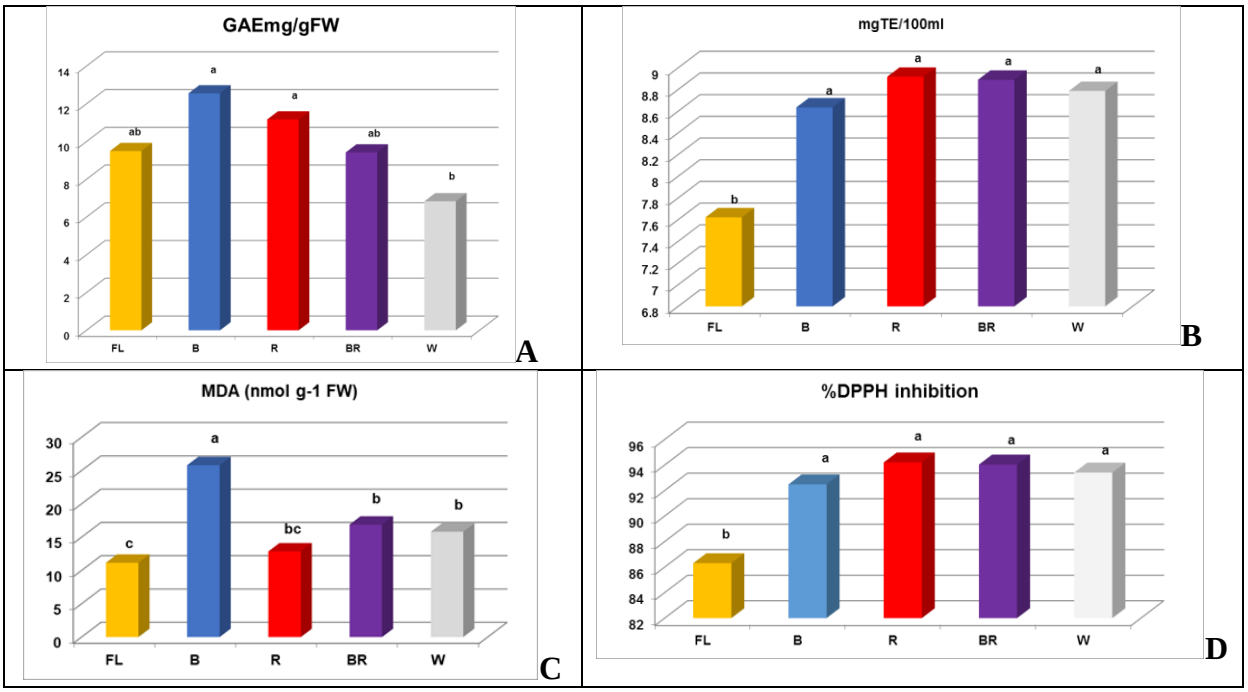

Figure 3. Effect of different light treatments on the content of some antioxidant substances in *in vitro* cultivated raspberry plantlets: A. total polyphenols (mgGAE/g FW); B. Antiradical activity (mg TE/100 ml) C. malondyaldehyde (MDA, nmol g^{-1}FW). D Antiradical activity (%DPPH/ g FW); FL - Fluorescence lamps (Control), W white LEDs, R – red LEDs, B – blue LEDs, RB – mixed LEDs.

The production of phenolics was influenced by the light spectral quality. Plantlets that were cultivated under the blue LED light had the highest phenolic content followed by those grown under red spectrum (Fig. 3A). Plantlets exposed to white LED light were characterized by the lowest phenol content.

The highest accumulation of MDA was observed in plants, grown under blue light (Figure 3C.). The control plants (FL) and plants grown under other LED treatments had a significantly lower level of lipid peroxidation (expressed as MDA content). Similarly, blue LED light enhanced total phenol level in leaf extracts of medicinal plant *Rehmannia glutinosa* Libosch. (Manivannan et al., 2015). Elevated levels of MDA and phenols in plantlets exposed to blue light could be attributed to the elevated production of reactive oxygen species (ROS). Confirmation of this could be the lowest FW measured in plants exposed to blue light, as well as the lowest values of ψ_{E0} and PI $_{ABS}$.

The results presented in our study showed that the combination of LEDs (variant BR) provided one of the highest values in most of the growth characteristics like FW, DW, net photosynthetic rate (Tables 2,4, Figure 1). Recent advances in LED technology in terms of plant growth optimization focus on mixed LEDs rather than monochromatic blue or red LEDs (Gupta and Jatothu, 2013). Studies of other researchers have shown that the combination of red and blue LEDs enhances *Mentha* and *Fragaria* growth as compared to other monochromatic spectra (Nhut et al., 2003; Gupta and Jatothu, 2013; Sabzalian et al., 2014). Also, the results presented by Pawlowska et al. (2018) and Cioć et al. (2019) showed that combination of red and blue LEDs (7:3) could be an effective and affordable tool for modifying the potential of *Gerbera jamesonii* Bolus plants for shoot multiplication

and for the control plant morphogenesis and photosynthetic pigment content.

As Muneer et al. (2018) noted, red and blue LEDs play a significant role in alleviating damage caused by hyperhydricity in carnation genotypes that was aggravated under the fluorescent light.

It is evident from these studies that the ideal light environment for each plant species is unique; i.e., the spectral composition and PPFD influencing the in vitro response of one plant species may not yield similar results for another plant species.

CONCLUSIONS

The present research demonstrates the potential of LEDs as an efficient lighting system for rapid in vitro micropropagation of raspberry (*Rubus idaeus* L.) plants. The combination of blue, red, far red and white light (1:1:1:1) stimulates their growth and biomass accumulation, as well as the intensity of net photosynthesis. For optimal results, it would be advisable, probably, to shorten the culture period to 3 weeks. This effective and affordable protocol would support the commercial micropropagation of raspberries and other soft fruits.

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