# PHOTOSYNTHETIC EFFECTS OF LIGHT-EMITTING DIODE (LED) ON IN VITRO-DERIVED STRAWBERRY (*FRAGARIA X ANANASSA* CV. FESTIVAL) PLANTS UNDER *IN VITRO* CONDITIONS

## EFECTELE FOTOSINTETICE ALE DIODEI ELECTRO-LUMINISCNTE (LED) ASUPRA PLANTELOR DE CĂPŞUN (*FRAGARIA X ANANASSA* CV. FESTIVAL) OBȚINUTE ŞI CRESCUTE ÎN CONDIȚII *IN VITRO*

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***Abstract.*** *Purpose of this study was to investigate effect of different photosynthetic photon flux density (PPFD) conditions using LED lamps on culture of shoot explants derived from in vitro shoots of Fragaria x ananassa cv. Festival. To examine the combined effect of 55% red LED, 15% far red LED, 10% blue LED, 15% green LED and 5% warm light LED light on in vitro growth of plantlets, fresh and dry plant biomass, plant height, leaf area, number of shoot, shoot length, number of root and percentage of rooting and various growth of micro-propagated plants were assessed under four light intensities (25, 50, 75 and 100 µmol $m^{-2}$ $s^{-1}$ PPFD; TRT2, TRT3, TRT4, TRT5, respectively). Un-rooted strawberry shoots were cultured in the "Culture Pack"-rockwool system with MS medium under $CO_2$-enriched condition. The best response for regeneration of shoots and root induction was observed for shoot explants obtained on MS supplemented with BAP 1mg/l and NAA 0.5 mg/l at TRT3 (50 µmol $m^{-2}$ $s^{-1}$ PPFD). Chlorophyll and net photosynthesis were optimal in plants grown under TRT4 (75 µmol $m^{-2}$ $s^{-1}$ PPFD). Stomatal resistance and Fv/Fm values were highest at low light irradiance (TRT2). The highest efficiency and high frequency of shoot formation occurred after 30 days. Elongation of shoot buds was obtained at TRT4 (75 µmol $m^{-2}$ $s^{-1}$ PPFD) and TRT5 (100 µmol $m^{-2}$ $s^{-1}$ PPFD) on the same MS medium. Regenerated shoots rooted best on the same medium of elongation. Irradiance at 75 µmol $m^{-2}$ $s^{-1}$ PPFD was suitable for the acclimatization of strawberry plants. We concluded that the present protocol can be efficiently used for mass propagation of the strawberry.*

**Key words:** Strawberry, *Fragaria*, LED lamp, light intensity, micro-propagation, direct organogenesis

***Rezumat.*** *Scopul acestui studiu a fost de a investiga efectul diferitelor densități ale fluxului fotonilor fotosintetici (PPFD) în condițiile folosirii lămpilor LED asupra lăstarilor transplantați care provin din lăstari "in vitro" de Fragaria x ananassa cv. Festival. Pentru a examina efectul combinat al luminii compuse din 55% LED roşu, 15% LED roşu pal, 10% LED albastru, 15% LED verde şi 5% LED cu lumină caldă asupra dezvoltării "in vitro" a plăntuțelor, a producției de biomasă proaspătă şi uscată, a înălțimii plantelor, a suprafaței frunzelor, a numărului de lăstari, a lungimii acestora, a numărului de rădăcini şi a procentului de înrădăcinare s-au folosit patru intensități luminoase diferite (25, 50, 75 şi respectiv 100 µmol $m^{-2}$ $s^{-1}$ PPFD; TRT2,*

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*TRT3, TRT4, TRT5). Lăstarii de căpşun fară rădăcină au fost cultivaţi folosind sistemul "Culture Pack"-cu vată minerală într-un mediu MS îmbogăţit cu $CO_2$. Cel mai bun răspuns pentru regenerarea lăstarilor şi a rădăcinilor a fost observat pentru lăstarii crescuţi într-un mediu MS suplimentat cu BAP 1mg/l şi NAA 0.5 mg/l pentru TRT3 (50 µmol $m^{-2}$ $s^{-1}$ PPFD). Clorofila şi fotosinteza au fost optime la plantele crescute sub TRT4 (75 µmol $m^{-2}$ $s^{-1}$ PPFD). Rezistenţa stomatelor şi raportul Fv/Fm au avut cele mai mari valori în condiţiile unei iradieri luminoase scăzute (TRT2). Cea mai mare mare eficienţă şi frecvenţă a formării lăstarilor a fost obţinută după 30 de zile. Elongaţia lăstarilor a fost obţinută pentru TRT4 (75 µmol $m^{-2}$ $s^{-1}$ PPFD) şi TRT5 (100 µmol $m^{-2}$ $s^{-1}$ PPFD) pe acelaşi mediu MS. Regenerarea rădăcinilor lăstarilor a fost cea mai bună la utilizarea aceluiaşi mediu ca şi în cazul elongaţiei. Iradierea la 75 µmol $m^{-2}$ $s^{-1}$ PPFD a fost benefică pentru aclimatizarea plantelor de căpşun. Concluzionăm că prezentul protocol poate fi utilizat cu eficienţă în cazul plantelor de căpşun.*

**Cuvinte cheie:** căpşun, *Fragaria*, lampă LED, intensitate luminoasă, micropropagare, organogeneză directă

## INTRODUCTION

According to Food and Agriculture Organization (Anonymous, 2012) of the United Nations, world production of strawberries has exceeded 4 million tons since 2007 (Wu *et al*., 2012). Currently, Turkey stands second in the world for strawberry production with an annual production of 353 173 tones (Anonymous, 2012). The future of strawberry production and sales is very positive. Increased demand in the future from European countries should increase export demand on Turkish growers. Strawberry production, sale price, trade (import and export), and consumption are expected to continue to increase over the next several years.

The conventionally propagated plants and propagated with tissue culture plants of differences in performance have been investigated for strawberry. The strawberry propagated plants with tissue culture were shown to have greater flower number and higher yield, smaller fruit size, more runners and increased vigor as compared to conventionally propagated plants. Methods to reduce input costs and increase productivity of in vitro propagation are needed to improve *in vitro* culture conditions for successful strawberry micropropagation. Numerous studies described an in vitro method for mass propagation of strawberry through axillary shoot multiplication (Boxus, 1974; Cameron and Hancock, 1986). The micropropagation of strawberry was achieved and now it seems to be a routine task in many commercial laboratories. Numerous studies have been published regarding field behaviour of micropropagated strawberry. Adventitious (stipular) shoots occurred spontaneously, and were common in strawberry *in vitro* shoot cultures. The special conditions during in vitro culture result in the formation of strawberry plantlets of normal anatomy, morphology and physiology.

Plants require temperature, light, $CO_2$, water and nutrients at optimal level to grow and develop (Yamori *et al*., 2010). The light is the factor influencing the growth of green plants in *in vitro* growing. If lighting is too excessive, light generates oxygen radicals and causes photoinhibition. However, if lighting is too

weak, etiolating symptoms appear and photosynthesis cannot work efficiently. Both phenomena strongly limit primary productivity (Barber and Andersson, 1992; Solymosi and Schoefs, 2010). Light intensity and quality have been characterized as important factor for plant development and growth *in vitro* (Macedo *et al*., 2011). At high light intensity, an increase in photosynthetic carbon fixation can occur, which varies depending on light intensity and may lead to different susceptibilities to photo-inhibition. But, under conditions of excess light absorption, the chloroplast lumen becomes acidic in nature, which reduces the electron transport chain, and excitation energy accumulates within the chloroplast which can lead to the generation of reactive oxygen species (ROS) such as super-oxide and hydrogen peroxide. The accumulation of ROS may lead to lipid peroxidation and to reduced accumulation of enzymatic antioxidants which exist as a defense system in plants (Asada, 1999). High photosynthetic flux of photoautotrophically micopropagated plant, cultured under high $CO_2$ concentration and high photosynthetic photon flux (PPF) has been observed in some plant species. Many studies have clearly showed that modulation in light photoperiod, quantity and quality can affect plant development and growth (Zuchi and Astolfi, 2012). Plants react to light mainly via photomorphogenetic, photosynthetic, and phototropic response (Kaur, 2015). These responses depend on the photoperiod, light quality and photon flux density (PFD) (Taiz and Zeiger, 1991). The Fv/Fm value, a measure of the intrinsic or maximum efficiency of PSII i.e. the quantum efficiency if all PSII centres were open, decreased significantly in plants grown and development. The decrease in this parameter indicates the down regulation of photosynthesis or photo-inhibition.

The fluorescent lamps were the main light source commonly used for in vitro cultivation of plants. However, conventional light sources (such as fluorescent light (especially cool-white), metal halide, high pressure sodium, incandescent and fluorescent lights) have some limitations due to their short lifetime, high electrical consumption and heat emission. Fluorescent lamps have fixed emission spectra composed of many bands in the wavelength range from 320 to 800 nm without the possibility of varying illumination of spectrum and time characteristic parameters. One of the disadvantages of fluorescent lamps is the difficulty of controlling light quality, which has been shown to have significant influences on plant morphogenesis.

To considerable interest for general plant photo-physiology, these responses are of commercial importance for *in vitro* plant cultivation. In terms of both sustainability and economics, new lighting technologies such as light-emitting diodes (LEDs) thus were necessary to be developed (Sheng, 2013). In recent years, the use of LEDs as an irradiation source for plants growing and propagation has attracted attention due to its vast potential for commercial application (Bula *et al*., 1991). LEDs are particularly suitable for plant growth chambers, because of their light weight, small volume and long life (Yeh and Chung, 2009). Narrow bandwidths and wavelength specificities of LEDs have been

used in morphogenesis (Robin *et al*., 1994), photosynthesis (Tennessen *et al*., 1994), photo-biological research (Tripathy and Brown, 1995) and algal photobioreactor (Lee and Palsson, 1994). Among artificial lighting systems, LEDs present the maximum photosynthetically active radiation (PAR) efficiency (80–100%). LEDs emitting blue, green, yellow, orange, red and far red are available and can be combined to provide either high fulence (Bula *et al*., 1991). LEDs, which generate virtually no heat, have very low energy consumption and estimated lifetimes of several years offer a simple and economic solution to the problem of creating controlled environments. Compared to conventional fluorescent lamps, LED based illuminators have improved features, including longer life-time, solid state construction, tailored spectrum, smaller mass and volume (Brown *et al*., 1995; Bula *et al*., 1991). Because of these culture of plants in a tightly controlled environment such as space-based plant culture systems. Thus, LEDs-based illuminators provide an alternative to fluorescent lamps as a light source with controllable spectrum that can be used for plant cultivation (Bula *et al*., 1991; Barta *et al*., 1992; Brown *et al*., 1995; Žukauskas *et al*., 2002; Tamulaitis *et al*., 2005).

The LEDs technology is predicted to replace HID and fluorescent lamps in horticultural systems and to revolutionize controlled growth environments (Eva *et al*., 2015). The LEDs have features which are far better than the commonly used irradiation source. Investigation of the effect of illumination spectrum and photon flux density (PFD) has been carried out by applying LED-based illumination to some species of plants on plant growth *in vitro*. The efficiency of 650–665 nm wavelengths of red LEDs on plant growth is fit with the absorption peak of chlorophylls (Schoefs, 2002) and phytochrome, while the supplemented blue light introduced the idea that growth under natural light could be mimicked using red and blue LEDs. In addition to providing an excitation of the different types of photoreceptors, the red+blue combination allowed a higher photosynthetic activity than that under either monochromatic light (Sabzalian *et al*., 2014). The supplementation of blue+red LEDs could also be complemented with green (460–475 nm) LEDs. Illumination with containing up to 24% green LED light enhanced in plant growth (Kim *et al*., 2006). Hypocotyl elongation could be prevented by adding at least 15 µmol $m^{-2}$ $s^{-1}$ of blue light (Hoenecke *et al*., 1992). Under red LEDs illumination, phytochrome stimulation is especially high as far red light is not provided.

The attractive features of the LEDs system, and the acquired knowledge about the effect of light quality on plant photosynthesis, morphogenesis and chlorophyll synthesis studies on growth and development of strawberry plants under different LEDs systems are very limited (Nhut *et al*., 2003), whereas are more advanced on horticultural plant species (Miyashita *et al*., 1995; Tanaka *et al*., 1998; Hahn *et al*., 2000; Lian *et al*., 2002; Nhut *et al*., 2003; Kim *et al*., 2004; Jao and Fang, 2004; Heo *et al*., 2006; Stutte *et al*., 2009; Li *et al*., 2012). Micro-propagation of strawberry shoots grown using LEDs were previously reported by Nhut *et al*. (2003). Nhut *et al*. (2003) showed that the best growth of strawberry plantlets

cultured in vitro was observed under LED-based illumination, and the optimal total photon flux density (PFD) were found to be 60 µmol $m^{-2}$ $s^{-1}$ with 70% red and 30% blue spectral components. Tripathy and Brown (1995) showed that wheat seedlings accumulated chlorophyll content under red LED light at 100 µmol $m^{-2}$ $s^{-1}$, but not at 500 µmol $m^{-2}$ $s^{-1}$. This inhibition of chlorophyll accumulation under high fluence red LED light could be avoided by the supplementation of blue LED light at 30 µmol $m^{-2}$ $s^{-1}$.

This research was aimed to examine the effects of LED lighting on the growth and development of strawberry plantlets in *in vitro* and on the changes in morphology and physiology of in vitro cultured strawberry explants, which were cultured in vitro under illumination system with four photon flux densities using LEDs. We tested the application of traditional fluorescent lighting and LED lighting in relation to growth in strawberry in *in vitro*.

## MATERIAL AND METHOD

For the experiment, the strawberry plantlets were multiplicated at the Plant Tissue Culture Laboratory of the Agricultural Biotechnology Department, Faculty of Agriculture, Süleyman Demirel University. Strawberry plantlets were micro-propagated from culture of shoot explants derived from *in vitro* shoots of *Fragaria x ananassa* cv. Festival.

The axillary shoots (micro-shoots) were obtained from a mass of shoots cultured in vitro on Murashige and Skoog (MS) basal medium. The axillary shoots were excised and placed on solid MS medium for *in vitro* culture. These axillary shoots were then transferred onto solid ½ MS medium supplemented with 6-benzylaminopurine (BAP; 2 ppm), indole-3-acetic acid (IAA; 0.1 ppm), ascorbic acid (80 ppm), TDZ (2.0 ppm), giberrellic acid ($GA_3$; 0.2 ppm), phloroglucinol (100 ppm), sucrose (30 g/l) and gelrite (3 g/l) at pH 5.6. The root medium was composed of 1/2 MS nutrients, supplemented with gelrite (3 g/l), sucrose (20 g/l), $GA_3$ (0.2 ppm) and IBA (1.0 ppm) at pH 5.6 before autoclaving at 121$^{o}$C for 20 min. Axillary shoots were transplanted into 250 ml Maganta vessels with 50 ml medium in each vessel. Five axillary shoots were placed in each vessel onto a fresh proliferation medium and subcultures were obtained in 30 days. Five axillary shoots cultured in one vessel and 9 vessels were used for each treatment. The experiment included 3 replicates with consisting of 15 axillary shoots per replication. Vessels were sealed with a rubber stopper that had a hole with permeable film to provide ventilation.

These culture vessels were placed in the grown chamber in which the $CO_2$ concentration was maintained at 1000 mg $m^{-3}$. For comparison, they were also placed on the shelf under PFD (45 µmol $m^{-2}$ $s^{-1}$) (control) and different photosynthetic photon flux density (PPFD) of LEDs light in the same grown chamber.

To elucidate the effect of different PPFD of LEDs light conditions at the in *in vitro* growth of strawberry plantlets, the cultures were established and grown under different light densities of PPFD. The light was provided by LED lamps and coold-white [photon flux density (PFD) of 45 µmol $m^{-2}$ $s^{-1}$ (for control)] (TRT1) fluorescent lamps placed under of shelf in the growth chamber. The cultures were kept in a growth chamber and the cultures of *in vitro* plantlets were illuminated using four different PPFD of LEDs light intensities in order to select the most suitable LED light intensity. Experiments conducted in growth chambers were programmed on a 24-h cycle; irradiance was set at a PPFD of LEDs light [25, 50, 75, 100 µmol $m^{-2}$ $s^{-1}$ PPFD (TRT2, TRT3, TRT4, TRT5, respectively)] and the combined effect

of 55% red LED (peak wavelength 640 nm), 15% far red LED (735 nm), 10% blue LED (450 nm), 15% green LED (510 nm) and 5% warm light LED and were tested. Irradiance was measured routinely at the centre of vessels (at the top of the plants of in the vessel) with a quantum sensor. The irradiation level measured at the center of vessel was kept constant at PPFD of LEDs light and PFD of fluorescent lamps light (control). The cultures were exposed in a photoperiod of 16 h light and 8 h dark. Culture vessels were transferred to grown chamber and maintained at 24±2$^{o}$C, and 60-70 % relative humidity (RH). RH was controlled by an ultrasonic fog system. Air temperature and relative humidity were measured with the JUMO Humitherm TDAc-70.

The apparatus which was used in LED experiments was an aluminium panel [width: 35 length: 90 cm; 3 pieces per panel: 25 (width), 30 (length) cm]. As a light source, the LED panel were arranged and were mounted on the 30 cm height from vessel. A total of seventy five culture vessels were placed on five layers of shelves in a growth chamber, 15 vessels per shelf. For fast micro-propagation, during cultivation, the explants were transferred to multiplication solid medium (same as above) by dividing the axillary shoot of each sample. After cultivation, axillary shoots of the surviving plantlets were allowed to elongate, and each axillary shoot was divided and transferred on to a proliferation medium. Multiple axillary shoots were produced one month. These axillary shoots were each separated, and sub-cultured onto a fresh solid MS medium. Finally, 45 axillary shoots (~2 cm long) were cut off and transferred onto a rooting medium. Rooted strawberry plantlets were transferred to growth chamber for acclimatization. The test was performed in an entirely randomized experimental design with three replications, each replication represented by vessels with fifteen axillary shoots and 45 axillary shoots per light intensity were used. Five axillary shoots was placed per vessel.

Three culture vessels were taken out to measure the growth characteristics of strawberry plantlets. The samples were obtained by random. For measure, the plantlets were harvested at 30 day in the middle of the light period, washed to remove medium particles and separated into leaves, shoot and roots. End of 30 days of cultivation were observed for their survival rate, total number of axillary shoots per explant, length of axillary shoots (cm), fresh and dry plant biomass, plant height, leaf area, number of shoot, shoot length, number of root and percentage of rooting. Plant growth at the coold-white fluorescent light *in vitro* derived axillary shoots was used as control. At the end of four week period, axillary shoots were assessed according to their regeneration capacity (number of axillary shoots). The samples were prepared from young leaves of *in vitro* propagated axillary shoots. Fifteen leaves from the top axillary shoots with a 2.5-5.0 cm length of axillary shoot were collected for each light intensity. Leaves were cut out from the axillary shoots for evaluating extended. PGF of coold-white fluorescent light on strawberry leaf samples were used as controls to compare with the LEDs light intensities material. The LEDs light assayed and non- assayed plantlets were used. The control consisted of untreated plantlets. Growth of plantlets were observed for their plantlets of the surviving (survival rate) data were recorded.

Microropogated strawberry plantlets in vitro conditions from axillary shoots were adapted under grown chamber conditions. After one month acclimatized plantlets were transferred to the in plug-mix compost (in 45 celled transplant trays, filled with equal parts by volume of vermiculite, polystyrene granules and peat) and maintained at 24±2$^{o}$C, 50-60 % relative humidity. The trays were placed on shelves in a growth chamber on a capillary mat and subirrigated. Strawberry plantlets were fertilized with a water-soluble fertilizer 20N-8P-16K at a rate of 2.5 g/l every four irrigation until approximately 30 days of cultivation in a grown chamber.

Five explant of each light treatment replication were harvested to calculate

mean of total plantlet height, fresh plantlets and dry plantlets biomass, leaf area, number of shoot, shoot length, number of root and percentage of rooting. Fresh weights of strawberry plantlets as well as those of stems, leaves and roots were measured. For determination dry weights of whole plantlet were dried at 65°C until constant weight was reached. The plantlets were dried in a hot air oven for three days and the weight at ambient temperature was recorded as the total dry weight.

Leaf area of the representative strawberry plantlets was measured by a digital planimeter (Placom KP-90N).

The chlorophyll *a+b* contents in the fully expanded young leaves of 4-week-old plants was measured. PFD of fluorescent lamps light treatment of leaf samples were used as control to compare with the PPFD of LEDs light treated material. Total chlorophyll *a+b* content were quantified in samples formed by five leaf discs (1,283 $cm^2$) cut from in the youngest fully expanded leaf (Porra *et al*., 1989; Wellburn, 1994; Schoefs, 2002). At the chlorophyll *a+b* contents of leaflets were calculated (in chlorophyll fluorescence) and determinations were subsequently expanded leaf using 100 mg fresh weight extracted with 10 ml of 80% cold acetone at $4^oC$ and determining the absorbance at 645 and 663 nm, calculations were made using the spectrophotometrically according in Serret *et al*. (1996) and Porra *et al.* (1989). The minimum chlorophyll fluorescence yield in dark (F0) was elicited. The maximum chlorophyll fluorescence yield in dark (Fm) and in light (Fm) were induced by a flash of intense white light from the light source PPFD of LEDs light and PFD of fluorescent lamps light. From chlorophyll *a+b* contents, fluorescence kinetic parameters, variable to maximum fluorescence ratio ($F_v/F_m$) was measured in strawberry plantlets grown in vitro conditions 30 days under 1000 mg $m^{-3}$ $CO_2$ concentration. The maximum photochemical efficiency was calculated in 30-min-dark-adapted leaves as (Fm−Fo)/Fm=Fv/Fm. Variable to maximum fluorescence ratio (Fv/Fm) measured originally photoautotrophically grown plantlets in vitro in irradiance leaves adapted Kadleček *et al*. (1998). [(Fv/Fm = Variable chlorophyll fluorescence / maximum chlorophyll fluorescence) [maximum photochemical efficiency Fv/Fm; Fv (Fm-Fo; variable fluorescence), Fo (minimum fluorescence), Fm (maximum fluorescence), and Fv/Fm (variable / maximum ratio fluorescence].

The photosynthetic rate ($P_G$) was measured at the strawberry plantlets grown in the in vitro conditions under 1000 mg ($CO_2$) $m^{-3}$ concentration of the in-flow air. $CO_2$ concentration was increased to 1000 mg $m^{-3}$ concentration during the light period. At the $CO_2$ level in the growth chamber was measured by the $CO_2$ supply apparatus. A portable LI-6400 apparatus (LI-COR Inc., Lincoln) was used to measure net assimilation of $CO_2$ ($ACO_2$) in response to increasing levels of light. The $CO_2$ was set to 1000 mg $m^{-3}$ $CO_2$ ($ACO_2$) during the light period in all treatments. Net photosynthetic rate ($P_N$) at the $P_{N1000}$ $CO_2$ concentration measured at 1000 mg ($CO_2$) $m^{-3}$ in strawberry plantlets grown *in vitro* conditions under 1000 mg $m^{-3}$ $CO_2$ concentration. CO2 concentration was increased to 1000 mg $m^{-3}$ during the light period. Adaxial stomatal resistance (cm s-1) was measured in strawberry plantlets grown *in vitro* conditions. Leaf temperature was $25^oC$ and RH was 70%.

The experiment had one factorial design with four PPFD of LEDs light intensities levels and one PFD of fluorescent lamps light intensity, each replication represented by 45 explants per light intensity treatment were used. The statistical one-way analysis of variance was used to analyze data. The mean values were subjected to analysis using the Tukey-Kramer (JMP, Version 5) to determine the PPFD of LEDs light intensities the above parameters for a comparison of the averages. Mean separation between treatments by LSD was 5 percent level.

## RESULTS AND DISCUSSIONS

Effects of LEDs light intensities on the morphology and physiology (leaf structure, root structure and photosynthesis) of strawberry plantlets grown *in vitro* were studied using an illumination system consisting of four different of light-emitting diodes (LEDs) intensities [25, 50, 75, 100 μmol $m^{-2}$ $s^{-1}$ PPFD of LEDs light] and the combined effect of 55% red LEDs (peak wavelength 640 nm), 15% far red LEDs (735 nm), 10% blue (450 nm) LEDs, 15% green LEDs (510nm) and 5% warm light LEDs light of LEDs. Taking into account all differences in number of the transplanted plantlet, survival rate number of the plantlet, usable plugs, plantlet height, number of root, percentage of rooting, leaf area, elongation of axillary shoot length, total number of axillary shoot, axillary shoot regeneration, number of leaf, net photosynthetic rate ($P_G$), stomatal resistance, Fv/Fm values, fresh plant biomass, total dry plant biomass, leaf chlorophyll content and elongation of root length from meristem of strawberry, observed while changing the PPFD of LEDs light intensities, the optimal total LEDs intensity for growth of strawberry plantlets in vitro was estimated. For 16 h photoperiod and the optimal total PPFD of LEDs light was found to be 75 μmol $m^{-2}$ $s^{-1}$.

During *in vitro* culture, strawberry plantlets grew under four different PPFD of LEDs light intensities conditions in relatively air-tight cultivation vessels. To determine the effects of LEDs irradiation level, cultures were compared irradiation level at 45 μmol $m^{-2}$ $s^{-1}$ coold-white fluorescent lamps. The strawberry (*Fragaria x ananassa* cv. Festival) plantlet growth was best at 75 μmol $m^{-2}$ $s^{-1}$ PPFD of LEDs light intensity. The optimal PPFD of LEDs light intensity was at 75–100 μmol $m^{-2}$ $s^{-1}$ PPFD of LEDs light favored the growth of strawberry plantlets in *in vitro* (tab. 1, tab. 2).

The axillary shoot regeneration, leaf area, percentage of rooting, plantlet height, usable plugs, survival rate number of the plantlet (tab. 1); fresh plant biomass, photosynthetic rate ($P_G$), net photosynthetic rate ($P_N$), stomatal resistance and leaf chlorophyll *a+b* content (tab. 2) of plantlets in in vitro growth of strawberry plantlets under 75–100 μmol $m^{-2}$ $s^{-1}$ PPFD of LEDs light were significantly greater than low PPFD of LEDs light (25 μmol $m^{-2}$ $s^{-1}$) of PPFD of LEDs light intensity (tab. 1, tab. 2). The number of root, elongation of axillary shoot length, total number of axillary shoot (tab. 1), total dry plant biomass, fluorescence ratio (Fv/Fm), number of leaf and elongation of root length (Table 2) of plantlets in *in vitro* growth of strawberry plantlets under 75 μmol $m^{-2}$ $s^{-1}$ PPFD of LEDs light was not significantly lower than low PPFD (25 μmol $m^{-2}$ $s^{-1}$ PPFD of LEDs light) of LEDs light intensity (tab. 1, tab. 22). A high PPFD of LEDs light at 100 μmol $m^{-2}$ $s^{-1}$ had severely negative effect on plantlets.

The exposure of strawberry plantlets to high irradiance may be more optimal for survival rate number of the plantlet (89.2 % at 100 μmol $m^{-2}$ $s^{-1}$) of strawberry plantlets than 87.1 % at 75 μmol $m^{-2}$ $s^{-1}$ level of PPFD of LEDs light. But, at 100 μmol $m^{-2}$ $s^{-1}$ light intensity level, the plantlets had smaller leaves (11.91 $cm^2$) than75 μmol $m^{-2}$ $s^{-1}$ level of PPFD of LEDs light (12.04 $cm^2$) and compact texture. At the 75 μmol $m^{-2}$ $s^{-1}$ level of PPFD of LEDs light had better quality of pot plants than at other light intensities. In the survival rate number of the plantlet and usable plugs (for *ex vitro*

acclimatization), most of the significant differences were found at the different PPFD of LEDs light intensity and PFD light (control) *in vitro* culture conditions in *in vitro* plantlets (day 30), from to in the grown chambers. Most of the strawberry plantlets survived until the end of the in vitro growth. By the end of *in vitro* culture, strawberry plantlets had developed 89.2 - 93.4 % survival rate. The usable plugs *in vitro* growth in the grown chambers was different for the different PPFD of LEDs light intensities. Irradiance at 75 μmol $m^{-2}$ $s^{-1}$ PPFD was suitable for the acclimatization (tab. 2).

Elongation of shoot buds was obtained at TRT4 (75 μmol $m^{-2}$ $s^{-1}$ PPFD of LED) and TRT5 (100 μmol $m^{-2}$ $s^{-1}$ PPFD of LED).

The strawberry plantlets were taller, had higher dry mass of leaves, axillary shoots and roots, and larger leaf area. The formation of percentage of rooting during in vitro growth in the grown chambers was different for the different LED light intensities. The best response for regeneration of shoots and root induction was 50-75 μmol $m^{-2}$ $s^{-1}$ PPFD (TRT3 and TRT4) of LED (tab. 1, tab. 2). The highest number of roots per plantlet (14.3 - 13.4) and percentage of rooting (65.4 - 68.6 %) were found under 50 – 75 μmol $m^{-2}$ $s^{-1}$ PPFD of LEDs (respectively), whereas a significantly lower number of roots per plantlet (13.2). Results showed that adventitious root formation was highest in plantlets cultured under 75 μmol $m^{-2}$ $s^{-1}$ PPFD of LEDs light (68.6 %), whereas poor rooting was observed in plantlets grown under 45 μmol $m^{-2}$ $s^{-1}$ coold-white fluorescent lamps light (45.4 %) (tab. 1). Similar results were reported in chrysanthemum (*Chrysanthemum morifolium*) (Kurilcik *et al.,* 2008) and anthurium (*Anthurium andreanum*) (Budiarto 2010), where red LEDs were also found to stimulate root formation.

The total dry plant biomass (plant dry) weight to leaf area increased in the PPFD of LEDs light intensities during in vitro grown in chambers while it remained more changed in the higher LED lights. Total dry mass of strawberry plantlets of grown PPFD of LEDs light intensities were higher than PGF light intensity. The total dry plant biomass (plant dry) weight of plantlets irradiated by the at 50 - 75 μmol $m^{-2}$ $s^{-1}$ PPFD of LEDs light intensity were significantly higher than these grown under the other light treatments. The stimulatory effect of red + blue LEDs on leaf growth is similar to those reported in strawberry (*Fragaria ananassa* cv. Akihime) (Nhut *et al.,* 2003). Total dry plant biomass were significantly higher in 1.6 g/plant (75 μmol $m^{-2}$ $s^{-1}$ PPFD of LED light) than 1.3 g/plant (control, 45 μmol $m^{-2}$ s−1 PGF light) indicating a lasting effect of the different LED light intensities availability during in the in vitro growth (tab. 2).

Leaves from *in vitro* plantlets of strawberry had a normal development in leaves. At the end of growth in the growth chambers, 6.2 number of leaf (75 μmol $m^{-2}$ $s^{-1}$ PPFD of LED light) had significantly higher leaf numbers than the 7.4 number of leaf (45 μmol $m^{-2}$ $s^{-1}$ PGF light) control plantlets, showing that this PPFD of LEDs light intensity treatment had been the most favourable for *in vitro* development (tab. 2).

Net photosynthetic rate ($P_N$) in strawberry plantlets increased in the *in vitro* grown. Similar results were found by Baroja *et al*. (1995) and Van Huylenbroeck and Debergh (1996). $P_N$ and in consequence biomass accumulation were increased at the 1000 mg ($CO_2$) $m^{-3}$ had effect on strawberry plantlets growth *in*

*vitro* conditions 30 days under; this increase was more marked under higher LED light intensities. Similar results were found by Desjardins (1995).

Stomatal resistance and Fv/Fm values were highest at low light irradiance TRT2 (25 μmol $m^{-2}$ $s^{-1}$ PPFD of LEDs light). Our study showed that the 100 μmol $m^{-2}$ $s^{-1}$ highest PPFD of LEDs light intensity increased the plantlet extension and formation of roots; and simultaneously stomatal resistance and the Fv/Fm ratio were decreased; and content of chlorophyll *a+b* were increased (tab. 2). The highest stomatal resistance (0.72 – 0.69 cm $s^{-1}$) was recorded at 50 - 75 μmol $m^{-2}$ $s^{-1}$ (respectively). On the other hand, low stomatal resistance observed in strawberry plantlets increased after transfer to *ex vitro* conditions (tab. 2).

Net photosynthetic rate ($P_N$) was increased in strawberry plantlets in the in vitro. The net photosynthetic rate was increased (tab. 2). Lee *et al.* (1985) reported that increasing PPFD of LEDs light to a high level could improve photosynthesis if other conditions necessary for a maximum photosynthetic rate were sustained, while a moderate PPFD of LEDs light level.

In strawberry plantlets cultivated to *in vitro* conditions under LEDs light intensity in an *in vitro* where irradiance varied during the daily illumination maximum was usually less than that needed for photosynthesis, no photoinhibition occurred: Fv/Fm was in the range typical for non-stressed plants and did not change during cultivation. Variable to maximum fluorescence ratio Fv/Fm were decreased at the highest PPFD of LEDs light intensities application of strawberry plantlets to *in vitro* conditions (tab. 2). The Fv/Fm value were decreased (0.554 - 0.539) significantly in strawberry plantlets grown and development in *in vitro* at 75 - 100 μmol $m^{-2}$ $s^{-1}$ (respectively) (tab. 2). The decrease in this parameter indicates the down regulation of photosynthesis or photoinhibition.

Changes in plant grown were dependent on the PPFD of LEDs light intensity in the grown chamber during in vitro culture. In plantlets grown *in vitro* PPFD of LEDs light intensity, chlorophyll *a+b* content increased, in plantlets grown *in vitro*. Strawberry plantlets in growth chamber at low irradiance (25 μmol $m^{-2}$ $s^{-1}$ PPFD) chlorophyll *a+b* content 279 mg $g^{-2}$ were found at low content, but chlorophyll *a+b* content was increased from the 50-100 μmol $m^{-2}$ $s^{-1}$ PPFD of LED light intensity. On the contrary, during cultivation at high irradiance (100 μmol $m^{-2}$ $s^{-1}$ PPFD of LED) plant growth were much higher. The changes were most pronounced for 287 mg $g^{-2}$ chlorophyll content high light plantlets which had a considerably increased chlorophyll *a+b* content. Chlorophyll *a+b* content (291 mg $g^{-2}$) and photosynthetic rate (PG) (15.18 μg($CO_2$) $m^{-2}s^{-1}$) were higher at 75 μmol $m^{-2}$ $s^{-1}$ PPFD of LEDs light than at low PPFD of LEDs light (tab. 2).

Our results suggest that 75 μmol $m^{-2}$ $s^{-1}$ PPFD of LEDs light intensity are suitable as a light source to promote root (elongation of root length and percentage of rooting), leaf formation (number of leaf and leaf area), fresh plant biomass, total dry plant biomass, photosynthetic rate, net photosynthetic rate, stomatal resistance, leaf chlorophyll *a+b* content, plantlet height, elongation of axillary shoot length and axillary shoot regeneration in strawberry plantlets in *in vitro*.

*Table 1*

**The effect of different LEDs light intensities on number of the transplanted plantlet, survival rate number of the plantlet, usable plugs, plantlet height, number of root, percentage of rooting, leaf area, elongation of axillary shoot length, total number of axillary shoot, axillary shoot regeneration from axillary shoots of strawberry (*Fragaria x ananassa* cv. Festival) at the *in vitro* conditions**

| Treatments | Number of the transplanted plantlet | Survival rate number of the plantlet (%) | Usable plugs (%) | Plantlet height (cm) | Number of root | Percentage of rooting (%) | Leaf area ($cm^2$) | Elongation of axillary shoot length (cm) |
|---|---|---|---|---|---|---|---|---|
| TRT1 | 100.0 | $93.4^{a}$ ±1.9 | $87.7^{a}$ ±1.4 | $6.5^{f}$ ±0.1 | $13.2^{b}$ ±2.1 | $45.4^{d}$ ±2.2 | $6.85^{h}$ ±1.5 | $1.6^{cc}$ ±0.6 |
| TRT2 | 100.0 | $88.4^{b}$ ±2.2 | $74.4^{d}$ ±1.7 | $7.4^{d}$ ±0.3 | $12.9^{b}$ ±1.9 | $59.2^{c}$ ±1.8 | $7.97^{d}$ ±1.3 | $1.7^{b}$ ±0.7 |
| TRT3 | 100.0 | $88.2^{b}$ ±1.5 | $77.6^{c}$ ±1.5 | $8.3^{b}$ ±0.4 | $14.3^{a}$ ±1.7 | $65.4^{a}$ ±1.7 | $9.19^{d}$ ±1.8 | $1.8^{a}$ ±0.5 |
| TRT4 | 100.0 | $87.1^{b}$ ±1.8 | $73.9^{d}$ ±1.3 | $8.9^{a}$ ±0.2 | $13.4^{ab}$ ±2.3 | $68.6^{a}$ ±1.5 | $12.04^{a}$ ±1.1 | $1.7^{b}$ ±0.5 |
| TRT5 | 100.0 | $89.2^{ab}$ ±2.1 | $70.5^{d}$ ±1.9 | $9.0^{a}$ ±0.4 | $13.9^{a}$ ±1.6 | $63.1^{ab}$ ±2.3 | $11.91^{a}$ ±1.4 | $1.5^{d}$ ±0.8 |

Significant differences between treatment effects were analyzed by regression analysis.
Values represent means ± SE followed by the different superscript letters show significant differences by Tukey HSD test (p≤0.05)
Means within a column followed by the same letters are not significantly different at P<0.05 according to Duncan fs multiple range test.

*Table 2*

**The effect of different LEDs light intensity on plant dehydrins, number of leaf, net photosynthetic rate ($P_N$), stomatal resistance, Fv/Fm values, fresh plant biomass, total dry plant biomass, leaf chlorophyll content and elongation of root length from axillary shoots of strawberry (*Fragaria x ananassa* cv. Festival) at the *in vitro* conditions**

| Treatments | Fresh plant Biomass (g $plant^{-1}$) | Total dry plant biomass (g $plant^{-1}$) | Photosynthetic rate ($P_N$) [$\mu g(CO_2)m^{-2}s^{-1}$] | Net photosynthetic rate ($P_N$) [$\mu g(CO_2)m^{-2}s^{-1}$] | Stomatal resistance (cm $s^{-1}$) | Fluorescence ratio (Fv/Fm) | Number of leaf | Leaf chlorophyll a+b content (mg $g^{-1}$) | Elongation of root length (cm) |
|---|---|---|---|---|---|---|---|---|---|
| TRT1 | $308.5^{a}$±0.3 | $1.3^{a}$±0.02 | $6.52^{h}$±7.05 | $237.75^{c}$±1.53 | $0.52^{f}$±0.07 | $0.589^{a}$±0.05 | $5.4^{c}$±0.1 | $279^{a}$±0.08 | $7.9^{ab}$±0.4 |
| TRT2 | $312.2^{a}$±0.2 | $1.4^{c}$±0.03 | $11.91^{f}$±5.22 | $252.34^{b}$±1.41 | $0.72^{a}$±0.05 | $0.583^{a}$±0.08 | $5.7^{b}$±0.1 | $284^{a}$±0.06 | $8.2^{a}$±0.3 |
| TRT3 | $314.7^{a}$±0.2 | $1.5^{b}$±0.04 | $12.75^{cb}$±7.48 | $261.20^{ab}$±1.58 | $0.69^{a}$±0.04 | $0.576^{a}$±0.07 | $5.9^{ab}$±0.2 | $289^{a}$±0.05 | $8.3^{a}$±0.4 |
| TRT4 | $317.9^{a}$±0.1 | $1.6^{a}$±0.02 | $15.18^{b}$±8.12 | $269.23^{a}$±1.03 | $0.63^{bc}$±0.05 | $0.554^{b}$±0.05 | $6.2^{a}$±0.3 | $291^{a}$±0.08 | $8.3^{a}$±0.5 |
| TRT5 | $318.6^{a}$ ± 0.2 | $1.6^{a}$± 0.03 | $16.41^{a}$ ± 6.31 | $275.14^{a}$ ± 1.60 | $0.58^{d}$ ± 0.06 | $0.539^{b}$±0.06 | $5.9^{ab}$± 0.2 | $287^{a}$±0.07 | $7.6^{b}$± 0.7 |

Values represent means ± SE followed by the different superscript letters show significant differences by Tukey HSD test (p≤0.05)
Means followed by the same letters do not differ significantly at 5% level of significance.

## CONCLUSIONS

For photosynthetic parameters it seems very important at which conditions *in vitro* plantlets have been grown.

According to this, PPFD of LEDs light intensities can be increased in photosynthetic parameters.

Further, an increase in chlorophyll content and net photosynthetic rate is usually observed in dependence on the PPFD of LEDs light intensities during in *in vitro* grown.

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