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Growth and chlorophyll fluorescence characteristics of *Sinningia speciosa* under red, blue and white light-emitting diodes and sunlight

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Key words: Greenhouse, growth chamber, light-emitting diodes, light spectra, transplant production.

Abstract: Determining the most reasonable LED spectral composition wavelengths on *Sinningia speciosa* transplants was the main focus of present experiment. Seeds were sown in cell trays under chambers with distinct spectral composition including white+blue+red (WBR), blue+red (BR) and white+red (WR) LEDs with equal light quality proportions (70 μmol $m^{-2}s^{-1}$ photon flux density) and under sunlight (400 μmol $m^{-2}s^{-1}$ photon flux density) in constant conditions of 14h photoperiod, 70% relative humidity and day/night temperature of 23/18°C for 50 days. In this stage, LED treatments led to higher germination percentage and better results in biomass, canopy width, leaf width and leaf area as well as chlorophyll and carotenoids accumulation were obtained in comparison with sunlight. Extracted and technical parameters of chlorophyll fluorescence induction kinetics and maximum quantum efficiency of photosystem II (F_v/F_m) were decreased by sunlight-grown seedlings. F_v/F_m was induced by WBR and BR treatments, correlated with maximum yield of primary photochemistry (φP_0). Quantum efficiencies (φP_0, φE_0 and ψ_0) and performance index of absorption energy flux (PI_{ABS}) were increased in BR-exposed transplants. In pot stage, LED-treated plants exhibited better results in morphological features with earlier marketable flowering stage especially under WBR, which can compensate costs of production in marketing stage.

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1. Introduction

Sinningia speciosa (Lodd.) Hiern. is a perennial potted flowering plant commonly known as Gloxinia, which is a herbaceous tropical species native to Brazil and belongs to Gesneriaceae family (Larson, 1992). Proper seasonally light adjustments are critical for production of Gloxinia, hence various source of artificial light has been effectively applied including fluorescent, high-pressure metal halide, high-pressure sodium with the optimal intensity of 45 to 70 μmol $m^{-2}s^{-1}$ (Larson, 1992; Dole and Wilkins, 2005). Even though aforementioned sources induce an increase in daily photosynthetic flux intensity, they are not energetically efficient as desired and there is no capability of spectral manipulation. Light-emitting diodes, including diverse size, long lifetime, solid state construction (Heo *et al*., 2002; Kim *et al*., 2004), low thermal output, specific wavelength, adjustable light quality and intensity (Okamoto *et al*., 1997) and high electrical efficiency (Bula *et al*., 1991), represent a promising technology for the greenhouse industry which has technical advantages over other artificial light sources (Mitchel *et al*., 2012). Capability of LED's spectrum adjustment results in better responses of photoreceptors, influencing plant physiology and morphology and ultimately enhances production (Morrow, 2008). Horticultural crop seedlings are intensively influenced by light spectrum which affects their morphological properties (McNellis and Deng, 1995). Production of transplants under desirable light spectrum and suitable control of environmental conditions can improve transplant quality compared to traditional greenhouse production conditions in which accordingly affects their growth and yield after transplantation (Oda, 2007). Producing a large number of seedlings in a small area justifies high electricity consumption that is economically advantageous. Developing various light spectral ratio recipes for different horticultural transplants based on their demand, would influence growth rate and improve quality (Hernández *et al*., 2016), as different wavebands were proved to have significant physiological effects on plants (Kim *et al*., 2004; Johkan *et al*., 2010) and can be assembled according to the light quality which plants need (Goins *et al*., 1997).

Detecting specific optimal light spectrum prevents energy loss for physiologically none-useful wavelengths (Kim *et al*., 2004; Johkan *et al*., 2010) and it can regulate a variety of plant development pathways from germination to flowering induction (Jiao *et al*., 2007).

Based on previous studies, it has been shown utilizing red (600-700 nm) and blue (400-500 nm) LEDs have the greatest impact on plant growth (Yorio *et al*., 2001) since they mainly contain range of wavelengths essential for plants photosynthesis (Cosgrove, 1981; Kasajima *et al*., 2008). Although blue light is photosynthetically less efficient than red light (McCree, 1972; Dougher and Bubgee, 2001), it has considerable photomorphogenic effects on chlorophyll biosynthesis, stomatal opening, enzyme synthesis, photosynthetic capacity on chloroplast (Tibbits *et al*., 1983), fresh and dry matter accumulation, flowering (Withelam and Halliday, 2007; Johkan *et al*., 2010), stem elongation and leaf expansion (Hoenecke *et al*., 1992; Dougher and Bubgee, 2001).

Researches have demonstrated that combinational light regimes may help to optimize growth (Brown *et al*., 1995). Various number of studies have suggested that combination of red, blue and white LED lighting in different ratios is a favorable lighting condition for plants in many aspects (Lin *et al.,* 2013; Ouzounis *et al.*, 2014). Combination of red-blue, red-white, red-blue-white provides the highest photon efficiency as compared to monochromatic LED illumination (Lin *et al*., 2013; Nelson and Bubgee, 2014; Ouzounis *et al.*, 2014; Nicole *et al*., 2016). Few studies on continuous-spectrum LED lamps fit to a theoretical model of the maximum photosynthetic response has been recorded since McCree's (1972) experiments on cultivated plants.

It has been shown that chlorophyll fluorescence data can help with analyzing energy flow and information related to the structure and function of photosynthetic apparatus (Brestic and Zivcak, 2013). The non-destructive analysis of polyphasic fast chlorophyll transient by the so-called OJIP test was developed for quick evaluation of biophysical aspects of photosynthesis (Strasser, 1995; Mathur *et al*., 2013). This test which is based on energy flow in thylakoid membranes provides detailed information about the biophysics of the photosynthetic system through measurement of fluorescence signals (Kalaji *et al*., 2017).

The main objective of this study was to investigate different ratios of blue and white with the red spectral composition (WR, BR, WBR) to determine the most effective combination of waveband in comparison with natural light condition.

2. Materials and Methods

Plants material and lighting treatments

This experiment was carried out in 2018-2019 in specialized chambers in Shahrekord University research greenhouses. *Sinningia speciosa* F1 (brocade blue) pelleted seeds were sown into 288 cell trays filled with a peat moss with the pH of 5.5-6 and EC of 1 dS/m^{-1} (1:2 dilution). Cell trays were placed inside three chambers with LED Lighting treatments and one cell tray under 50% shaded sunlight in greenhouse, as control, using a completely randomized design. Day/night temperature (23/18°C), relative humidity (70%) and photoperiod (14-hour) were maintained constant in all (LED and sunlight) treatments. Plants were grown under LED modules

(Shezhen Sunled Lighting Co., Ltd, CN Manufacturer, China) yielding approximately 70 μmol m^{-2}s^{-1} measured and adjusted using PARmeter (Apogee Quantum meter, MQ500, USA). The peak emissions of blue (460 nm), red (620 nm) and white (in the range of 380-750 nm) were measured and recorded using spectrometer (BLACK-Comet CXR-SR-50, StellarNet, Inc., USA) with range of 300-800 nm (Table 1). During transplant production cell trays were exposed to 50% white + 50% red (WR), 50% blue + 50% red (BR) and 33.3% white + 33.3% blue + 33.3% red (WBR) LED and 50% shaded sunlight (SL) treatments (Table 1). The relative spectral distribution of light treatments are presented in figure 1. Cell trays were rotated frequently in order to ensure equal growth conditions. Subsurface irrigation was applied every 3 days and plants were irrigated as needed with a 500-1000 ppm water-soluble Radixol fertilizer (N:P:K + microelements; 15:17:15 + 0.12% Mg, 0.02% B, 0.0075% Cu, 0.04% Mn, 0.01% Mo, 0.012% Zn). Measurements were conducted in both transplant and flowering stages.

Transplant stage measurements

Germination and morphological characteristics. After 50 days under light treatments (10 days for germination + 40 days for growth till four fully expanded leaves observed), germination percentage was calculated. Plugs with four fully expanded true leaves, were then transplanted to 12 cm pots, in a completely randomized design with five replications (n=5). Morphological traits of five randomly selected plants from each replicate including shoot fresh and dry weight, root fresh and dry weight, leaf area, leaf width and canopy width was measured. Shoots and roots were dried in a drying oven at 72°C for 24 hours

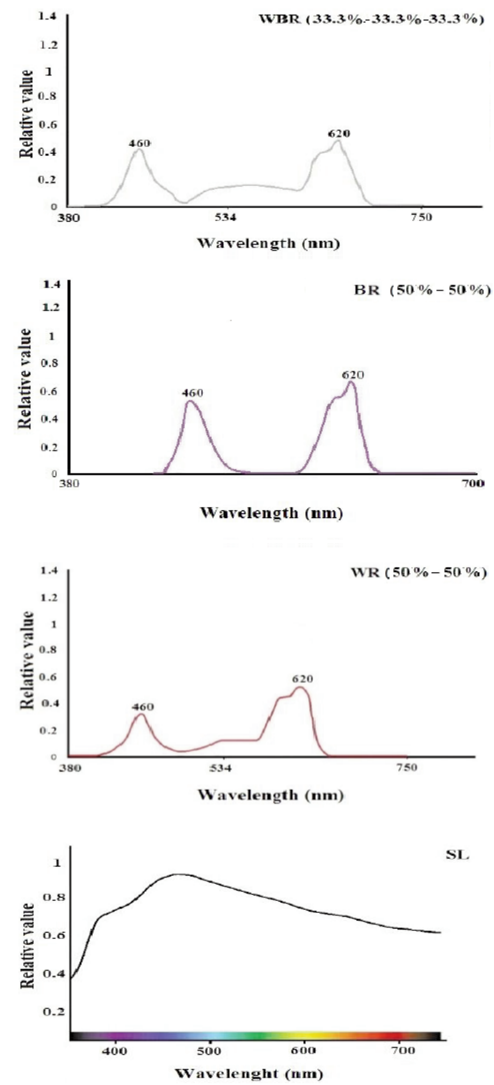

Fig. 1 - Relative distribution spectra of white+blue+red (WBR), blue+red (BR), white+red (WR) and sunlight (SL) treatments. Wavelength peaks are indicated by values above each peak.

Table 1 - Light intensity, different light spectra and light quality were used for growing *Sinningia speciosa* transplants

LED treatment	Proportion (white: blue: red)	Light intensity (mmol m^{-2}.s^{-1})
WBR	33.3% : 33.3% : 33.3%	70 ± 3.1
BR	0% : 50% : 50%	70 ± 1.0
WR	50% : 0% : 50%	70 ± 2.1
Control light treatment	*Average light intensity (mmol m^{-2}.s^{-1})*	
%50 Shaded sunlight (SL)	400 ± 50	
Light quality	*Light spectrum (nm)*	
White	380-750	
Blue	460	
Red	620	
SL (control)	400-700*	

*: For comparing sunlight quality with LEDs, sunlight has purple/blue : green/yellow : orange/red (400-492 : 493-597 : 598-700 nm) with the relationship of 23 : 28 : 39 according to Aphalo *et al*. (2012).

to determine dry weight. The leaf area of plants was measured by Digimizer V. 5.4.6 software.

Biochemical measurements. Biochemical measurements included chlorophyll a, chlorophyll b, total chlorophyll (a + b), carotenoid and total soluble sugar contents. Chlorophyll and carotenoid contents were extracted from 0.5 g fresh leaf tissue from five randomly selected transplants (n=5) and the pigments were eluted with 10 ml of 80% acetone centrifuged at 4000 *g* for 10 min and the amount of chlorophyll was estimated spectrophotometrically (using PG instruments T80+) at 470, 646 and 663 nm by the method of Lichtenthaler and Welburn (1983).

Total soluble sugars was quantified in 95% ethanol extracts of leaf tissue from five randomly selected transplants (n=5). A sample of 0.5 g of freshly harvested leaves was crushed in 5 ml of 95% (V/V) ethanol. The insoluble fraction of the extract was washed twice with 5 ml of 70% ethanol. All soluble fractions were centrifuged at 3500 *g* for 10 min. The supernatants were collected and stored at 4°C for TSS determination. TSS were analyzed by reacting 0.1 ml of the alcoholic extract with 3 ml freshly prepared anthrone reagent (150 mg anthrone + 100 ml 72% [W/W] H_2SO_4) and placed in a boiling water bath for 10 min. After cooling, the absorbance at 625 nm was determined (Irrigoyen *et al.*, 1992) in a PG instruments T80+ spectrophotometer and the data was pooled and extracted with standard curve ($y = 0.002x - 0.009$, $R^2 = 0.992$).

Chlorophyll fluorescence and OJIP test parameters

After 50 days of growth (at four true leaves stage), a portable PAR-FluorPen FP 100 max (Photon System Instrument, PSI, Czech Republic) was used to measure maximal quantum efficiency of Photo System II (F_v/F_m) photochemistry. The most recent fully expanded leaf attached to plants of five randomly selected transplants (n=5) in each treatment were used for this measurement. A custom- made method (Genty *et al.*, 1989; Aliniaeifard *et al.*, 2014; Aliniaeifard and van Meeteren, 2014) was used for the calculation of F_v/F_m. After reaching steady state fluorescence, during short measuring flash in darkness and during saturating light flash (by exposing to 3900µmol $m^{-2}s^{-1}$ saturating light pulse) F_0 and Fm were digitized and averaged, respectively. These two images were applied to obtain maximal variable fluorescence ($F_v = F_m - F_0$). F_v/F_m was calculated using expression $(F_m - F_0)/F_m$. The average value and standard deviation of F_v/F_m per image were calculated by Fluor Cam V.7.0 software.The same device (PAR-FluorPen FP 100 max, Photon System Instrument, PSI, Czech Republic) with the same method (20 minutes dark adaption) was used to measure OJIP-test whilethe last fully expanded intact leaf of randomly selected transplants was used to investigate biophysical and phenomenological parameters of Photo System II status (Strasser, 1995). The transient fluorescence measurement was induced by a saturating light of 3000 µmol $m^{-2}s^{-1}$. The OJIP transients were done according to the JIP test (Strasser *et al.*, 2000). F_0, F_i, F_j, F_m, F_v, V_j, V_i, F_m/F_0, F_v/F_0, F_v/F_m, φP_0, φE_0, φD_0, ABS/RC, TR_0/RC, DI_0/RC, ET_0/RC, PI_{ABS} and ψ_0 were extracted using FluorPen software. More information on formulas are presented in Table 2.

Table 2 - Summary of OJIP-test formula using data extracted from OJIP chlorophyll fluorescence transient. Formulas

Formula abbreviation	Formula explanation
F_0	$F_0 = F_{50\mu s}$, fluorescence intensity at 50 µs
F_j	F_j= fluorescence intensity at J-step (at 2 ms)
F_i	F_i= fluorescence intensity at i-step (at 60 ms)
F_m	F_m= maximal fluorescence intensity
F_v	$F_v = F_m - F_0$ (maximal variable fluorescence)
V_j	$V_j = (F_j - F_0)/(F_m - F_0)$
V_i	$V_i = (F_i - F_0)/(F_m - F_0)$
F_m / F_0	
F_v / F_0	
F_v / F_m	
φP_0	$\varphi jP_0 = 1-(F_0/F_m)=F_v/F_m$
ψ_0	$\psi_0 = 1-V_j$
φE_0	$\varphi E_0 = [1-(F_0/F_m)] \times \psi_0$
φD_0	$\varphi D_0 = 1-\varphi P_0 - (F_0/F_m)$
PI_{ABS}	$PI_{ABS} = (RC/ABS)\times[\varphi P_0/(1-\varphi P_0)]\times[\psi_0/(1-\psi_0)]$
ABS / RC	$ABS/RC = M_0 \times (1/Vj) \times (1/\varphi P_0)$
TR_0 / RC	$TR_0/RC = M_0 \times (1/V_j)$
ET_0 / RC	$ET_0/RC = M_0 \times (1/V_J) \times \psi_0$
DI_0 / RC	$DI_0/RC = (AB/RC)-(TR_0/RC)$
M_0	$M_0 = (TR_0/RC)-(ET_0/RC)=4(F_{300} - F_0)/(F_m - F_0)$

ABS= absorption energy flux; CS= excited energy cross-section of leaf sample; DI= dissipation energy flux at the level of the antenna chlorophyll; ET= flux of electron from Q_A^- into the electron transport chain; φD_0= quantum yield of dissipation; φE_0= probability that an absorbed photon will move an electron into electron transport further than Q_A^-; φP_0= maximum quantum yield of primary photochemistry; PI_{ABS}= performance index; ψ_0= efficiency by which a trapped exaction, having triggered the reduction of Q_A to Q_A^-, can move an electron further than Q_A^- into the electron transport chain; RC= reaction center of PSII; RC/CS= fraction of active reaction centers per excited cross-section of leaf; TR, PSII; RC/CS= fraction of active reaction centers per excited cross-section of leaf; TR= excitation energy flux trapped by a RC and utilized for the reduction of Q_A to Q_A^-.

Pot stage (mature plants) measurements

Time to flowering and morphological characteristics. During pot stage, morphological traits such as number of flowers, flower diameter, number of leaves and number of days to flowering were measured and recorded.

Statistical analysis

Analysis of variance (ANOVA) was performed using SPSS (SPSS 15.0, SPSS Inc.) software and the means were compared with Tukey's test at $p \leq 0.05$.

3. Results

Transplant stage

Germination. Seeds grown under all LED lighting treatments performed better germination rate compared to SL. WBR, BR, WR and SL treatments had 96%, 94%, 96% and 87% germination, respectively (Table 3).

Morphological characteristics. Forty-five days after sowing seeds when all transplant had four fully expanded leaves, growth parameters were measured and analyzed (presented in Table 3). Seedlings grown under WBR and WR LED light exhibited significantly higher shoot fresh weight compared with control. Furthermore, shoot dry weight of plants grown under WBR and SL treatment had the highest and lowest average values, respectively. Average root fresh and dry weight values were maximum in WBR, however in the absence of blue LED, root biomass in WR-grown transplants was greatly reduced. All three LED lighting treatments had significantly greater canopy width than SL. Leaf area and leaf width of plants were significantly influenced by WBR light, though the SL treatment had the least average values. This prominence of WBR (with 33% blue LED ratio compared to 0% and 50%) was visible on leaf features and canopy width (Table 3).

Biochemical measurements

Plants grown under sunlight had the lowest chlorophyll a content while WBR and WR lighting treatments resulted in the highest content of chlorophyll a. LED light composed of blue and red had most profound effect on chlorophyll b synthesis. Control treatment and WR LED treatment had the lowest amount of chlorophyll b content. Additionally, identical proportion of white, red and blue light (WBR) led to the highest total chlorophyll (chl a+b) content among all the other treatments whereas SL had the lowest values. Carotenoid content was highly affected by WBR and WR LED lighting treatments while SL-treated plants showed the lowest carotenoid content (Table 3). Transplants grown under sunlight in greenhouse condition (SL) exhibited higher total soluble sugar content in comparison with all LED treatments (Table 3).

Chlorophyll fluorescence parameters

Measurements of chlorophyll fluorescence parameters were used to study the photosystem II activi-

Table 3 - Influence of light quality of white+blue+red (WBR), blue+red (BR), white+red (WR) and sunlight (SL) on germination, morphological and physiological characteristics of *Sinningia speciosa* transplants represented by means values ± standard deviation (n=5)

Parameters	Light quality			
	WBR	BR	WR	SL (as control)
Germination (%)	96 a	94 a	96 a	87 b
Shoot fresh weight (g)	1.122 ± 0.08 a	0.862 ± 0.05 b	1.084 ± 0.14 a	0.608 ± 0.12 c
Shoot dry weight (g)	0.065 ± 0.004 a	0.044 ± 0.003 bc	0.053 ± 0.01 ab	0.037 ± 0.012 c
Root fresh weight (g)	0.148 ± 0.008 a	0.088 ± 0.008 bc	0.080 ± 0.01 c	0.098 ± 0.008 b
Root dry weight (g)	0.0102 ± 0.0024 a	0.0084 ± 0.0011 ab	0.006 ± 0.0007 c	0.0078 ± 0.0013 bc
Canopy width (cm)	7.6 ± 0.3 a	7.9 ± 0.3 a	7.7 ± 0.02 a	6.1 ± 0.4 b
Leaf width (cm)	2.6 ± 0.1 a	2.4 ± 0.1 ab	2.5 ± 0.3 ab	2.2 ± 0.1 b
Leaf area (cm^2)	4.8 ± 0.3 a	3.6 ± 0.2 c	4.2 ± 0.5 b	3.2 ± 0.2 c
Chlorophyll a ($mg.g^{-1}$ FW)	0.122 ± 0.002 a	0.091 ± 0.026 ab	0.114 ± 0.031 a	0.077 ± 0.009 b
Chlorophyll b ($mg.g^{-1}$ FW)	0.089 ± 0.011 ab	0.099 ± 0.024 a	0.064 ± 0.022 b	0.064 ± 0.005
Chlorophyll a + b ($mg.g^{-1}$ FW)	0.212 ± 0.013 a	0.194 ± 0.027 ab	0.1946 ± 0.057 ab	0.141 ± 0.008 b
Carotenoid ($mg.g^{-1}$ FW)	2.840 ± 0.23 a	2.038 ± 0.47 ab	2.470 ± 0.77 a	1.580 ± 0.18 b
Total soluble sugar ($mg.g^{-1}$ FW)	49.42 ± 2.05 c	61.81 ± 1.98 b	47.05 ± 1.65 c	94.80 ± 0.65 a

Values followed by the same letter within a row do not significantly differ (by the tukey'stest, p≤0.05).

ty (Table 4). Based on this result, the fluorescence signal intensity of transplants grown under WR LED light, increased from F_0 to F_j and then to Fm, however, SL treatment showed the lowest values of extract and technical fluorescence parameters (F_0, F_i, F_j, F_m, F_v, V_i, F_m/F_0, F_v/F_0) as well as F_v/F_m. Transplants grown under WR LED lighting and control condition (SL) showed the highest and lowest values as for F_0, respectively. WBR and WR lighting treatments led to the significantly highest F_j value while it had the lowest significant value in SL treatment. All LED treatments (WBR, BR, WR) exhibited higher fluorescence yield at F_i, F_m, F_v compared to SL. The Highest V_j obtained in WBR and WR-grown plants and the lowest values were detected in BR-grown seedlings. The highest V_i value was from plants grown under WR lighting; however, SL treatment had the lowest value among the treatments. Plants exposed to BR LED light resulted in higher F_m/F_0value than other plants grown under different lighting conditions. F_v/F_0 was also decreased for all except BR-grown plants. The F_v/F_m ratio was higher in plants exposed to BR and WBR LED lights compared to SL which had the greatest decrease. Analyzed parameters for specific energy fluxes per reaction center (ABS/RC, TR_0/RC and DI_0/RC) increased in WR treated plants, in contrast BR light treatment had the greatest decrease among lighting treatments in DI_0/RC ratio while SL treatment had the highest increase of the same ratio. Also, SL and WBR showed the highest and lowest values of ET_0/RC, respectively. By analyzing the parameters that estimate quantum efficiencies or flux ratio (yields and efficiency of electron transport chain) the highest calculated values for φP_0, φE_0, PI_{ABS} and ψ_0 were obtained under BR treatment. In addition, plants grown under BR and WBR treatments had similarly the highest significant value in φP_0. Plants of WBR and BR treatments had the lowest values in φD_0 in compare with SL which had the highest values.

Pot stage (mature plants)

Morphological characteristics

Based on the results, there was a significant effect of LED lighting treatments on flower quantity where-

Table 4 - Chlorophyll fluorescence of transplants grown under white+blue+red (WBR), blue+red (BR), white+red (WR) and sunlight represented by means values ± standard deviation (n=5)

Parameters	Light quality			SL (as control)
	WBR	BR	WR	
F_0	9802 ± 1083 ab	8794 ± 663.5 bc	10517 ± 649 a	8031 ± 750 c
F_i	39013 ± 3447 a	36843 ± 2126 a	41150 ± 2688 a	29808 ± 3230 b
F_j	27919 ± 3109 a	23611 ± 2439 b	28439 ± 1890 a	20392 ± 2299 b
F_m	42752 ± 2254 a	39793 ± 2655 a	43443 ± 3162 a	32758 ± 3599 b
F_v	32949 ± 1755 a	30999 ± 2222 a	32925 ± 2530 a	24798 ± 2661 b
V_j	0.5508 ± 0.0628 a	0.4768 ± 0.0310 b	0.5448 ± 0.0236 a	0.4856 ± 0.018 ab
V_i	0.913 ± 0.022 ab	0.905 ± 0.011 bc	0.931 ± 0.013 a	0.886 ± 0.006 c
F_m/F_0	4.228 ± 0.171 b	4.535 ± 0.242 a	4.128 ± 0.069 b	3.944 ± 0.169 b
F_v/F_0	3.230 ± 0.171 b	3.535 ± 0.242 a	3.128 ± 0.069 b	2.944 ± 0.169 b
F_v/F_m	0.771 ± 0.021 a	0.778 ± 0.012 a	0.758 ± 0.004 ab	0.746 ± 0.011 b
φ_{P0}	0.771 ± 0.021 a	0.778 ± 0.012 a	0.758 ± 0.004 ab	0.746 ± 0.011 b
φ_{E0}	0.348 ± 0.059 b	0.408 ± 0.028 a	0.345 ± 0.019 b	0.387 ± 0.008 ab
φ_{D0}	0.229 ± 0.021 b	0.221 ± 0.012 b	0.242 ± 0.004 ab	0.254 ± 0.011 a
ABS/RC	3.449 ± 0.271 b	3.133 ± 0.170 c	3.771 ± 0.095 a	3.477 ± 0.038 b
TR_0/RC	2.654 ± 0.147 b	2.439 ± 0.128 c	2.857 ± 0.072 a	2.594 ± 0.01 bc
ET_0/RC	1.217 ± 0.070 b	1.273 ± 0.037 ab	1.301 ± 0.082 ab	1.337 ± 0.04 a
DI_0/RC	0.796 ± 0.127 ab	0.697 ± 0.060 b	0.914 ± 0.028 a	0.887 ± 0.048 a
PI_{ABS}	0.700 ± 0.149 c	1.163 ± 0.161 a	0.696 ± 0.072 c	0.895 ± 0.001 b
ψ_0	0.449 ± 0.063 c	0.523 ± 0.031 a	0.455 ± 0.024 bc	0.515 ± 0.018 ab

Values followed by the same letter within a row do not significantly differ (by the tukey's test, p≤0.05).

as the SL treatment resulted in the lowest number of flowers (presented in figure 2A). Largest flowers, in terms of flower diameter was detected in plants grown under WBR treatment, however WR treatment resulted in smallest dimeter of flowers (Fig. 2B). The results also indicated that treatments had significantly different number of days to flowering and plants grown under partial sunlight (SL) in greenhouse had longer time to flowering in pot stage in comparison with WBR, BR and WR-treated plants (Fig. 2D). The transplants grown under LED treatments had greatly higher number of leaves than SL at flowering stage (Fig. 2C).

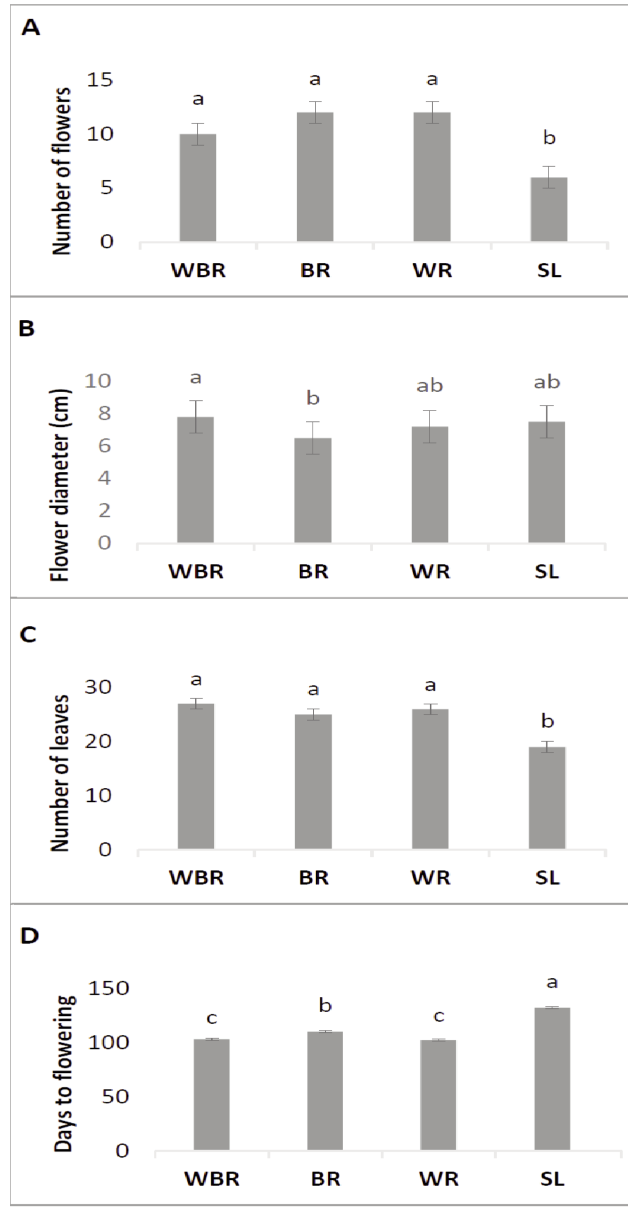

Fig. 2 - Morphological characteristics and time to reach flower of transplants grown under white+blue+red (WBR), blue+red (BR), white+red (WR) and sunlight in pot stage. The mean of values ± Standard deviation (n=5) is displayed. Values with the same letter in column do not significantly differ (by the tukey's test, p≤0.05). The explanations for the treatment abbreviations are provided in Table 1.

4. Discussion and Conclusions

In this experiment, significantly higher percentage of germination under LED light treatments, highlighted the effect of light quality on germination rate. It is known that orange/red and blue regions of light spectrum are most effective in germination process (Tozzi *et al*., 2005). Germination rate was satisfactory in absence of both blue and white LED lighting in WR and BR treatments, respectively. Overall, germination rate was highly affected under LED treatments compared to SL and it can be derived that presence of red light in all treatment resulted in a partially better germination in *Sinningia speciosa.*

Using different light spectra for tomato seedlings revealed that exposing seedlings to monochromatic red light showed higher shoot dry weight than 80% RB but small proportion of blue (95% RB) contributes getting more shoot dry weight (Gómez and Mitchell, 2015). Moreover, it was reported that lamps with substantial red but small blue waveband radiation energy, produced more weight yields in tomato compared to high blue and less red biased lamps (Warrington and Mitchell, 1976). Furthermore, among sodium lamp (1:1 RB), 100% R, 50% RB, 70% RB, 90% RB and white LEDs lighting treatments 90% RB led to significantly higher dry matter content (Wojciechowska, 2015). In another study with RW, RB, RBW and FL (fluorescent lamp) lighting treatments, WBR enhanced yield of lettuce plants including shoot FW, shoot DW, root FW, root DW and Leaf area (Lin *et al*., 2013). Our Results on fresh and dry weight and leaf features (leaf area and leaf width) were consistent with quoted reports and it can be concluded that a small blue proportion in combination with red and white spectrum will result in a higher biomass. Blue light is considered to be a substantial stimulator for leaf expansion which enhances leaf area and biomass production (Li *et al*., 2010; Cope ad Bugbee, 2013), on the other hand it has been proved that plant growth typically tends to decrease as the fraction of blue photons exceeds 5-10%, high levels of blue light in the spectrum results in inhibition of cell expansion, cell division, and leaf area expansion, which ends up in less photon capture and diminished growth (Bugbee, 2016). Exposing to red light results in enhanced stem elongation (Hoenecke *et al.*, 1992), also absorbing high ratio of red light by plant photoreceptors can lead to production of a plant hormone called metatopolin (Steele, 2004), which can stimulate cell division as well as leaf expansion. Addition of white LED light may further increase plant growth, as white light might penetrate deeper in to the canopy and enhance photosynthesis compared to combination of red and blue light. Perhaps the combination of white, blue and red light perform a balanced spectral environment by providing a favorable amount of white light to plants (Lin *et al.*, 2013).

White light is more capable of increasing chlorophyll than blue light, however some reports stated that blue light had significant effect on chlorophyll a synthesis (Wynne and Rhee, 1986; Rivkin, 1989; Aidar *et al.*, 1994; Sanchez-Saavedra and Voltolina, 1994; Mercado *et al.*, 2004; Hogewoning *et al.*, 2010; Vadiveloo *et al.*, 2015), also it was reported that red light plays an important role in chlorophyll content enhancement (Kubota *et al.*, 1996). In addition, it has been reported combinational red light with blue and white light can increase carotenoid content accumulation (Lefsrud *et al.*, 2008; Kopsell *et al.*, 2014; Chen *et al.*, 2016; Kopsell *et al.*, 2016), however Lin *et al.* (2013) reported versus result, claiming that WBR LED has no effect on carotenoid content compared to RB LED and FL light. It can be concluded that white, red and blue light conjointly can enhance chlorophyll and carotenoid content but plant exact response to light quality varies with species and cultivars.

In present experiment, the maximum yield of primary photochemistry (φP_0) was in correlation with maximum quantum efficiency of photosystem II (F_v/F_m) which confirmed the enhancement of chlorophyll concentration under WBR treatment. We would suggest that transplants under WBR light treatment contained more chloroplast which maximized light capture for photosynthesis. Based on our results, we would suggest that the lowest photosynthetic rate in plants grown under SL is the result of low means of chlorophyll a, chlorophyll b, total chlorophyll and carotenoid content which was confirmed by the decline in F_v/F_m and φP_0 and increase in φD_0 and it can be concluded that increased φD_0 in SL treated transplants explains that due to highest amount of quantum yield of energy dissipation (φD_0), the major of natural light absorbed by the plant in high intensity was not used for the photochemical yield of electron transport chain and excess light dissipated as heat from the electron transport system (Aliniaeifard *et al.*, 2018).

The higher total soluble sugar content of plants grown under SL compared to other plants grown under artificial LED lights, suggesting that under higher light intensity elevated level of soluble sugar helps transplants to avoid excessive light intensity and unlike LED treatments, they had less utility for photosynthesis (Ciereszko *et al.*, 2001; Havaux and Kloppstech, 2001).

In this study, WR treatment showed the highest rate of increase in minimal fluorescence intensity (F_0) and this value decreased asproportion of blue lightbut the opposite trend was observed as white proportion of light increased. This was also observed in F_i, F_j, F_m, F_v and $V_i$parameters. The maximum efficiency of photosystem II (F_v/F_m) increased in BR followed by WBR, however it was reduced in WR treatment (in the absence of blue LED). In an experiment using 32% BW and 40% BR lighting treatments on Phalaenopsis 'purple star 'showed higher amount in F_v/F_m in comparison with 0% BR (Ouzounis *et al.*, 2014) which necessitates certain amount of blue light for proper photosynthesis (Hogewoning *et al.*, 2010). F_v/F_m value ranges between 0.72-0.84 in many plants (Maxwell and Johnson, 2000), although the F_v/F_m of SL-grown transplants was in this range but it showed the least efficiency among other treatments, which is not surprising as this value changes with environmental conditions (Ouzounis *et al.*, 2014). Furthermore, it was shown that existenceof UV and yellow light in sunlight reduce photosynthesis efficiency (Takashi *et al.*, 2010). In addition, *Sinningia speciosa* has no optimum photosynthetic activity under sunlight (Larson, 1992; Dole and Wilkins, 2005).

Transplants under highest blue proportion (BR) had the highest value in PI_{ABS} and φE_0 and the lowest value of DI_0/RC. The WR exposed transplants (which had no high ratio of blue light but high red ratio) showed the lowest PI_{ABS} and φE_0 and higher ABS/RC, TR_0/RC and DI_0/RC as the result. In an experiment investigating on photosynthetic and growth responses of purple variety of basil under white, blue and red LED lamps results shown that red light had the highest increase in ABS/RC, TR_0/RC and DI_0/RC and this amount was decreased under blue light (Hosseini *et al.*, 2019). Inactivation of reaction centers and a decrease in active Q_A reducing centers occur as ABS/RC increases (Strasser and Stirbet, 1998). WR and WBR-grown transplants (with higher white and red ratios) represented lower ET_0/RC which indicates that absorbed energy is briefly conveyed to the electron transport chain (Sarkar and Ray, 2016). This confirms that plants grown under BR light are more capable of transporting electrons from absorbed photons into electron transport chain and beyond $Q_A^{-1}\psi_0$ which could efficiently regulate energy level in the center of R reaction (Strasser *et al.*, 2004). SL-grown transplants showed highest soluble sugar content; however they had the second increase in PI_{ABS}, it is possible that in case of an environmental stresssuch as high light intensity, in which excess energy beyond photosynthetic capacity is existing, led to production of ROS which results in oxidative damage to photosystem II (Pospíšil, 2016). In transplants grown under WBR and BR LED lighting, an increment in φP_0 value was observed, while there was no increase of the same value in transplants grown under sunlight. The results for φP_0 were in correlation with F_v/F_m which impacted chlorophyll and leaf area as explained in aforementioned chlorophyll measurements.

At flowering stage, transplants grown under LED lighting treatments resulted performed better ornamental criteria including number of flowers, flower diameter and number of leaves. Also it could be suggest that, those plants grown under LED lighting treatments could reach flowering stage sooner which will result in higher profit especially in commercial scale. Totally, in this experiment, this scenario was the case for plants grown under WBR treatment. Application of LED light in greenhouse in combination of red, blue and white wavelengths with a high photon efficiency are suitable for the production of horticultural plants (Kozai *et al*., 2015; Nicole *et al*., 2016). Our results are consistent with previous studies findings which indicate thatlighting source composed of red, blue and white light spectrum enhance morphological development of seedlings compared to monochromic light of each waveband (Brown *et al*., 1995; Gómez and Mitchell, 2015; Hogewoning *et al*., 2010; Ouzounis *et al*., 2014).

Desirable morphological and physiological characteristics in *Sinningia speciosa* transplants achieved under LED lighting treatments with identical proportion of white, blue and red led to enhanced morphological and physiological features at marketing stage including higher number of flowers, flower diameter, number of leaves as well as fewer days to flowering. Shorter time interval to flowering may help commercial growers to save time and costs of production while enhancing *Sinningia speciosa*plants quality in comparison with sunlight-grown transplants in conventional greenhouse condition. Moreover, it should be noted that using LEDs have higher expenses due to the cost of providing LEDs and growth chambers and also high consumption of electricity. Additional investigation is required to evaluate different ratios of spectral composition to optimize environmental condition for Gloxinia transplant production.

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