

Red light optimized physiological traits and enhanced the growth of ramie (*Boehmeria nivea* L.)

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Abstract

Light is an important variable affecting the plant growth. In present study, the effects of different color light-emitting diodes (mixed colors, red, blue, and orange light) on plant growth, gas exchange, and oxidative stress were investigated in *Boehmeria nivea* L., by means of measuring growth, photosynthesis, chlorophyll (Chl) content, reactive oxygen species (ROS), and activity of antioxidant enzymes under controlled conditions. Comparing to the mixed colors light, red light significantly increased shoot and leaf biomass, plant height, number of leaves per plant, and stem diameter by increasing the Chl content and therefore promoting the highest photosynthetic capacity. This might partially be explained by the decrease of malondialdehyde and proline contents as well as the activities of superoxide dismutase and peroxidase under red light, to keep a better internal environment of the cell. However, blue and orange light decreased plant growth, and increased the activities of antioxidant enzymes which suggest an environmental stress on plants. These results suggest that red light can enhance *B. nivea* growth by activating photosynthesis and reducing ROS accumulation.

Additional key words: antioxidant system; gas-exchange parameters; light quality; morphological traits.

Introduction

Boehmeria nivea L. (ramie) is an ancient perennial plant with a rapid growth, nutritional value, and multifunctional applications in textiles, livestock feed, medicine, and environmental conservation (Rehman *et al.* 2019). This light-demanding species is widely grown as a bast fiber crop all over the China. It can be used as fresh fodder, or can be artificially dried to produce a leaf meal. Its farming, industry, and trade provide living support to about 5 million people (An *et al.* 2017). Ramie fiber has a good quality, color, and appearance, which can give strength to textile industry (Rehman *et al.* 2019). However, it has been reported that stress conditions before harvest can inhibit the growth of *B. nivea*. Thus, it is essential to find out the most advantageous environmental conditions for better growth of *B. nivea*.

Light wavelengths have varying effects on the growth of plants. During photosynthesis in green plants, light energy is captured and used to convert into energy-rich

organic compounds (Fukuda *et al.* 2008). However, quality of light or shifting wavelengths can affect morphology, anatomy, physiology or development and is identified by phytochromes in plants (Haliapas *et al.* 2008, Kami *et al.* 2010). For example, red light contributed to higher plant biomass and starch contents, while blue light caused higher contents of Chl and vitamin C in *Brassica campestris* (Li *et al.* 2012). According to Landi *et al.* (2020) monochromatic light affects not only plant photosynthetic performance but also the 'quality' of plants by modulating the biosynthesis of photoprotective compounds. In a previous study, Simlat *et al.* (2016) reported that blue light resulted in an increased germination rate, number of leaves and roots, higher stomatal frequency and pigment contents, as well as antioxidant enzyme activities in *Stevia rebaudiana* Bertoni. Orange light increased the germination of *Bletilla ochracea* Schltr. seeds and induced the formation of rhizoids (Godo *et al.* 2011). Meanwhile, previous literature on different colors of light suggests that plant's response to light stress is species specific

Received 6 February 2020, accepted 4 May 2020.

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Abbreviations: BL – blue LED light; C_i – intercellular CO₂ concentration; CK – mixed colors LED light as control (white); DM – dry mass; E – transpiration rate; FM – fresh mass; g_s – stomatal conductance; LED – light-emitting diodes; MDA – malondialdehyde; OL – orange LED light; P_N – net photosynthesis; POD – peroxidase; RL – red LED light; ROS – reactive oxygen species; SOD – superoxide dismutase; SPAD – Soil Plant Analysis Development.

Acknowledgements: This work was supported by Fundamental Research Funds for the Central Universities (2662015PY059), China Agriculture Research System project (CARS-16-E12), and the National Natural Science Foundation of China (31571717).

(Hogewoning *et al.* 2010, Cope and Bugbee 2013), mediated by different photoreceptors. For example, cucumbers are known to be more responsive towards light spectral distribution than other greenhouse plants, such as pepper and tomato (Hemming *et al.* 2008, Trouwborst *et al.* 2010, Hernández and Kubota 2012). Chen *et al.* (2014) showed that lettuce plant biomass and stem diameter were lower under monochromatic blue as compared to monochromatic red light.

Stress environment causes generation of reactive oxygen species (ROS), such as superoxide radical ($O_2^{\cdot-}$), H_2O_2 , singlet oxygen (1O_2), and hydroxyl radicals (OH) (Fahad and Bano 2012, Fahad *et al.* 2013, 2014, 2015a,b,c; 2016a,b,c,d; 2017, 2018; Saud *et al.* 2014, 2016, 2017a,b; Liu *et al.* 2015, Ma *et al.* 2019, Turan 2019, 2020). In order to prevent oxidative stress, the plants respond to ROS by activating an antioxidant defense system in their cells (Kurutas 2016). Deng *et al.* (2012) reported that plants under stressful environment can augment lipid peroxidation as malondialdehyde (MDA), in their tissues, indicating the occurrence of oxidative stress. Proline accumulation is also an adaptive behavior in plants against stressful conditions. Proline is a signal molecule activating physiological and molecular responses (Szabados and Savouré 2010). Accumulation of proline due to increased synthesis and decreased catabolism under stress conditions has been documented in several plants (Mani *et al.* 2002).

Plants have an efficient antioxidant defense system including superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) which is involved in protecting plant cells by scavenging of ROS (Lall *et al.* 1999, Procházková *et al.* 2001, Alici and Arabaci 2016). However, antioxidant enzymes activity in plants under different light spectra is more complex. For example, ascorbate peroxidase and SOD were notably influenced by light intensity in different ways (Bayat *et al.* 2018). Manivannan *et al.* (2015) reported that blue light improves antioxidant enzyme activities in *Rehmannia glutinosa* cultured *in vitro*. Similar results were reported for tomato leaves (Kim *et al.* 2013). Conversely, the antioxidant activity of pea and *Dendrobium officinale* seedlings was improved under red light (Wu *et al.* 2007, Wang *et al.* 2017). These findings suggest that light spectrum distribution evokes diverse morphological, photosynthetic, and antioxidative responses in different plant species with contrasting results. Therefore, it is requisite to find out appropriate light quality for plants, grown under artificial conditions.

Therefore, the main objective of present study was to investigate the effects of different light quality on growth, Chl content, leaf gas-exchange characteristics, and antioxidant capacity of *B. nivea* tissue, to explore the relationship between growth, photosynthesis, and antioxidant capacity, and to screen out the most favorable light spectrum for standardized production of *B. nivea*. This study would provide reference for the regulation of light quality environment of *B. nivea* and other crop plants.

Materials and methods

Plant material and growth conditions: The pot experiment was carried out under controlled conditions in a

glasshouse, at Huazhong Agricultural University Wuhan, China in 2018. Physicochemical properties of soil were: pH 6.0, EC of 2.0 dS cm^{-1} , $22.2 \text{ g(organic matter kg}^{-1})$, $23.1 \text{ mg(exchangeable potassium kg}^{-1})$, $19.9 \text{ g(total nitrogen kg}^{-1})$, $0.25 \text{ g(total phosphorous kg}^{-1})$. Uniform rhizome segments of 15 cm were taken from roots of *B. nivea*, cultivar ‘Zhongsizhu 1’, and planted in pots of 30-cm length and 40-cm width. Each pot contained 14.0 kg of soil. After planting, pots were placed in natural light for seven days, and then moved under color LEDs. Treatments of different light quality included mixed colors light as white light (CK), red light (RL), blue light (BL), and orange light (OL) (Fig. 1). LEDs light wavelengths are given in Fig. 2. Each treatment was set in a completely randomized design (CRD). Fertilizer was applied at a recommended dose. Weeding and irrigation was done with metal-free water when needed. Pots were placed within different color LED cabins covered with porous black sheet under a glasshouse. Analytical grade chemicals obtained from Sinopharm Chemical Reagent Co., Ltd. (China) were used in this study.

Sampling and data collection: During the rapid growth period of crop, 5th fully expanded and healthy leaf from top (functional leaf) for each treatment was picked at 09:00–10:30 h, cleaned, and immediately frozen in liquid nitrogen before being stored in -80°C until further analysis. Before harvesting, three plants from each treatment were sampled and then plants were carefully harvested after 30 d from shifting under light quality treatments, by cutting the stems at a height of 5 cm from the soil surface. Total number of leaves and stems per plant was counted and plant height [cm] was measured by meter scale from soil surface to the plant tip. Stem diameter [mm] at a height of 15 cm from root neck was measured using a digital vernier caliper (ST22302, SG Tools, Hangzhou, China). Fresh shoot biomass and fresh leaf biomass were determined by weighing aboveground plant parts and leaves, respectively. These plant parts were then oven dried at 70°C for 72 h until constant mass and then weighed to get their dry mass.

Chl content and gas exchange: The Chl content of the fully expanded and healthy leaf at the stage of rapid plant growth was measured for each treatment during 09:00–10:30 h using a Soil Plant Analysis Development meter SPAD-502 plus (Konica Minolta, Inc., Japan). Nine leaves were selected from each treatment for the measurement of gas-exchange attributes, such as net photosynthesis (P_N), transpiration rate (E), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i), from 9:00–11:00 h using a portable photosynthesis system Li-6400 (Li-COR, Lincoln, NE, USA).

Lipid peroxidation and proline content: Lipid peroxidation in ramie leaves was measured by thiobarbituric acid (TBA) test, which determines the content of malondialdehyde (MDA), an end product of lipid peroxidation (Heath and Packer 1968). Leaf material (0.5 g) was homogenized in 5 ml of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged for 5 min at $10,000 \times g$ and 1 mL of supernatant was added to 4 mL of 20% TCA

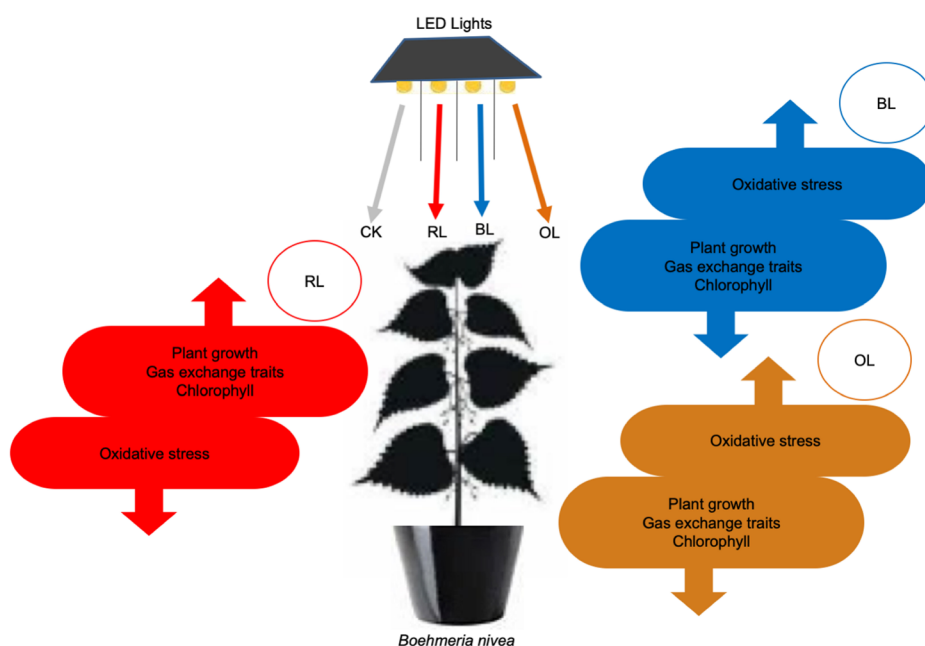


Fig. 1. A schematic diagram representing different color light treatments and their effects on *Boehmeria nivea* L. BL – blue LED light; CK – mixed colors LED light as control (white); OL – orange LED light; RL – red LED light.

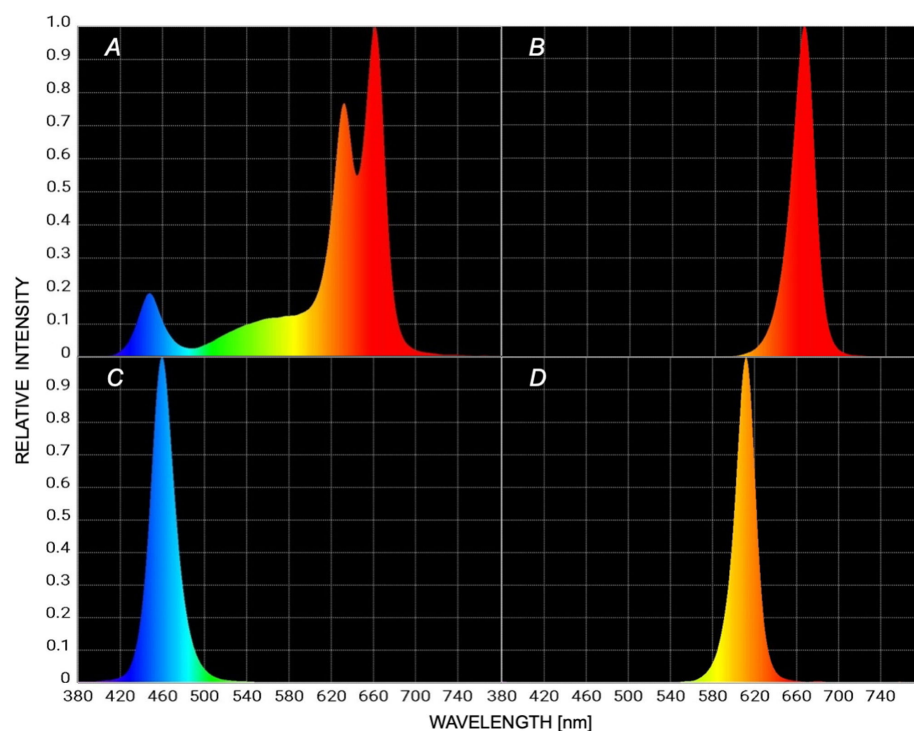


Fig. 2. Wavelengths and relative intensity of CK (mixed color LED light), RL (red LED light), BL (blue LED light), and OL (orange LED light).

containing 0.025 mL of 0.5% TBA. The mixture was incubated at 95°C in a hot water bath for half an hour and placed in an iced-water bath to stop the reaction. Later the material was centrifuged for 15 min at $10,000 \times g$ and the absorbance of the supernatant was read at 532 and 600 nm using a microplate absorbance spectrophotometer (*xMarkTM*, Bio-Rad, United States). Read for nonspecific absorption at 600 nm was subtracted. The amount of MDA was calculated from the extinction coefficient 155 mM cm^{-1} .

Leaf proline content was measured according to the

method of Bates *et al.* (1973). The leaf material was homogenized in sulphosalicylic acid. Then the homogenate was filtered by filter paper (*Whatman's* no. 1). The obtained filtrate was boiled for 60 min after adding acetic acid and acid ninhydrin, then absorbance was taken at 520 nm using spectrophotometer (*xMarkTM*, Bio-Rad, United States) and expressed in $\mu\text{g g}^{-1}(\text{FM})$.

Antioxidant enzymes activity: Superoxide dismutase (SOD, EC 1.15.1.1) and peroxidase (POD, EC 1.11.1.7.) were determined in crude extracts extracted by crushing

0.5 g of fresh leaves using liquid nitrogen followed by homogenization in 5 ml of 50 mM sodium phosphate buffer pH 7.0, containing 0.5 M methylene-diaminetetraacetic acid and 0.15 M NaCl. Then the homogenate was centrifuged for 10 min at 4°C and $12,000 \times g$. The supernatants were used for assay of SOD and POD.

Activity of SOD enzyme was determined in a reaction mixture consisting of 50 mM sodium phosphate buffer of pH 7.0, 10 mM methionine, 1.17 mM riboflavin, 56 mM nitroblue tetrazolium (NBT), and 100 μ l of enzyme extract. The solution absorbance was tested by measuring its capacity of inhibiting the photochemical reduction of NBT at 560 nm using microplate absorbance spectrophotometer (*xMarkTM*, *Bio-Rad*, United States). One unit of SOD was defined as the enzyme activity that reduced the photoreduction of nitroblue tetrazolium (NBT) to blue formazan by 50% (Chen and Pan 1996). SOD activity was expressed as enzyme U g^{-1} (FM).

POD activity was determined following the method of Sakharov and Aridilla (1999) by measuring the increase in absorbance at 470 nm using microplate absorbance spectrophotometer (*xMarkTM*, *Bio-Rad*, United States). The mixture consisted of 2.8 mL of guaiacol (3%), 0.1 mL of H_2O_2 (2%), and 0.1 mL of enzyme extract. One unit of POD activity is the increase in absorbance of 1.0 per min. The POD activity was expressed as enzyme U g^{-1} (FM).

Statistical analysis: The data were subjected to one-way analysis of variance (ANOVA), followed by Tukey's HSD (Honestly significant difference) test to avoid a type I error, using *Statistix 8.1* (Analytical Software, Tallahassee, United States). Testing showed that all data were approximately normally distributed. Statistical variations of data were represented as standard deviation and $p \leq 0.05$ was

used to denote statistical significance. *GraphPad Prism 6* software and *R studio* were used for the graphical presentation.

Results

Plant growth: Shoot fresh mass, leaf fresh mass, shoot dry mass, and leaf dry mass, plant height, number of leaves, and number of stems per plant were greatest for the plants grown under RL, and minimum values for above parameters were observed under OL (Table 1). Shoot and leaf fresh mass decreased by 33.6 and 27.5% under BL, by 61.9 and 46.4% under OL, and increased by 10.1 and 14.3% under RL, respectively, compared with the plants under control (CK). In the same way, shoot dry mass and leaf dry mass decreased by 24.9 and 27.1% under BL, by 43.8 and 34.4% under OL, but increased by 12.6 and 8.6% under RL, respectively, compared with CK. Plant height increased by 27.2% under RL, which was statistically similar with plant height under BL, whereas OL caused a decrease of 45.1% in plant height, compared with the CK (Table 2). Number of leaves per plant also decreased by 18.8% under OL, but increased by 6.9% under RL, compared with CK (Table 2).

Chl content and gas-exchange traits: A change in SPAD (Chl) was evaluated in *B. nivea* leaves under different color LED lights (Fig. 3). RL enhanced by 10.3% the Chl contents, however, Chl contents were reduced by 12.0 and 22.9% under BL and OL, respectively, compared with CK. RL improved photosynthesis in *B. nivea* as indicated by significantly higher values for P_N , E , g_s , and C_i . However, BL and OL showed decreased P_N , E , g_s , and C_i compared with CK (Fig. 4).

Table 1. Changes in fresh and dry mass of *Boehmeria nivea* grown under varying colors of light emitting diodes. Values are means $\pm$ SD ($n = 3$). Different letters within a column indicate significant difference between the treatments ($p \leq 0.05$). CK – mixed color LED light; RL – red LED light; BL – blue LED light, OL – orange LED light.

Treatment	Shoot fresh mass [g per plant]	Leaf fresh mass [g per plant]	Shoot dry mass [g per plant]	Leaf dry mass [g per plant]
CK	64.58 $\pm$ 3.03 ^b	29.51 $\pm$ 0.95 ^b	12.91 $\pm$ 0.32 ^b	7.01 $\pm$ 0.23 ^b
RL	71.09 $\pm$ 2.31 ^a	33.73 $\pm$ 1.70 ^a	14.53 $\pm$ 0.59 ^a	7.61 $\pm$ 0.26 ^a
BL	42.91 $\pm$ 2.14 ^c	21.39 $\pm$ 1.07 ^c	9.70 $\pm$ 0.66 ^c	5.11 $\pm$ 0.06 ^c
OL	24.60 $\pm$ 0.66 ^d	15.81 $\pm$ 0.64 ^d	7.25 $\pm$ 0.16 ^d	4.60 $\pm$ 0.14 ^d

Table 2. Changes in morphological traits of *Boehmeria nivea* grown under varying colors of light emitting diodes. Values are means $\pm$ SD ($n = 3$). Different letters within a column indicate significant difference between the treatments ($p \leq 0.05$). CK – mixed color LED light; RL – red LED light; BL – blue LED light, OL – orange LED light.

Treatment	Plant height [cm]	Number of leaves per plant	Number of stems per plant	Stem diameter [mm]
CK	52.13 $\pm$ 3.36 ^b	33.67 $\pm$ 1.15 ^b	2.67 $\pm$ 0.58 ^{ab}	6.19 $\pm$ 0.31 ^a
RL	66.30 $\pm$ 3.40 ^a	36.00 $\pm$ 1.00 ^a	3.33 $\pm$ 0.58 ^a	6.68 $\pm$ 0.23 ^a
BL	60.67 $\pm$ 3.79 ^a	31.33 $\pm$ 0.58 ^c	2.67 $\pm$ 0.58 ^{ab}	5.40 $\pm$ 0.28 ^b
OL	28.63 $\pm$ 2.18 ^c	27.33 $\pm$ 0.58 ^d	2.00 $\pm$ 1.00 ^b	3.71 $\pm$ 0.18 ^c

Lipid peroxidation, proline content, and antioxidant enzymes: BL and OL resulted in severe lipid peroxidation in *B. nivea*, compared with CK (Fig. 5). MDA and proline contents were reduced by 12.5 and 12.6% under RL; but increased by 27.7 and 21.4% under BL, and by 47.3 and 39.4% under OL, compared with CK (Fig. 5A,B). The activities of antioxidant enzymes SOD and POD were also affected by differential light quality. SOD activity increased by 14.1% under OL, but decreased by 8.9% under RL;

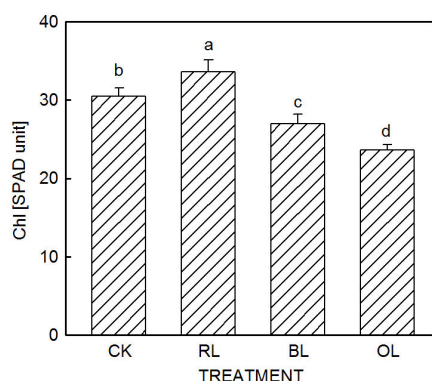


Fig. 3. Effects of differential light quality on SPAD (Soil Plant Analysis Development) value of *Boehmeria nivea* grown under different color light emitting diodes. CK, RL, BL, and OL designated as mixed color LED light, red LED light, blue LED light, and orange LED light, respectively. Bars indicated the mean $\pm$ SD ($n = 3$). Different letters on bars indicated significant difference between treatments at $p \leq 0.05$.

however, there was no difference in SOD activity under BL, compared with CK (Fig. 5C). POD activity increased by 21.5 and 47.7% under BL and OL respectively, but was reduced by 35.1% under RL, compared with CK (Fig. 5D).

Correlation analysis: Correlation between different morphological traits and gas-exchange attributes of *B. nivea* grown under differential light quality is shown in Fig. 6. According to this correlation, fresh and dry shoot and leaf biomass, plant height, and number of leaves per plant were positively correlated with Chl content, P_N , E , and g_s (Fig. 6A). However, these studied growth attributes were negatively correlated with MDA, and proline contents as well as SOD and POD activity in *B. nivea* plants (Fig. 6B). This correlation reflected a close link between studied traits of *B. nivea*.

Discussion

Light is not only a source of energy, but also an essential signal for plant growth and development (Chory and Li 1997, Kim *et al.* 2002). Plants require light for whole life span from germination to production of seeds. However, different wavelengths of light have diverse effects on the growth of plants (Fukuda *et al.* 2008). Several processes in plants, for example, germination, flowering, photosynthesis, biomass accumulation, and stomatal regulation can be controlled and optimized by adjusting light wavelengths (Taylor and Assmann 2001, Taiz and Zeiger 2002, Kim *et al.* 2004, Pinho 2008, Yeh and Chung 2009, Vänninen *et al.* 2010, Liu *et al.* 2016).

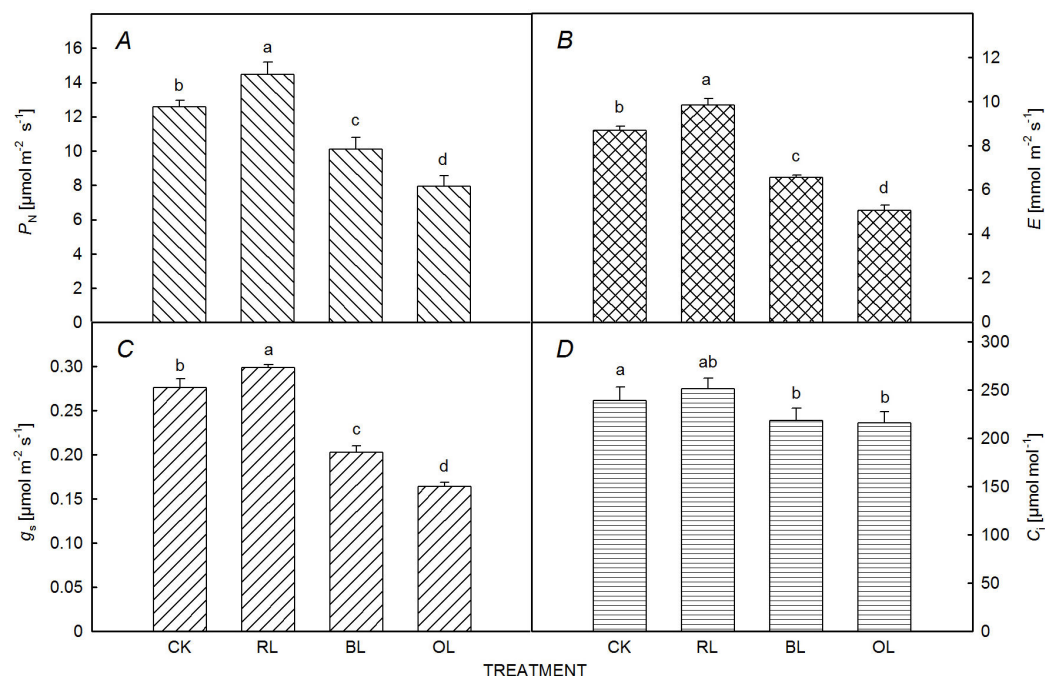


Fig. 4. Effects of differential light quality on net photosynthesis (P_N), transpiration rate (E), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) in *Boehmeria nivea* grown under different color light emitting diodes. CK, RL, BL, and OL designated as mixed color LED light, red LED light, blue LED light, and orange LED light, respectively. Bars indicated the mean $\pm$ SD ($n = 3$). Different letters on bars indicated significant difference between treatments at $p \leq 0.05$.

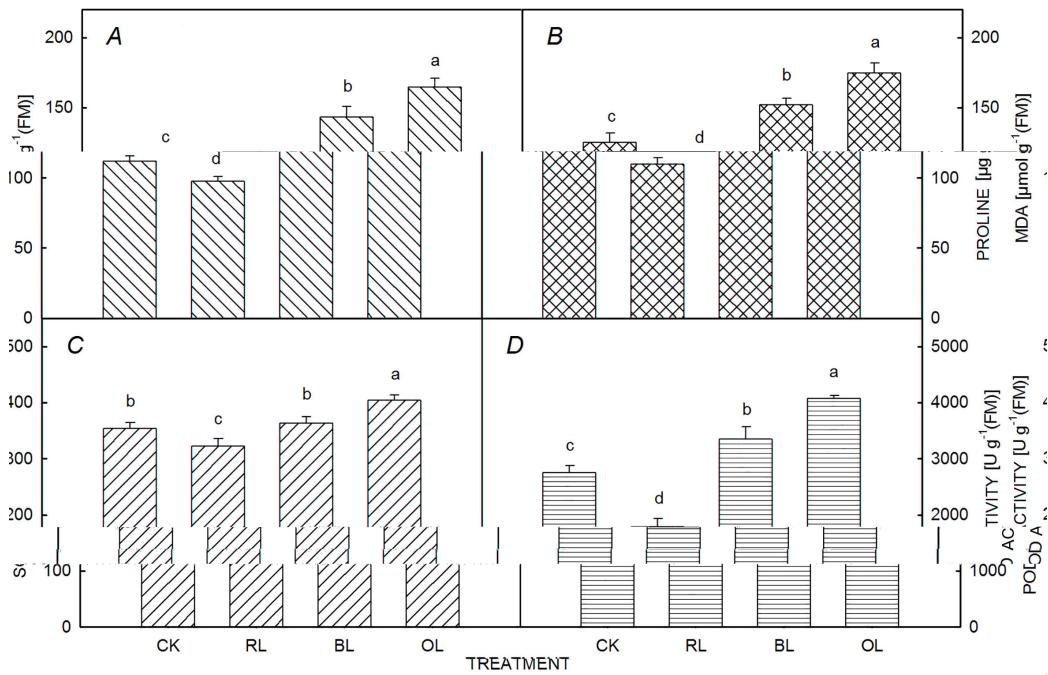


Fig. 5. Effects of differential light quality on malondialdehyde (MDA) content (A), proline content (B), superoxide dismutase (SOD) activity (C), and peroxidase (POD) activity (D) in the leaves of *Boehmeria nivea* grown under different color light emitting diodes. CK, RL, BL, and OL designated as mixed color LED light, red LED light, blue LED light, and orange LED light, respectively. Bars indicated the mean $\pm$ SD ($n = 3$). Different letters on bars indicated significant difference between treatments at $p \leq 0.05$.

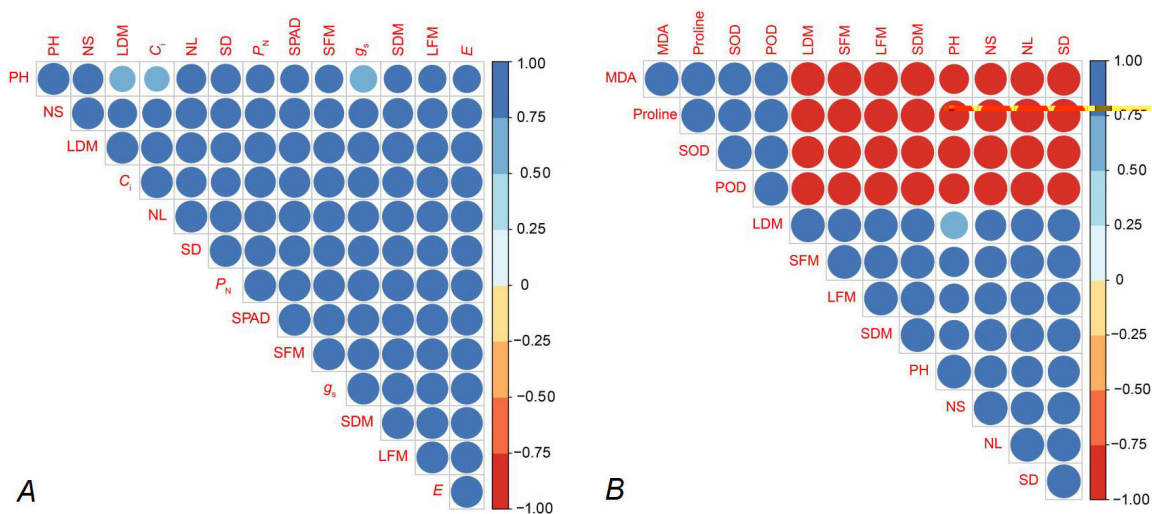


Fig. 6. Correlation of morphological traits with gas-exchange attributes and chlorophyll content (A); correlation of morphological traits and antioxidant system in *Boehmeria nivea* (B). SFM – shoot fresh mass; LFM – leaf fresh mass; SDM – shoot dry mass; LDM – leaf dry mass; PH – plant height; NL – number of leaves per plant; NS – number of stems per plant; SD – stem diameter; P_N – net photosynthesis; E – transpiration rate; g_s – stomatal conductance; C_i – intercellular CO_2 concentration; MDA – malondialdehyde; Pro – proline; SOD – superoxide dismutase; POD – peroxidase.

Frequently used lights for *in vitro* plant culture, such as fluorescent lamps, high-pressure sodium lamps, metal halide lamps, and incandescent lamps, are of poor light quality with unnecessary wavelengths for improving plant growth. In addition, these light sources require high electrical energy and generate heat in closed chambers

(Kim *et al.* 2004, Dutta Gupta and Jatothu 2013). However, light emitting diode (LED) is an environment friendly technology for *in vitro* plant culture with more advantages over traditional light sources (Mitchell *et al.* 2012). Thus, LEDs appear to be a suitable strategy for improving light-use efficiency for higher plant growth. In present study,

we made an effort to investigate the effects of light quality using varying color LEDs (mixed color, red, blue, and orange light) on growth, Chl content, gas exchange, and oxidative stress in *B. nivea*, in order to determine the optimal light spectra for maximal plant growth. Light spectral distribution also has an effect on plant shape, development and flowering (Singh *et al.* 2015). In present study, BL and OL reduced the plant growth in terms of plant height, plant biomasses, number of leaves per plant, and number of stems per plant as well as stem diameter, however, RL appeared to increase plant growth as shown by higher shoot and leaf biomass, plant height, and number of leaves per plant, compared with control (Tables 1, 2). In our previous study, we observed that RL increased plant height, biomass, photosynthetic pigments, while reduced ROS production in the cell/tissues of *Corchorus capsularis* plants (Saleem *et al.* 2019). Similar findings were reported by Dong *et al.* (2014) in *Triticum aestivum* L.; the red light thickened the stem and increased the overall ground biomass of plants. Kurepin *et al.* (2007) reported that red light, mediated by phytochrome, is involved in leaf morphogenesis, photosynthetic apparatus development, and accumulation of carbohydrates. According to Gautam *et al.* (2015), a reduced quantity of red light results in delay or prevention of flowering, blue and orange lights induced stress which decreased plant growth and biomass; however, red light might increase plant biomass (Li and Kubota 2009).

In present study, the increase in *B. nivea* biomass grown under RL might be associated with the increased rate of photosynthesis. RL increased the gas exchange in terms of P_n , E , g_s , and C_i (Fig. 4), signifying an increase in the photosynthesis in *B. nivea* plants under RL. Similar results have previously been observed by Shimizu *et al.* (2011) who reported an increase in photosynthetic pigments and carotenoid contents in lettuce under red light, compared with the plants grown under control conditions. This may clarify that red light was more effective than blue light for Chl synthesis (Eskins and McCarthy 1987). In a previous study, Yanagi *et al.* (1996) compared the rate of photosynthesis in strawberry (*Fragaria × ananassa* L.) leaves under red and blue lights and found higher quantum efficiencies under red light. The role of red-LED light to drive photosynthesis has been widely accepted due to that (1) red wavelengths (600–700 nm) are efficiently absorbed by photosynthetic pigments and (2) early LEDs were red close to an absorption peak of Chl (Sager and McFarlane 1997). Chloroplast is an organelle that conducts photosynthesis, where the photosynthetic pigment chlorophyll captures the energy from sunlight, converts it, and stores it in the energy-storage molecules (Kirchhoff 2019). However, chloroplast ultrastructure, number of thylakoids and thylakoid area is strongly affected by light exposure (Allen *et al.* 2011, Chen *et al.* 2018). The present study showed increased plant height under RL and BL, compared with control. Nanya *et al.* (2012) studied the effects of red, blue, and combination of red and blue LED lights on morphology of tomato seedlings and found that stem height depended on quantity of blue light. Whereas, according to Hernández and Kubota (2016), blue radiation reduced the

length of stem in cucumber, but improved plant extension growth; thus, the response is species specific.

Abiotic stress can promote ROS production; however, plants possess a well-organized protective system to detoxify the ROS (Sharma *et al.* 2012, Das and Roychoudhury 2014). In current study, plants under BL and OL maintained higher production of MDA and proline, indicating oxidative damage to lipid membranes (Fig. 5), while RL facilitated a reduction of oxidative stress in *B. nivea*. In a recent study, Adil *et al.* (2019) also reported that red light treatment was most favorable for high biomass accumulation and antioxidant activity in calli of *Withania somnifera* L. It has been established that LED light wavelengths can enhance not only the antioxidants contents but also the activities of antioxidative enzymes. *B. nivea* plants grown under BL and OL showed the higher activity of antioxidant enzymes, SOD and POD, whereas RL reduced SOD and POD activities, compared with control (Fig. 5). Simlat *et al.* (2016) reported that the POD activity was enhanced by blue LED light in *Stevia rebaudiana* Bertoni, whilst red LED light exerted an opposite effect. These results are in agreement with our research. Kim *et al.* (2013) also reported that higher accumulation of proline in tomato leaves was observed under blue LED, however, the lower content of proline was observed under red LED. Similarly, ROS scavenging enzyme activities also increased under blue LED. This variable antioxidant response might be associated with changes in gene expression and protein function in different plant tissues. Thus ROS-scavenging enzymes, *i.e.*, SOD and POD performance in *B. nivea* serves as an approach to reinforce cell protection system and to reduce ROS production due to light stress.

Conclusion: Our results demonstrated that red LED promoted *B. nivea* growth by improving leaf gas exchange and chlorophyll content, and by reducing ROS accumulation, while blue LED and orange LED both reduced *B. nivea* growth and photosynthesis with raised contents of MDA and proline contents, which may damage membranes in organelles. Furthermore, these results showed that light quality was linked to measured morphological and physiological parameters in *B. nivea*. These results depicted that red light can be successfully implemented for production of *B. nivea*. Furthermore, our study provides a theoretical base for consistent cultivation of *B. nivea* on commercial level for getting quality products.

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