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Comparative Study of Color, Pungency, and Biochemical Composition in Chili Pepper (*Capsicum annuum*) Under Different Light-emitting Diode Treatments

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Abstract. **Color and pungency are the two main factors of chili peppers that determine their quality and final market price. In this study, we investigated the effect of light-emitting diodes (LEDs) on fruit color and primary and secondary metabolites (capsaicinoids) in *Capsicum annuum* L. cv. Cheonyang. High-performance liquid chromatography analysis of acetonitrile extract of chili fruits revealed enhanced capsaicinoid contents in blue LEDs (180 ± 6.32 mg/100 g) when compared with fluorescent light (54 ± 3.12 mg/100 g). However, color of chili pepper was remarkably changed under different light treatments. Among these, high ASTA color values and chromatic parameters (L*, a*, b*, C*, and H°) were recorded under red plus blue LED, further indicating red plus blue LED is responsible for most vivid color development compared with monochromatic red or blue LEDs. In addition, we found total carbohydrate [22.32 mg·g^{-1} fresh weight (FW)], reducing sugars (8.27 mg·g^{-1} FW), starch (14.05 mg·g^{-1} FW), carotenoids (6.15 mg·g^{-1} FW), and protein (4.83 mg·g^{-1} FW) were significantly high under red plus blue LED. These results showed that there were significant differences of plant growth and production of various metabolites among the different LEDs. Furthermore, blue LED showed a relatively higher rate of capsaicinoid production and red plus blue LED produced striking color development in chili peppers. Therefore, the results presented here might serve as an alternative strategy for nutritional improvement (color, oleoresin, and pungency) of chili peppers.**

Environmental conditions have always been critical factors for plant growth and development. Among them, three primary and ecologically important factors for plant growth are light, temperature, and water. Light, being the sole source of energy, plays a major role in plant growth and development. Hence, it is not surprising that plants have developed the capability to sense various factors of ambient light signals, including quality and quantity of light (Hoenecke et al., 1992; Saebo et al., 1995). Plant responses to various effects of light occurs in terms of multiple developmental processes such as seed germination, seedling photomorphogenesis, leaf size, leaf anatomy, plant height, flowering and fruit development, and altered primary and secondary metabolite biosynthesis (Chory et al., 1996; Dale, 1988). The conventional light sources used for greenhouse and in vitro studies are fluorescent lamps, metal halide, high-pressure sodium, and incandescent lamps. However, these light sources have certain limitations like mixture of impractical wavelengths for promoting plant growth (Brown et al., 1995). In recent days, LEDs are in the process of replacing these obsolete light sources for plant growth under controlled conditions. LEDs are gaining importance as an ideal light source for plant culture systems because of the following features: DC power, small mass/volume, specific wavelength, adjustable light intensity/quality, low thermal energy output, and long life (Goins et al., 1997; Tanaka et al., 1998).

Various effects of different LEDs have been studied in some food and horticultural crops such as *Cymbidium* (Tanaka et al., 1998), lettuce (Hoenecke et al., 1992), and wheat (Goins et al., 1997). Most of these studies emphasized the role of blue light on chloroplast development, chlorophyll formation, and stomatal opening. Red light was found to be critical for induction of stem elongation, leaf expansion, and photosynthesis. It was also reported that red and blue light have an effect in seedling photomorphogenesis in lettuce, wheat, and cymbidium (Goins et al., 1997; Hoenecke et al., 1992; Tanaka et al., 1998).

Although previous studies were able to determine the physiological and morphological effects of light quality, responses vary according to plant species. One cannot determine specific effects of light quality and, therefore, this could not be quantified. Still, there is a report affirming the positive influence of continuous light and temperature on capsaicinoid accumulation in chili (Murakami et al., 2006), but no extensive studies have been carried out on the effect of LEDs on chili pepper. The present study was carried out to understand the effect of different LEDs (blue, red, red plus blue) on plant growth and development, metabolites (total sugar, reducing sugar, starch, protein, and free amino acid), color, and pungency of fruit in chili pepper.

Materials and Methods

Plant material. Seeds of commercial chili pepper (*Capsicum annuum* L. cv. Cheonyang) were first surface-sterilized with 70% ethanol for 1 min followed by 4% (v/v) sodium hypochlorite for 20 min and the seeds were thoroughly washed with sterile distilled water. The capsicum seeds were germinated on Whatman No. 1 filter paper. Seedlings were then planted in pots containing a peat:soil (1:1) mixture and shifted to a growth chamber. The seedlings were watered once a day with Hoagland's solution to prevent mineral deficiency and moisture. At the eight-leaf stage, the plantlets were transferred to a growth chamber illuminated with different LED lights.

Lighting system and culture conditions. The LED-based light was tested in chili pepper growth experiments with different ranges of illumination. Plants at the eight-leaf stage were transferred to a growth chamber maintained at 25 °C and 70% relative humidity. There were four LEDs/fluorescence lights installed in the growth chamber, namely: 1) fluorescent lamps (Phillips, Fluotone 40 W; as control); 2) red LEDs (peak wavelength: 660 nm); 3) blue LEDs (peak wavelength: 460 nm); and 4) red plus blue LEDs (1:1). The LED system was installed by Dyne Bio, Seoul, Korea. The spectral distributions of each LED were set with the help of a spectroradiometer (Li-1800; LI-COR, Lincoln, NE). The photoperiod of LEDs was set as 16 h light and 8 h dark. The photosynthesis photon flux level was adjusted to 70 μmol·m^{-2}·s^{-1} for all lighting systems.

Sampling procedure and data observation. The experiment was conducted in a growth chamber and the first experiment began 10 Dec. 2010 and second 10 June 2011. For each LED treatment, 24 plants were maintained up to 120 d. From each light treatment, randomly five plants were harvested in 2, 4, and 6 weeks for morphological and biochemical analysis. The morphological characteristics like plant height, leaf length,

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leaf width, leaf area, and leaf (fresh and dry) weight were observed. The dry weight was determined after drying the sample for 48 h at 70 °C. The chili fruits under each LED treatment were tagged after anthesis and data recorded date by date. After the 45 d of postanthesis, the same age group pepper fruits were harvested, marked, and collected in different polyzip bags with date. The morphological characters of each fruit like average size, weight, and surface color were recorded. For the further biochemical analysis of primary and secondary metabolites, 20 g of fruit from each treatment was stored in –20 °C. For the extractable color analysis, 50 g of chili fruit from light treatment was cut and mixed in the same quantity, dried at 50 °C, ground into fine powder, and kept at room temperature in an airtight container before extraction.

Chlorophyll fluorescence measurements. Chlorophyll fluorescence was measured from dark-adapted intact leaves of the chili pepper plants exposed to different LED treatments using a Pocket PEA (Plant efficiency Analyzer, Hansatech, U.K.) 1 h after the light period (16:8 light:dark). We made eight fluorescence measurements per plant from marked leaves of five different plants under each LED treatment and fluorescent light. The data collected from the various treatments were downloaded to a computer and analyzed using the BIOLYZER program (Rodriguez, R.M. Bioenergetic Laboratory, and University of Geneva) according to (Strasser and Tsimilli-Michael, 2001) based on O-J-I-P analyses according to (Strasser and Tsimilli-Michael, 2001), which indicates the analysis of structural integrity of chloroplasts and function based on fluorescence emission data. From this we calculated initial fluorescence (F_o), maximum Fluorescence (F_m), variable fluorescence ($F_v = F_mF_o$), and photochemical efficiency of PS II (F_v/F_m). We also calculated Performance Index (PI), which indicates the physiological performance of plants under each LED treatment according to Strasser and Tsimilli-Michael (2001) (Supplementary Table 1).

Color value measurements. The surface color of chili fruit of different light treatment was measured by reflectance using a colorimeter (Model CE 310; Macbeth, Tokyo, Japan). A rectangular CIELab system (L*, a*, and b*) was measured, and chroma (C*), the cylindrical hue angle (h°ab) system, and color difference (ΔE*ab) were calculated according to Kim et al. (2002). From each fruit, a total of five readings was recorded to avoid any miscalculation.

Estimation of total carbohydrates and reducing sugar. For estimation of carbohydrate and reducing sugar, extractions were prepared from the second, fourth, and sixth weeks. A leaf sample (0.5 g) from each light treatment was washed with distilled water, ground with 5 mL of 95% ethanol, and filtered under vacuum. Furthermore, 3 mL of distilled water plus 4 mL of chloroform were added in the filtrate. After centrifugation, the upper ethanolic phase was used for analysis. The total sugars were estimated by the phenol sulfuric acid method (Dubois et al., 1956). To 100 mL of dry ethanol extract, we added 0.5 mL distilled water and 0.5 mL 5% phenol. After a brief shaking, 2.5 mL of concentrated H_2SO_4 was added and the mixture was allowed to stand for 30 min and absorbance was read at 490 nm. Pure D-glucose was used as a standard. Reducing sugars concentration was determined by the method described by Somogyi (1952). A solution of 200 mL distilled water plus 200 mL of Somogyi reagent were added to 100 mL of ethanolic extract and dried once. The mixture was boiled for 20 min and after it cooled, 200 mL of Nelson reagent plus 2.4 mL of distilled water were added. Furthermore, this mixture was shaken to eliminate CO_2 and absorbance was measured at 540 nm. The results were determined according to the standard line obtained with different concentrations of pure D-glucose as the standard.

Determination of starch. Starch was estimated by according to Sadasivam and Manickam (1992). Leaf samples of each light treatment (0.5 g) were homogenized in 5 mL of hot 80% ethanol. The homogenate was incubated in a water bath at 60 °C for 30 min to remove sugars and then centrifuged at 5000 rpm for 15 min and residue was collected. Furthermore, residue was repeatedly washed with hot 80% ethanol and the residue dissolved in 5 mL of distilled water plus 6.5 mL of perchloric acid. After extract was kept at 4 °C for 20 min, supernatant was retained after brief centrifugation. Extraction was repeated with fresh perchloric acid and supernatant was pooled and a 100-mL volume was made. A 100-mL starch extract was made to 1 mL with distilled water; to this mixture, 4 mL of fresh anthrone reagent was added and the mixture was heated for 8 min in a hot water bath. The mixture was rapidly cooled and intensity of green to dark green color was measured at 630 nm.

Determination of amino acids. For the estimation of amino acid, 0.5 g of a leaf sample was taken and ground in a small quantity of acid-washed sand according to Moore and Stein (1948). To this homogenate, 10 mL of hot 80% ethanol (60 °C) was added and centrifuged at 5000 rpm for 15 min and the supernatant was collected. This step is repeated two to three times and supernatants were pooled. To 100 µL of extract, 1 mL of ninhydrin solution was added and the final volume was made to 2 mL using distilled water and incubated for 20 min in a hot water bath (80 °C). After incubation, 5 mL of diluents (equal volumes of water and N-propanol) was added and allowed to stand for 15 min. Absorbance of purple color was recorded at 570 nm using leucine as the standard.

Estimation of total protein content. Total protein content of plant leaf was estimated according to Lowry et al. (1951). The 100-mg leaf sample was homogenized in ice-cold extraction buffer (50 mM potassium phosphate, pH 7.4, 1 mM EDTA). The extracts were centrifuged at 3000 rpm for 10 min and the resulting supernatants were used for estimation of soluble protein contents. Protein contents were assayed by Lowry et al. (1951) method with bovine serum albumin as the standard.

Estimation of chlorophyll and carotenoid content. The amount of chlorophyll was estimated from plants grown under different LEDs using the method reported by Arnon (1994). One hundred milligrams of leaf sample was ground in 10 mL of 80% acetone, mixed well, and stored at 4 °C overnight in the dark. Supernatant was withdrawn after centrifugation at 5000 rpm for 10 min. The supernatants were collected and absorbance was recorded at 663, 646, 652, 510, and 480 nm with a spectrophotometer (Shimadzu, ultraviolet 160A) and the amount of Chl a, Chl b, Chl a/b ratio, total Chl, and total carotenoids was estimated.

Chlorophyll content: (20.2 × OD 645 nm + 8.02 × OD 663 nm) × Dil. factor

Biochemical estimation from chili pepper fruits. Samples obtained from eight fruits grown under each LED treatment were ground in liquid nitrogen and the final sample made up 1 g FW. The finely powdered sample was extracted in 5 mL of 80% ethanol at 80 °C for 20 min and resultant mixture was centrifuged at 5000 rpm for 15 min. The extraction step was repeated twice. After the third exaction, all the three supernatants were combined and vacuum-dried, the resultant mixture was used for estimation of total sugars, reducing sugars, total protein, and amino acids. The final pellet was used for determining starch content in chili pepper fruits. The total sugars were estimated by the phenol sulfuric acid method according to Dubois et al. (1956), reducing sugars by Somogyi (1952), starch by Sadasivam and Manickam (1992), amino acids by Moore and Stein (1948) and proteins by Lowry et al. (1951).

Extraction of oleoresin. Oleoresin content of the fruit sample was estimated by the method of the American Spice Trade Association (ASTA, 2004). Five grams of chili pepper fruit samples from each light treatment were homogenized using a pestle and mortar to a fine powder. The resultant chili pepper powder was taken into a chromatographic column, plugged with cotton, and 50 mL of acetone was added and allowed to stand overnight. The slurry was collected in a pre-weighted beaker. This step is repeated two to three times every 2- to 3-h intervals and slurry was completely evaporated to dryness. The collected slurry was cooled and weighed. The difference in weight over the sample weight gives percent oleoresin content.

American Spice Trade Association analytical method for color estimation in capsicum. The ASTA value of chili pepper samples was evaluated according to the ASTA method. Five grams of chili pepper powder was added to 100 mL acetone and the mixture intermittently stirred and after 16 h of extraction at room temperature in the dark; absorbance of an aliquot was measured at 460 nm in comparison with a standard glass reference. The

absorbance was measured by a spectrophotometer (Shimadzu, ultraviolet 160A). The ASTA color value was calculated from the equation ASTA color value = A × 16.4 × C/w, where A is absorbance of the extract, C is the correction factor of the spectrophotometer, which was calculated by dividing the theoretical absorbance by the real absorbance of standard color solution [0.001 M K_2SO_4 and 0.09 M $(NH_4)_2$ Co (SO4)2·$6H_2O$ in 1.8 M H_2SO_4] at 460 nm, and w is the sample weight (g) on a dry basis. The absorbance of each sample solution was measured five times in two parallel trials.

Estimation of pungency level. The concentration of capsaicinoids in capsicum fruit was estimated by high-performance liquid chromatography (HPLC; Agilent 1100 Series, Palo Alto, CA). For capsaicinoid extraction, 1.5 g (FW) pepper fruit was extracted with 15 mL of acetonitrile and then placed in an 80 °C water bath for 4 h with intermittent stirring every 1 h. Then the mixture was allowed to cool and filtered through a 0.45-µm membrane filter into glass vials. Capsaicinoid content was estimated by HPLC equipped with a solvent delivery system, an autosampler, an ultraviolet detector, and a Chemstation data acquisition system. Degassed 150 isocratic mobile phase, 40% acetonitrile, and 1% acetic acid (40:60) was used at a flow rate of 1.2 mL·min^{-1} using a reverse phase ODS column C-18 (250 × 4.6 mm; Eclipse, USA) with a 5-µm particle size and with an injection volume of 20 µL. Each injection was repeated three times. Chromatograms were recorded at 280 nm with an ultraviolet detector. The ASTA (2004) method was used for the estimation of capsaicin content and pungency.

Statistical analysis. The experiment was conducted in a randomized block design. A total of three replicates for each treatment was taken. Treatment means were compared by analysis of variance using SPSS.10 (SPSS, Chicago, IL). The treatment means were separated by different letters.

Results

Growth and accumulation of metabolites as affected by light-emitting diode light sources

Morphological changes induced by light-emitting diode light. The LEDs consisting of red, blue, and red plus blue LEDs along with fluorescent light had shown a significant influence on growth and morphology of *Capsicum annuum* cv. Cheonyang plants. Different plant growth parameters like plant height, leaf area, leaf fresh, and dry weight were recorded after 2, 4, and 6 weeks of light treatment. After 6 weeks of continuous LED light treatment (16 h day and 8 h night), plant height was significantly increased under red LED (15.70 ± 0.15 cm) in comparison with fluorescent (13.40 ± 0.11 cm) and red plus blue (9.25 ± 0.20 cm) LEDs (Table 1). Maximum leaf length was recorded under red LEDs (6.69 ± 0.07 and 7.29 ± 0.06 cm, respectively) and significantly low leaf length was recorded under blue LEDs (6.01 ± 0.07 and 6.24 ± 0.13 cm, respectively) after 4 and 6 weeks of light treatment, although the maximum leaf width was recorded under red plus blue LEDs (3.92 ± 0.08 and 4.48 ± 0.04 cm) and the minimum under red LEDs (3.14 ± 0.02 and 3.22 ± 0.04 cm). Interestingly, highest leaf area was recorded under blue LEDs (27.54 ± 0.21 cm^2) followed by red plus blue LEDs (25.56 ± 0.13 cm^2), fluorescent light (20.32 ± 0.18 cm^2), and red LEDs (21.37 ± 0.19 cm^2) after 6 weeks of treatment (Table 1). However, in case of biomass, leaf fresh and dry weight varied significantly under different LEDs (after 6 weeks) and recorded a maximum in red plus blue LEDs (76.1 ± 10.44 and 63.4 ± 1.00 mg·g^{-1} FW, respectively).

Chlorophyll and carotenoid formation. The chlorophyll contents like total Chl, Chl a and b, Chl a/b ratio, and carotenoid in leaves of capsicum plants grown under different LEDs condition were shown to have significant differences (Table 2). After 6 weeks of

Table 1. Effect of red, blue, red plus blue LEDs, and fluorescent light on plant height, leaf length, leaf width, leaf area, leaf fresh weight, and leaf dry weight at different time interval.[z]

Days of treatment	Types of LED/light	Plant ht (cm)	Leaf length (cm)	Leaf width (cm)	Leaf area (cm^2)	Leaf fresh wt (mg)	Leaf dry wt (mg·g^{-1} FW)
15 DAT	Fluorescent	6.21 ± 0.04 b	4.56 ± 0.10 c	2.67 ± 0.02 c	12.17 ± 0.09 c	472 ± 16.17 b	37.7 ± 0.55 b
	Red	6.33 ± 0.05 b	5.12 ± 0.10 b	2.84 ± 0.02 b	14.54 ± 0.13 bc	354 ± 19.63 c	29.5 ± 0.76 c
	Blue	5.97 ± 0.07 c	5.34 ± 0.10 b	2.91 ± 0.05 b	17.34 ± 0.13 a	474 ± 10.08 b	39.5 ± 0.69 b
	RB	6.98 ± 0.06 a	5.56 ± 0.10 a	3.21 ± 0.02 a	15.53 ± 0.12 b	587 ± 12.25 a	46.9 ± 1.17 a
30 DAT	Fluorescent	9.16 ± 0.09 b	6.26 ± 0.06 b	3.24 ± 0.03 c	20.32 ± 0.10 c	570 ± 12.34 b	47.5 ± 0.46 c
	Red	10.85 ± 0.05 a	6.69 ± 0.07 a	3.14 ± 0.02 c	20.66 ± 0.09 c	557 ± 11.55 c	46.8 ± 0.55 c
	Blue	9.475 ± 0.10 b	6.01 ± 0.07 b	3.84 ± 0.05 b	25.03 ± 0.25 a	589 ± 11.05 b	49.7 ± 0.46 b
	RB	8.825 ± 0.09 c	6.25 ± 0.07 b	3.92 ± 0.08 a	24.75 ± 0.13 b	667 ± 19.95 a	56.5 ± 0.75 a
60 DAT	Fluorescent	13.40 ± 0.11 b	6.46 ± 0.14 b	3.44 ± 0.06 c	20.32 ± 0.18 c	657 ± 10.08 c	54.5 ± 0.47 c
	Red	15.70 ± 0.15 a	7.29 ± 0.06 a	3.22 ± 0.04 c	21.37 ± 0.19 c	648 ± 11.82 c	52.6 ± 0.58 c
	Blue	14.90 ± 0.11 b	6.24 ± 0.13 b	4.10 ± 0.07 ab	27.54 ± 0.21 a	683 ± 9.68 b	56.9 ± 0.58 b
	RB	9.25 ± 0.20 c	6.30 ± 0.08 b	4.48 ± 0.04 a	25.56 ± 0.13 b	761 ± 10.44 a	63.4 ± 1.00 a

[z]Values represent mean ± SE (n = 5) followed by different letters within a column are significantly different at the 1% level by Duncan's multiple range test.
LED = light-emitting diode; FW = fresh weight; DAT = days of treatment; RB, red and blue.

Table 2. Effect of red, blue, red plus blue, and white light on total chlorophyll, chlorophyll a, b, a/b, and carotenoids at different time intervals.[z]

		Chlorophyll (mg·g^{-1} FW)				
Days of treatment	Type of LED/light	a	b	Total	Chlorophyll (a/b ratio)	Carotenoids (mg·g^{-1} FW)
15 DAT	Fluorescent	4.36 ± 0.03 a	3.30 ± 0.04 a	7.66 ± 0.02 b	1.32 ± 0.02 bc	1.14 ± 0.02 c
	Red	3.78 ± 0.11 c	2.82 ± 0.06 b	6.6 ± 0.03 a	1.34 ± 0.01 b	1.28 ± 0.03 b
	Blue	4.02 ± 0.04 b	3.07 ± 0.04 ab	7.09 ± 0.02 a	1.30 ± 0.02 b	1.85 ± 0.04 a
	RB	4.24 ± 0.12 a	2.99 ± 0.06 b	7.23 ± 0.03 b	1.41 ± 0.02 a	1.21 ± 0.03 b
30 DAT	Fluorescent	13.88 ± 0.04 c	9.45 ± 0.03 b	23.33 ± 0.04 b	1.47 ± 0.03 bc	4.26 ± 0.05 c
	Red	11.89 ± 0.06 c	10.03 ± 0.05 a	21.92 ± 0.06 c	1.18 ± 0.03 c	4.14 ± 0.08 c
	Blue	16.11 ± 0.07 a	9.41 ± 0.07 b	25.52 ± 0.06 a	1.71 ± 0.03 a	4.78 ± 0.10 b
	RB	15.23 ± 0.08 b	10.16 ± 0.05 a	25.39 ± 0.07 b	1.49 ± 0.04 b	5.15 ± 0.09 a
45 DAT	Fluorescent	17.82 ± 0.07 b	11.52 ± 0.07 b	29.34 ± 0.08 c	1.54 ± 0.02 b	8.87 ± 0.10 c
	Red	15.01 ± 0.07 c	12.29 ± 0.03 a	27.3 ± 0.08 b	1.22 ± 0.03 c	8.74 ± 0.13 c
	Blue	21.91 ± 0.07 a	11.21 ± 0.06 b	33.12 ± 0.06 a	1.95 ± 0.06 a	9.48 ± 0.17 b
	RB	18.83 ± 0.05 b	10.66 ± 0.05 c	29.49 ± 0.08 c	1.76 ± 0.03 b	10.15 ± 0.01 a

[z]Values represent mean ± SE (n = 5) followed by different letters within a column are significantly different at the 1% level, by Duncan's multiple range test.
LED = light-emitting diode; FW = fresh weight; DAT = days of treatment; RB, red and blue.

LED treatment, the chlorophyll contents like Chl a, total chlorophyll, and chlorophyll a/b ratio estimated significantly high under blue LED (21.91 ± 0.07, 33.12 ± 0.06, and 1.95 ± 0.06 mg·g^{-1} FW, respectively) followed by red plus blue light (18.83 ± 0.05, 29.49 ± 0.08, and 1.76 ± 0.03 mg·g^{-1} FW, respectively). However, there was no significant change in the levels of major chlorophyll content under red LED except Chl b (12.29 ± 0.03 mg·g^{-1} FW), which recorded maximum compared with blue and red plus blue LED (11.21 ± 0.06 and 10.66 ± 0.05 mg·g^{-1} FW, respectively). In case of other carotenoids, maximum amounts of carotenoids were estimated in red plus blue LED (10.15 ± 0.01 mg·g^{-1} FW) and the minimum was observed in red light (8.74 ± 0.13 mg·g^{-1} FW) (Table 2).

Chlorophyll fluorescence measurements. To evaluate the photosynthetic performance, we measured F_v/F_m values, which indicate the maximum quantum yield of primary photochemistry or maximal relative electron transport rate of PS I, and theses values are also correlated to quantum yield of net photosynthesis. We found that F_v/F_m values were found to be highest in plants grown under red plus blue LED (0.93 ± 0.03) followed by blue LED (0.91 ± 0.05) and notably least was recorded from plants under fluorescent light(0.78 ± 0.02) compared with red (0.82 ± 0.01). PI values were also recorded as being highest for plants grown under a combination of red plus blue LED (30.45 ± 3.34) followed by blue LED (30.12 ± 3.33), whereas these values were recorded least in red (24.78 ± 2.89) and fluorescent light (22.56 ± 2.43) (Supplementary Table 1).

Total amino acids and proteins, reducing sugar, and starch contents. After 2 weeks of LED treatment, the reducing sugar and starch content was found maximum in plants grown under red plus blue (0.692 ± 0.012 mg·g^{-1} FW; 10.70 ± 0.288 mg·g^{-1} FW) LED. However, after 4 weeks, reducing sugar increased significantly in plants grown under red plus blue LEDs (5.27 ± 0.22 mg·g^{-1} FW), but starch concentration was increased slightly (12.05 ± 0.13 mg·g^{-1} FW). Similarly, after 6 weeks of LED treatment, the maximum amount of the reducing sugar and starch was observed in red plus blue light (8.27 ± 0.12 and 14.05 ± 0.18 mg·g^{-1} FW, respectively). The total carbohydrate content was also found to be maximum in plants grown under red plus blue light in comparison with other LED lights (Fig. 1). Similarly, highest levels of total proteins were recorded in plants of red plus blue (4.83 ± 0.08 mg·g^{-1} FW) after 6 weeks. However, the maximum amount of total amino acids (2.93 ± 0.07 mg·g^{-1} FW) was recorded in blue and closely followed by red plus blue LED-grown plants (2.15 ± 0.05 mg·g^{-1} FW; Fig. 1).

Fruit yield, pungency, and fruit color affected by led light source

Fruit yield. The chili plants varied significantly in major phenological characters such as fruit number, fruit size, fruit yield, and its quality grown under monochromatic and a combination of LEDs. We harvested fruits of the same age group from the plants under each light condition after 45 d of post-anthesis. Chili plants grown under a combination of red plus blue LED showed outstanding performance in terms of total yield (23.3 ± 0.12) and fruit number (35) in comparison with other LEDs and fluorescence light (Table 3). However, no significant differences in fruit length and fruit diameter were observed among monochromatic red/blue and a mixture of red plus blue LEDs.

Biochemical estimation of chili pepper fruits. The amount of total sugars was significantly higher in plants grown under red plus blue LED (23.35 ± 0.23 mg·g^{-1} FW) followed by blue (22.25 ± 0.21 mg·g^{-1} FW), while significantly low was recorded under red (20.25 ± 0.25 mg·g^{-1} FW) and fluorescent light (19.37 ± 0.38 mg·g^{-1} FW). Similarly, plants grown under red plus blue accumulated a higher amount of starch and reducing sugars (12.74 ± 0.18 and 3.54 ± 0.153 mg·g^{-1} FW, respectively) compared with other LEDs or fluorescent light (Fig. 2). Again, fruits harvested from red plus blue LED showed

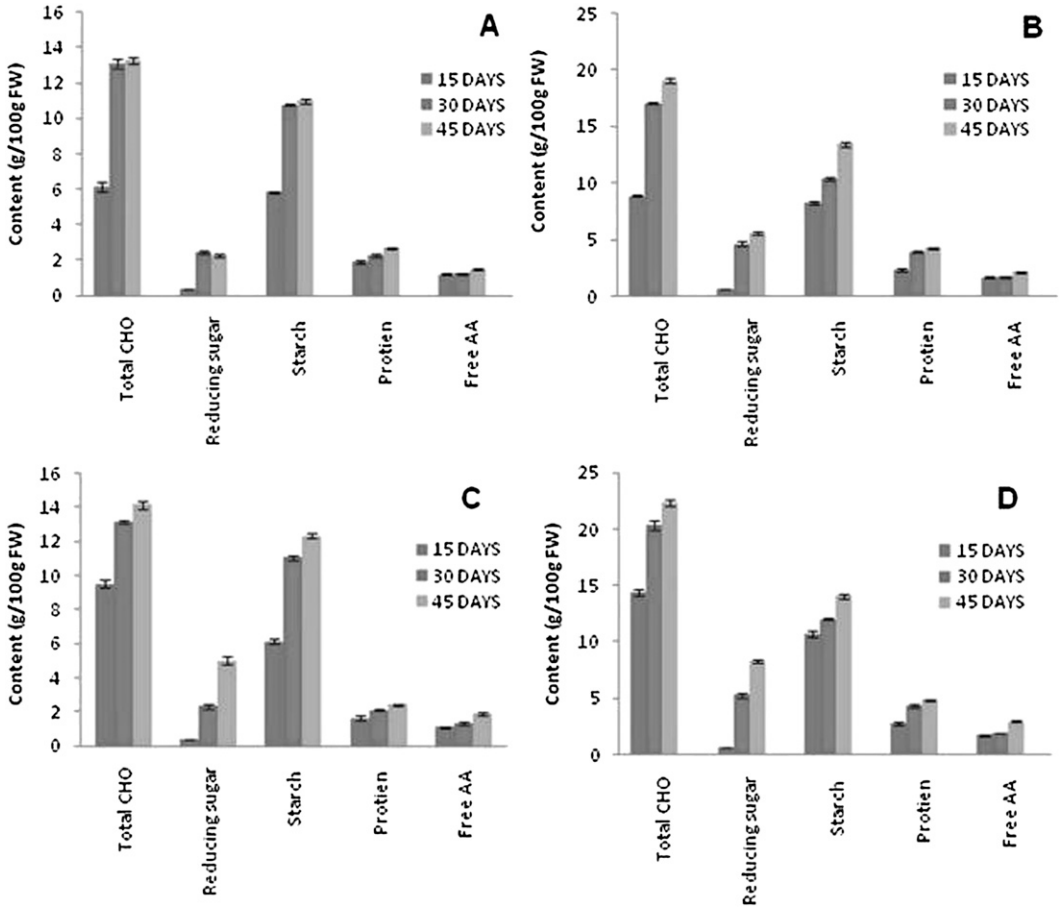

Fig. 1. Content of total carbohydrates, reducing sugar, total protein and total amino acids in capsicum under different LED/light. (**A**) Fluorescent light; (**B**) blue LED; (**C**) red LED; (**D**) red plus blue LED. LED = light-emitting diode.

Table 3. Effect of the different LED treatment on total marketable fruit yield and fruit quality of pepper.[z]

Type of LED/light	Total no. of plants in each LED	Total no. of fruits in all plants	Avg no. of fruits per plant	Fruit diam (cm)	Fruit length (cm)	Avg yield of fruits per plant (g)
Fluorescent	9	30	3.3 ± 0.03 b	1.4 ± 0.01 b	4.9 ± 0.03 c	20.5 ± 0.59 b
Red	9	27	3 ± 0.02 b	1.6 ± 0.04 a	5.6 ± 0.04 ab	17.2 ± 0.64 bc
Blue	9	19	2.1 ± 0.02 c	1.2 ± 0.02 c	5.8 ± 0.03 a	10 ± 0.06 c
Red plus blue	9	35	3.8 ± 0.01 a	1.6 ± 0.01 a	5.5 ± 0.02 b	23.3 ± 0.12 a

[z]Values represent mean ± SE (n = 9) followed by different letters within a column are significantly different at the 1% level by Duncan's multiple range test. LED = light-emitting diode.

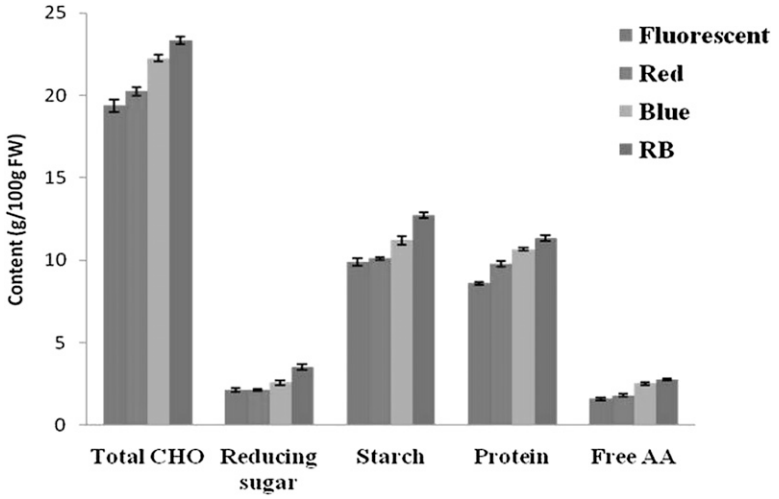

Fig. 2. Biochemical constituents of chili pepper fruits grown under fluorescent light, blue LED, red LED, red plus blue LED. LED = light-emitting diode.

maximum protein and amino acids (11.32 ± 0.17 and 2.77 ± 0.05 mg·g^{-1} FW, respectively) followed by blue LED (10.65 ± 0.08 and 2.52 ± 0.07 mg·g^{-1} FW, respectively; Fig. 2).

Capsaicinoid analysis. Important factors to be considered for the market value chili peppers are pungency and color. For the estimation of capsaicinoid contents under different light condition, acetonitrile extract of the same age group of chili fruits was prepared. The capsaicinoid content was analyzed by HPLC; interestingly, we observed prominent changes of capsaicinoid levels in fruits developed under different LEDs. The capsaicinoid content of fruits (180 ± 6.32 mg/100 g) developed under blue LEDs was much higher in comparison with fruits from under fluorescent light (54 ± 3.12 mg/100 g). Interestingly, in red light, the capsaicinoid concentrations (26 ± 3.43 mg/100 g) were two times lower in comparison with fluorescent light. The fruits from red plus blue LEDs (90 ± 4.86 mg/100 g) recorded substantial levels of capsaicinoids in comparison with fluorescent light (Table 4).

Extractable and surface color analysis. After pungency, the second most important character for chili peppers is color. Color of chili peppers can be measured either as extractable red color or surface color. Among the fruits harvested from different LEDs, ASTA color values of fruits grown under red plus blue LEDs recorded the maximum (113.16 ± 0.76), whereas fruits from blue and red LEDs were on par with 87.24 ± 0.92 and 86.92 ± 0.94 ASTA units, respectively (Table 4). In the present study, ASTA units showed a significant difference among the fruits grown under different LEDs. In case of surface color estimation of chili fruit, redness (a*) and yellowness (b*) were found to significantly increase in fruits from blue (31.10 ± 0.2, 10.49 ± 0.06) and red plus blue LEDs (30.79 ± 0.34, 10.28 ± 0.06) in comparison with red LEDs (25.08 ± 0.42b, 8.14 ± 0.03b) and fluorescent light (21.81 ± 0.39, 6.99 ± 0.05). The C* value of fruits harvested from blue and red plus blue were similar (32.82 ± 0.68, 32.45 ± 0.57), but it varied significantly in relation to red LEDs (26.30 ± 0.27) and fluorescent light (22.89 ± 0.59). Δ E*ab values were increased significantly under red plus blue (11.11 ± 0.05) and blue (10.13 ± 0.08) LEDs, whereas there was a substantial change under red LEDs (3.88 ± 0.06c). On the other hand, increase in h*ab values under red plus blue (18.41 ± 0.18) and blue LEDs (18.62 ± 0.11) reflects that color stability varied significantly from other light treatments (Table 4). Similar to color values, oleoresin content was significantly elevated under red plus blue LEDs (46.2%) and blue LEDs (38.1%); intriguingly, no significant variation of oleoresin content was observed under red and fluorescent lights. These results suggest that development of better pigment of chili pepper can be achieved under red plus blue LEDs.

Discussion

Light quality regulates plant growth and development through various photoreceptors, which stimulate signal transduction systems by various mechanisms to change the plant morphology (Ward et al., 2005). The present study demonstrates that plants grown under red plus blue LED show a significant superiority in plant growth. Although monochromatic blue LEDs and fluorescent lights were significantly similar in promoting plant growth, these similar effects of LEDs on capsicum plants in this study have also been observed in other plants such as *Triticum aestivum* and *Cucumis sativus* (Goins et al., 1997; Wang et al., 2009). Furthermore, plant height and leaf length were observed maximum under red LED treatment compared with other treatment and is in close agreement with previous reports (Poudel et al., 2008). Leaf area was observed maximum under blue LED treatment indicating that blue LED light is beneficial for better leaf development of chili peppers. These results are consistent with Saebo et al. (1995), who showed that blue light enhances the leaf area of birch. In case of leaf biomass, leaf fresh and dry weight was increased in plants grown under red plus a blue combination of LEDs (Table 1) after 45 d of plant growth, whereas these values were comparatively low in the plants grown under other treatments. Our investigations revealed that fruit yield characteristics such as fruit length, size, number, and yield were highest in plants grown under red plus blue LED. This may be the result of the maximum photosynthetic efficiency of plants grown under red and blue LED, because the spectral energy distribution of red and blue light coincided with that of chlorophyll absorption (Lin and Jolliffe, 1996).

Light radiation from different LEDs on chili pepper greatly enhanced the expression of chlorophyll and significantly differed from the control plants under fluorescent light (Table 2). The present results indicated that blue LED light is beneficial for total Chl and Chla accumulation, whereas monochromatic red LED is responsible for Chlb accumulation. This might be the resultof positive and synchronized influence of blue light on both nuclear and plastid genomes and could have played a vital role in the formation of chlorophyll and chloroplast development (Akoyunoglou and Anni, 1984; Pushnik et al., 1987). These results seemed to be close to previous reports on spinach and lettuce (Tibbitts et al., 1983). However, the other important photosynthetic pigment carotenoid also helps Chl to receive light energy (Zheng et al., 2008). High carotenoid content recorded in plants cultivated under red plus blue LEDs was similar to previous reports (Tanaka et al.,

Table 4. Extractable and surface color values, oleoresin, and pungency of pepper fruit (*Capsicum annuum*, L. cv. Cheonyang) under different LED treatments.[z]

Type of LED/light	Extractable colors (ASTA)	L	C	h	a	b	ΔE*ab	Oleoresin (%)	Total capsaicinoids (mg/100 g)
Fluorescent	86.92 ± 0.94 b	25.95 ± 0.65 b	22.89 ± 0.59 b	17.77 ± 0.63 b	21.81 ± 0.39 b	6.99 ± 0.05 bc	—	36.80 ± 0.28 ab	54 ± 3.12 c
Red	78.72 ± 0.87 b	27.71 ± 1.32 b	26.30 ± 0.27 b	17.95 ± 0.06 b	25.08 ± 0.42 b	8.14 ± 0.03 b	3.88 ± 0.06 c	35.21 ± 0.25 b	26 ± 3.43 c
Blue	87.24 ± 0.92 b	28.01 ± 0.63 b	32.82 ± 0.68 a	18.62 ± 0.11 a	31.10 ± 0.2 ab	10.49 ± 0.06 a	10.13 ± 0.08 b	38.14±.22 ab	180 ± 6.32 a
Red plus blue	113.16 ± 0.76 a	31.61 ± 0.91 a	32.45 ± 0.57 a	18.41±.18 a	30.79 ± 0.34 a	10.28 ± 0.06 a	11.11 ± 0.05 a	46.28 ± 0.26 a	90 ± 4.86 b

[z]Values represent mean ± SE (n = 5) followed by different letters within a column are significantly different at the 1% level by Duncan's multiple range test.
LED = light-emitting diode.

1998; Tibbitts et al., 1983). We also noted clear differences in the accumulation of chlorophyll in chili pepper growing under different light treatments (Fig. 1) and conclude that blue LEDs might be essential for chlorophyll synthesis in chili pepper. To understand the growth performance and to choose the best LED suitable for better yield performance in chili pepper under each light treatment, chlorophyll content and chlorophyll fluorescence were measured. Chlorophyll fluorescence parameters such as F_v/F_m and PI values indicated red plus blue LED is essential for maximizing plant photosynthetic performance, growth, and eventually their yield performance of chili pepper (Supplementary Table 1).

Light quality also regulates carbohydrate metabolism of plants and thereby affects growth of plants (Kowallik, 1987). In the present study, we investigated the effect of spectral quality of different LEDs on primary metabolites. We observed a significant accumulation of total carbohydrates, reducing sugar, and starch under red plus blue LEDs. Previously, it was reported that red light could promote accumulation of photosynthetic products of plants; however, supplemental red light with blue light was more effective to the accumulation of these compounds (Zheng et al., 2008). Other primary metabolites like total protein and amino acid also estimated from leaves of capsicum plants grown under the different LEDs showed varied results. In our study, we found the maximum amount of protein was estimated under red plus blue LEDs. However, maximum amino acid was observed under blue LEDs. Protein and free amino acid production are regulated by various abiotic and biotic environmental factors and depend on how these factors affect photosynthesis and growth (Estrada et al., 1999). Under stress conditions, the free amino acids will be high to prevent cellular oxidative damage (Moyer et al., 2002). These results further demonstrate that nutritional quality of plants could be varied by selecting special light sources under controlled growth environments.

To further test our hypothesis concerning modulation of color and pungency of the capsicum fruits by LEDs, we measured various color characteristics of capsicum fruit. Previously it was reported in chili pepper that fruit color depends on the carotenoid content, because it is a primary component, which dictates the quality of capsicum fruit. The amount of carotenoids in fruit tissue depends on factors such as cultivar, maturity stage, and growing conditions (Reeves, 1987). Based on extractable and surface color measurements, red plus blue LED was found to be most effective to improve color development in chili pepper fruits. The extractable measurement of fruits color under red plus blue LEDs (113.16 ± 0.76) is in close agreement with that of Korean red pepper (ASTA color value of 107 to 114) (Rhim and Seok, 2011). However, ASTA color value does not provide the L*, a*, and b* (CIELab), which are an essential parameter for assessing surface color of fruit pepper (Kim et al., 2002). Hence, we measured visual light (L*) and chroma (C*) that indicates saturation of color and hue angle (h°ab) that represents a relative increase of yellowness and color differences between two samples (ΔEab*). In the present studies, redness (a*) and yellowness (b*) and C* value were recorded maximum in blue and red plus blue LEDs, but it varied significantly in relation to red LEDs. The mentioned results indicating redness of fruits (a*) and C* values point out vivid color development of chili pepper under red plus blue LEDs. Δ E*ab values were significantly increased under red plus blue LEDs and blue LEDs, further indicating color change was prominent with supplementation of blue with red LEDs in comparison with monochromatic red LEDs and fluorescent light. However, it can be predicted that vivid color development under red plus blue LED is the result of high photostability of carotenoids (especially capsanthin and capsorubin) in comparison with other light treatments. Similar to our results, previously it was reported in cucumber that high light intensity, under low light conditions. and high red/far red ratio can improve fruit color (Lin and Jolliffe, 1996). Similar to surface and extractable color values, oleoresin content was significantly elevated under red plus blue LEDs and blue LEDs; intriguingly, no significant variation of oleoresin content was observed under red and fluorescent lights. This finding confirms that fruit color of chili pepper could differ according to environmental factors like LEDs.

The pungency of capsicum depends on capsaicinoid compounds, primarily capsaicin (8-methyl-N-vanillyl-trans-6-nonenamide) and dihydrocapsaicin, which are the major pungent principals of chili pepper. However, capsaicin content determines commercial quality of chili pepper fruit because of its major presence in capsaicinoids. The capsaicin level in chili pepper fruits is determined by two factors: genotype of plant and environment interactions (Iwai et al., 1979). The accumulation of capsaicin in a chili pepper fruit also depends on the intensity of light, temperature, and age of the fruit. Previously it was reported in chili pepper fruit that different light intensity (40%, 60%, 80% and natural light) has a significant effect on capsaicin concentration (Song et al., 2008). However, the effect of a monochromatic and combination of LED sources on capsaicinoid concentration has not yet been reported. In the present study, we investigated whether different spectral quality affects accumulation of major capsaicinoids in capsicum fruit. Interestingly, chromatographic analysis of chili pepper fruit extract showed highest concentration of capsaicinoids from monochromatic blue LED-treated fruit after 45 d of post-anthesis. However, the low amounts of capsaicinoids were recorded under red LED-treated fruits. In addition, the combination of red and blue LEDs' effect on capsaicinoids reported relatively similar to florescence light. These results are closely related to previous studies in *Brassica oleracea*, in which it was reported

that LED can determine variation in the accumulation of glucosinolates and maximum accumulation of glucosinolates occurred at the high wavelength (Lefsrud et al., 2008). Similar to our result, it was reported previously that light illumination could affect accumulation of secondary metabolites (Endress, 1994). Although very few studies are reported about the stimulatory effect of LED light on production of secondary metabolites, several reports have been published in which the influence of light on primary and secondary metabolites was demonstrated in plants like *Zingiber officinale* (Anasori and Asghari, 2008) and *Artemisia annua* (Liu et al., 2002).

Conclusions

LEDs used in the present study were proven to be a promising light source in comparison with the reported artificial sources. In the current study, chili pepper plants exhibited a strong morphological plasticity with special reference to leaf architecture under LED lights. Our results suggests that red LED is useful in regulating plant height and leaf length, whereas blue LED is effective in increasing leaf area and a combination of red plus blue LED might be effective in modulating leaf width and fresh/dry weight of chili pepper. In addition, red plus blue LED is necessary for a substantial increase of total carbohydrates, reducing sugars, and starch, whereas blue LED is important for chlorophyll synthesis in chili pepper. Furthermore, we measured the effect of LEDs on capsaicinoid synthesis and found that capsaicinoid concentration was induced under blue LEDs compared with other light treatments. On the other hand, LEDs has produced a profound effect on parameters of fruit color. Among the different light treatments, red plus blue LEDs remarkably increased color values such as extractable colors and surface color. Additionally, we observed no relation between oleoresin and pungency, whereas variation in oleoresin content was directly correlated to color content of chili peppers. Our results demonstrated that the blue and red plus blue LEDs were certainly more effective than a conventional light source in altering the accumulation of capsaicinoids and carotenoids and maintaining fruit color stability. Therefore, LED lights can be practically used to modulate the accumulation of various bioactive compounds present in chili pepper. However, future research on genetic and molecular analysis of light-regulated alteration in biosynthetic pathways will provide better insight for nutritional improvement of chili pepper.

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