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LED SUPPLEMENTATION MODULATES MORPHO-PHYSIOLOGICAL FRAMEWORK, FLOWERING ASPECTS AND GIBBERELLIC ACID LEVELS IN *CHRYSANTHEMUM MORIFOLIUM* CV. ZEMBLA

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ABSTRACT

A set of experiments was conducted at CPCT, IARI New Delhi to assess the impact of LED lights on morphological and physiological attributes in *Chrysanthemum morifolium* cv. Zembla, as well as their effect on gibberellic acid levels. Different LED treatments, i.e. red (100% R), blue (100% B), red+ blue (80%R :20%B), white (100% W) and ambient natural light source (under greenhouse) and fluorescent light (FL) as control were used as treatments. The flower buds exposed under photo-synthetically active radiation (PAR) were subjected to HPLC to analyze the levels of gibberellic acid hormone. Morphological and physiological parameters were augmented with supplemental dichromatic 80%R 20%B light. Fresh and dry weight of leaf and stem were observed highest in the plants raised under 80%R 20%B. Physiological parameters, viz., chlorophyll content, NPR, stomatal conductance, NRR, NAR, RGR were observed elevated under 80%R 20%B LEDs. Bud diameter, flower diameter, flower weight was observed highest in 80%R 20%B LEDs, while these parameters were recorded minimum in the plants raised under blue (100% B) LEDs. Earliest bud induction (66.6 days) was observed in plants exposed with a mixture of 80%R 20%B LEDs lights, while it got delayed (102.6 days) in Blue (100% B) LEDs. Gibberellic acid levels were recorded to be highest in plants under 80%R 20%B LEDs and minimum in Blue (100%) LEDs.

Key words : LED, Chrysanthemum, Bud induction, GA_3.

Introduction

Chrysanthemum morifolium Ramat. (florists' daisy) is a dicotyledonous, perennial sub-shrub, which belongs to the family Asteraceae, can be grown in open fields as well as in the protected structures for loose and cut flowers respectively. An obligate short day plant, flower induction takes place only when short days (SD) commence, otherwise remain vegetative under long days (LD). As the source of energy for plant life, light is one of the most crucial environmental variables for plants. By causing modifications that change the photoreceptors' cellular location, light can modify the activity of photoreceptors. Photoperiod is a major determinant in the seasonal control of flowering in most of the short-day plants (Osnato *et al*., 2022). Phytochromes (one of the major photoreceptors) are initially generated in the inactive Pr form, which is converted to the physiologically active Pfr form upon light absorption (Deng *et al*., 2007). Plants respond to light signals by growing and developing through a process called photomorphogenesis. A complicated network of photoreceptors, including phytochromes, mediates this process (Han *et al*., 2007). A soluble coloured protein called phytochrome can exist in two spectrally different, photointerconvertible forms: Pr, which absorbs red light, and Pfr, which absorbs far-red light. The phytochrome's "Pr" form has an absorption maximum that is similar to that of chlorophylls (red light), while the "Pfr" form has a longer wavelength (far-red light) (Mathews, 2010). Chrysanthemum is climatically adapted crop to express a strong diurnal response, as it

attains a minimum vegetative growth before being generative. Hence, it has two different phases that can be distinguished from one another. First, a period of long day (day length more than 12 h) is maintained so that the plants grow vegetatively. Depending on the season, this period may last between 10–25 days (Carvalho, 2003). The formation of flowers is then induced by growing plants under short-day (day length less than 12 h).

Production of quality chrysanthemums involves growth in greenhouses where climate and plant density play a major role in determining the flowering period. Stem elongation is of primary importance for cut flowers, since it ultimately determines the overall grade of flowers and the final price. In view of environmental concerns, use of growth retardants has to be replaced with other alternatives for regulating plant height.

Supplemental lighting in form of LEDs is a recent trend due to availability of a suitable light spectrum (quality and duration) and have been used as photoperiodic, supplemental or photomorphogenic lighting system for numerous plant species (Kozai, 2016). LEDs have been observed to have a higher photosynthetic rate than the monochromatic light and R and FR combinations have led to an increased stem length in chrysanthemum (Kim *et al*., 2004). In a study conducted with various treatments of R:FR LED lights in chrysanthemum, chlorophyll *a* content, SPAD value, net photosynthetic rate CO_2-saturated carboxylation rate were the highest for R:FR ratio of 2.5 (Yang *et al*., 2013), that might be due to increase in calvin benson cycle (Wang *et al*., 2009). Smart LED combinations of 80% Red and 20% Blue @ 100 ì mol m^{-2} s^{-1} in chrysanthemum cv. Zembla has been observed to produce flowers even under LD conditions upto 61 days (Singh *et al*., 2013), because blue LED exposure is equivalent to the dark phase or has a weak long day signalling (Lopez *et al*., 2020). Some studies suggest that blue light inhibits flowering in chrysanthemum, e.g. when given as night break, inhibits flowering in certain short day plants (Park and Jeong, 2020) but the inhibition efficiency is dependent on the duration of lighting, *i.e*. shortened illumination time for blue light was ineffective in preventing flowering (Nissim-Levi *et al*., 2019). Flowering in chrysanthemum can also occur with long day conditions, under exposure to dichromatic red and blue LEDs, alongwith a supplemental 4- hour blue LED treatment (SharathKumar *et al*., 2021). An increase in stem length was also observed as a result of blue light mediated response (Ying *et al*., 2020).

The analysis of endogenous hormones showed that Gibberellic Acid (GA_3) had an important role in flowering. It has been suggested that GA_3 is required for flowering of a short-day chrysanthemum plant (Sumitomo *et al*., 2009). An appropriate amount of IAA appears to be necessary for inflorescence differentiation, and a stable GA_3 and ABA level for crown formation (Jiang *et al*., 2010). Internodal elongation under blue light has been observed under the influence of sole blue light due to increase in active gibberelins (Fukuda *et al*., 2016). Quality cut flower production still poses a major challenge for Indian growers in commercial market due to longer winter days and shorter summer days. Hence, it can be concluded that there is a need to observe effect of PAR light and photoperiod on the floral and vegetative characteristics and to clearly understand the physiology of hormonal mechanism involved in chrysanthemum in order to develop a working model for achieving continued production in terms of flower quality and stem length. 'Zembla' variety (Standard type) was considered for this study to regulate the growth cycle of commercial cut chrysanthemum using smart LEDs and elaborating role of gibberellic acid in flower induction for stem and flower quality parameters.

Materials and Methods

Planting material and pre-cultivation

Terminal cuttings (5-6 cm length) were obtained from the mother plants of the *Chrysanthemum* cultivar, i.e., Zembla during the months of July-August. These cuttings were planted in the plug pro-trays containing a soil less media composed of coco-peat, perlite and vermiculite in a ratio of 3:1:1. The rooted plants were transplanted after 30 days into pots of 10 cm diameter with developed roots and 5-7 pair of leaves. The UV stabilized, plastic pots were filled with soil less media, with the same ratio of coco-peat, perlite and vermiculite. 150 uniform sized plants were selected for transplanting into the growing media filled-in pots, and thereafter kept for healing for 30 days under semi-automated greenhouse conditions.

Transplanting and pre-treatment of pots

The plastic pots were subjected to sterilization using 0.25% phosphoric acid by immersing them in a bucket of treated water for 3 days. Then the prepared media was lightly filled into the pots followed by a light irrigation using a rose can. Transplanting was done during evening at 7.3 cm depth and placed under climate-controlled greenhouse. 19:19:19 NPK at 2g/*l* and micronutrients (2.5g/*l* multiplex solution) were provided through fertigation via foliar spray at 2-3 days interval. The transplanted potted plants were kept for healing in the greenhouse for 30 days, before starting the exposure towards photoperiodic and long day treatments using smart LED lights.

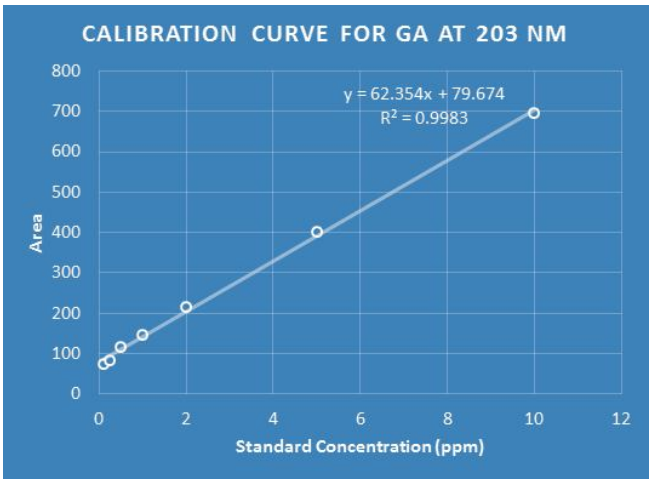

Fig. 1 : Linear equation derived between the Standard GA concentrations and area obtained via calibration curves using HPLC-MS.

Stages of observation

After the completion of LED treatments, potted plants were placed on growth benches in the greenhouse. Fertigation was provided with 2g/l of 19:19:19 NPK on a daily basis. Adequate plant protection measures were applied throughout the growth phase of plants. Staking was performed by using bamboo sticks to prevent lodging off. Observations on plant growth, morphology and physiological parameters were taken at different time intervals. At the time of flower bud initiation and flower development, flowering observations were taken and recorded. Observations on gibberellic acid levels were recorded after the completion of growth phase in the respective laboratories.

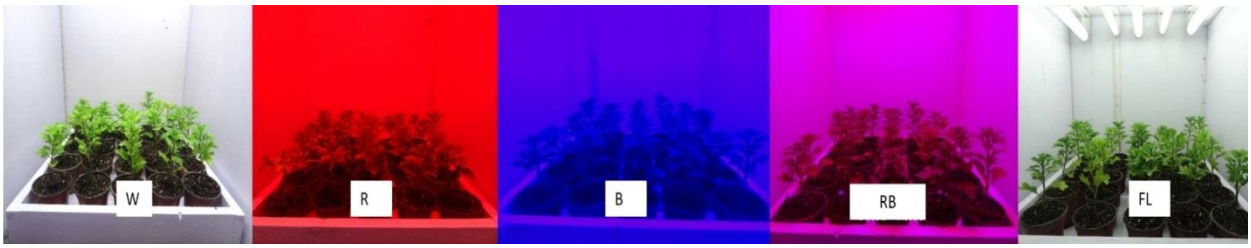

Fig. 2 : Chrysanthemum cv 'Zembla' plants kept under individual light treatments in growth chambers. **FL** : Fluorescent light, equivalent to natural daylight, **RB**: 80% Red mixed 20% Blue light, **B**: 100% Blue light, **R**: 100% Red light, **W**: 100% White light.

Plants were kept under different LED lights at different light intensities in the growth chambers, on October, 2020. The LED lights used were Red (100%), Blue (100%), White (100%), RB (80% Red + 20% Blue) and Fluorescent (Control). The five different LED panel dimensions were 1.0m × 1.0m area each, to avoid any kind of light interference with each other. In addition to this, a light dimming function with four different levels was also provided using a regulator. After determining the light intensities at different levels, using the quantum light sensor, plants were placed under artificial long day conditions of 15 h photoperiod and 120 ± 5 µmol m^{-2} s^{-1} LED irradiance of different treatments of light along with control i.e., 11 h under fluorescent light and 110 µmol m^{-2} s^{-1} irradiance (Fig. 2). The light intensities were reduced with increase in plant height, through the dimming function. Light intensity was measured on a weekly basis and pots were re shuffled at every 4-5 days for uniform distribution of light to each plant. Fertigation was done manually as and when required at every 4-5 days. Constant room temperature and humidity were maintained and checked using sensors. After completion of the light exposure treatments, plants were transferred to the greenhouse and observations were recorded.

Morphological observations

Leaf fresh and dry weight (g)

Five plants were randomly selected from each replicated treatment and a pan balance of 0.01g accuracy was used to measure fresh weight of leaves. After taking the fresh weight, leaves were dried in hot oven at 60^0C for 24 h and a pan balance of 0.01g accuracy was used to measure dry weight of leaves. The mean values of these leaf weights were ascertained and recorded.

Stem fresh and dry weight (g)

Five plants were randomly selected from each replicated treatment and a pan balance of 0.01g accuracy was used to measure fresh weight of stem. After measuring the fresh weight, stems were dried in hot oven at 60^0C for 24 h and a pan balance of 0.01g accuracy was used to measure dry weight of stem. The mean values of these stem weights were ascertained and recorded.

Flower weight (g)

Weight of the fresh fully opened flowers was recorded without stem from five randomly selected plants under each replicated treatment with a pan balance of 0.01g accuracy.

Time taken for bud induction (days)

It is counted as the number of days taken from placing plants in the growth chamber to visual initiation of flower bud. Five plants were randomly selected from each treatment and bud initiation time is recorded in days. Then the mean value was calculated and recorded.

Bud diameter (cm)

Bud diameter was measured at the fully turgid stage of bud when it has attained the full size before the opening of florets. Five plants were randomly selected from each treatment and measurement is taken at 60 days s using vernier calliper. The mean value was calculated and recorded.

Time taken for flower opening (day)

It is counted as time taken from placing plants in the growth chamber to the full bloom stage was recorded from five randomly selected plants from each replicated treatment. The mean values were calculated and recorded.

Flower diameter (cm)

The diameter of flower was measured using vernier callipers at fully opened stage from five randomly selected plants from each treatment. Mean value of flower diameter was calculated and recorded.

Physiological Observations

Relative growth rate (g g^{-1} day^{-1})

Dry weight of whole plants above ground level was measured at 0, 15th, 20th and 45th days after planting from randomly selected five plants from each replicated treatment. Relative growth rate is calculated by using following formula (Fisher, 1920).

$RGR = \log eW_2 - \log eW_1/T_2-T_1$

(W_1, W_2 - weight of sample during a period; T_1, T_2 - Time period)

Net photosynthetic rate (µmol $m^{-2}s^{-1}$) and Stomatal conductance (µmol $m^{-2}s^{-1}$)

Net photosynthetic rate and stomatal conductance rate were measured on 5 plants per treatment at 15th, 30th and 45th day after planting using an infrared gas analyzer (LI-COR, Biosciences, USA, Model LI 64000) equipped with air supply unit and a broad leaf chamber (aperture area 6.25cm^2). Fully expanded leaves at an identical location on the plant (4th leaf from the apical terminal) were used for the measurements. During the measurements, the light condition (light intensity and quality) of the leaf chamber was calibrated to compare real-time light conditions by adjusting the LEDs back panel. The CO_2 concentration of the air entering the leaf chamber was adjusted to 400 $mmol^{-1}$ by using a CO_2 gas container and leaf temperature was maintained at 22^0C. The data were logged at every 30 seconds for 30 min.

Net Assimilation Rate (mg cm^{-2} day^{-1})

Dry weight and leaf area of five randomly selected plants from each replicated treatment was measured at 0, 15th, 30th and 45th and Net Assimilation Rate was calculated at 15 days interval by using following formula as per the method suggested by Gregory (1926).

$NAR = W_2-W_1 (InLA_2-LA_1)/T_2-T_1 (LA_2-LA_1)$ mg $cm^{-2}day^{-1}$

(W_1, W_2 - weight of sample during a period; LA_1, LA_2 - leaf area during a period; T_1, T_2 - Time period)

Net Respiration Rate (µmol $m^{-2}s^{-1}$)

Five plants were randomly selected from each replicated treatment and net respiration rate was measured by using an infra-red gas analyzer (LI-COR, Biosciences, USA, Model LI 64000) at 0, 15th, 30th and 45th days after planting.

Chlorophyll content (mg g^{-1})

Chlorophyll content in the leaves was measured by DMSO method. In this method, 50mg of finely chopped fresh leaf was taken and filled in test tubes poured with 10ml of dimethyl sulphoxide (DMSO). The filled tubes were covered with aluminium foil and kept in an oven at 65^0C for 4h. Subsequently, the tubes were shaken to allow the pigment to distribute uniformly and the absorbance was read at 645 nm, 663 nm and 470 nm wavelengths in a spectrometer using DMSO as a blank reading. The following formulas were used for estimation of chlorophyll-a, chlorophyll-b and total chlorophyll.

Chlorophyll a (mg g^{-1}fw) = (12.7xA663)-(2.69xA645) x V / (1000xW)

Chlorophyll b (mg g^{-1}fw) = (22.9xA645)-(4.68xA663) x V / (1000xW)

Chlorophyll a + b (mg g^{-1}fw) = (20.2xA645)-(8.02xA663) x V / (1000xW)

(Where, A = absorbance at given wave length; V = final volume of solvent in ml; W = weight of plant sample in g).

Gibberellic Acid levels

Phosphate Buffer preparation

To prepare phosphate buffer solution, 0.2 M solution of monobasic sodium phosphate was made by dissolving 27.8 g of monobasic sodium phosphate in 1000 ml of water. Then, a 0.2 M solution of dibasic sodium phosphate

was created by dissolving 53.6 g of $Na_2HPO_4.7H_2O$ in 1000 ml of water. To make a 0.05 M phosphate buffer solution, 16 ml of the monobasic salt solution was combined with 84 ml of the dibasic salt solution, and the mixture was diluted to a total volume of 400 ml using water. This solution yields a 0.05 M phosphate buffer, suitable for further procedure.

Procedure

Initially, 0.02 g of the sample was weighed and grounded using liquid nitrogen, followed by homogenization. Subsequently, 100 ml of buffer solution containing 0.02% Na-diethyl di thio-carbamate antioxidant was added to 15 ml falcon tubes containing the sample. The solution was then kept at 4^0C in a shaker at 150 rpm overnight. Afterwards, the samples were centrifuged at 10,000 rpm at 4^0C for 10 minutes, and the supernatant was collected. The volume was adjusted to 10 ml with sodium phosphate buffer. The solution was subjected to partitioning using a separating funnel with 5 ml of diethyl ether, discarding the upper phase and retaining the lower phase, and the process was repeated twice. Subsequently, the pH was adjusted to 2.5 using 1 N HCL, followed by partitioning with 10 ml of petroleum ether. The solution was partitioned three times again using diethyl ether, and the upper phase was retained. The samples were left overnight, covered with aluminum foil with holes. The following day, a mixture of 65% methanol and 35% water (HPLC grade) was added to the dried samples. Different standard solutions were prepared using GA_3 along with the samples and stored in glass vials. Finally, the prepared samples underwent analysis using the HPLC-MS facility available in the department of plant physiology.

Statistical analysis

The experiment was conducted under growth chamber and climate-controlled greenhouse where homogenous conditions maintained. Experimental set-up was a complete randomized block design (CRBD). The recorded data was analyzed for analysis of variance (ANOVA) to explore the main and interaction impacts of treatments were tested at the 5% level of significance. The statistical package OPSTAT version was used for analysis and required graphs were drawn using MS Excel software.

Results and Discussion

Fresh and dry weight of leaves (g)

The fresh and dry weight of leaves in chrysanthemum cv. Zembla plants was investigated at 15 days and at an interval of 15, 30 and 45 days, respectively. The obtained results were statistically significant at different days interval (Table 1). An overall increase among fresh and dry weight of leaves was observed due to all the artificial light treatments. It was observed that the maximum leaf fresh weight (12.4 g) was attained in the treatment with R+B, LED combination at 45 days interval. However, white LED treatment (control) demonstrated a minimum increase in leaf fresh weight (8.06 g) among rest of the treatments. Similarly, the maximum leaf dry weight (3.1 g) was obtained in treatment with R+B, LED combination, while minimum increase in dry weight (2.01 g) was seen under white LED treated plants.

Table 1 : Effect of LED lights on leaf fresh weight (gm) and dry weight at 15, 30 and 45 DAP.

Treatment	Leaf fresh weight (gm)			Leaf dry weight (gm)		
	15 d	30 d	45 d	15 d	30 d	45 d
Fluorescent	7.5	10.08	10.14	0.92	2.52	2.53
Red	8.7	10.16	11.2	2.17	2.54	2.8
Blue	6.94	9.76	12.2	1.73	2.44	3.05
80%R+20%B	7.84	9.05	12.4	1.96	2.26	3.1
White	3.66	6.05	8.06	1.87	1.51	2.01
CD 0.05	0.58	1.01	2.08	0.67	0.62	0.58
S.E(m) ±	0.19	0.34	0.7	0.23	0.21	0.19

Fresh and dry weight of stem (g)

The fresh and dry weight of stem in potted chrysanthemum plants was investigated at a 15 days interval at 15, 30 and 45 days respectively. The obtained results were statistically significant at different days interval. An overall increase among fresh and dry weight of stem was observed due to all the artificial light treatments (Table 2). It was observed that the maximum stem fresh weight (4.6 g) was attained in treatment with R+B LED combination at 45 days interval. However, white LED treatment (control) demonstrated a minimum increase in stem fresh weight (3.43 g) among rest of the treatments. Similarly, the maximum stem dry weight (1.18 g) was obtained in treatment with R+B LED combination,

Table 2 : Effect of LED lights on stem fresh weight (gm) at 15, 30 and 45 DAP

Treatment	Stem fresh weight (gm)			Stem dry weight (gm)		
	15 d	30 d	45 d	15 d	30 d	45 d
Fluorescent	2.48	3.34	3.8	0.63	0.64	0.96
Red	2.3	3.19	4.08	0.63	0.85	1.08
Blue	3.28	4.01	4.42	0.72	0.97	1.08
80%R+20%B	2.74	3.46	4.6	0.65	0.88	1.18
White	2.82	2.53	3.43	0.75	0.85	0.95
CD 0.05	NA	0.66	0.71	NA	0.12	0.2
S.E(m) ±	0.25	0.22	0.24	0.05	0.04	0.06

while minimum increase in dry weight (0.95 g) was seen in white LED treatment.

Higher biomass accumulation was also reported by Ozounis *et al*. (2014) due to RB, LED (100%) in chrysanthemum. Red light affects photomorphogenetic responses, thus affecting growth by influencing the red and far-red ratio (Sager and McFarlane, 1997). On the other hand, low photon intensity can lead to a lesser accumulation of dry matter and RB lighting leads to increase in dry matter production by enhancing the net assimilation rate (Goins *et al*., 1997).

Chlorophyll content (mg g^{-1})

The chlorophyll content in chrysanthemum 'Zembla' kept under artificial lights were recorded at 15, 30 and 45 days after planting. The results were statistically significant at all day intervals (Table 3). The chlorophyll content was observed to increase under the effect of LED lights and greenhouse. It was recorded maximum in 80% Red and 20% blue treatment combination (10.94 mg g^{-1}) at 45 day's interval, while white LEDs registered a minimum increase (3.82 mg g^{-1}). Similar findings were reported by Kim *et al*. (2004), where the use of RB, LED lighting modules resulted in elevated chlorophyll levels in chrysanthemum *in-vitro* plantlets. Shin *et al*. (2008) also documented increased chlorophyll content when using a combination of red and blue LED lighting systems. The increase in chlorophyll content might be due to maximum photosynthetic efficiency of plants grown under RB LEDs and their wavelengths coinciding with absorption peaks of chlorophyll.

Net Photosynthetic Rate (µmol CO_2 m^2 sec^{-1})

The net photosynthetic rate in chrysanthemum potted plants was recorded at a 15 day's interval at 15, 30 and 45 days, respectively. An overall increase in NPR was observed with the progression of days (Table 3). It was observed that the maximum NPR (32.12 µmol CO_2 m^2 sec^{-1}) was attained with treatment R+B, LED combination at 45 day's intervals. However, white LED treatment demonstrated a minimum increase in NPR (13.84 µmol CO_2 m^2 sec^{-1}) among all other treatments. The table indicates that net photosynthesis rate results are statistically significant at all day intervals.

Stomatal Conductance (mol H_2O m^2 sec^{-1})

The stomatal conductance in chrysanthemum cv. Zembla plants was investigated at a 15 days interval at 15, 30 and 45 days respectively. The obtained results were statistically significant at different day's interval. An overall increase among stomatal conductance was observed due to all the artificial light treatments (Table 4). It was observed that the maximum stomatal conductance (0.33 mol H_2O m^2 sec^{-1}) was attained in the treatment with treatment with R+B LED combination at 45 days intervals. However, white LED treatment (control) demonstrated a minimum increase in stomatal conductance (0.07 mol H_2O m^2 sec^{-1}) among rest of the treatments. Kim *et al*. (2004) has used red and blue mixed LED treatments tends to increase the stomatal conductance in chrysanthemum *in-vitro* plantlets. Stomatal conductance is influenced by both red and blue light, where blue spectrum of radiation acts as an energy source through photosynthesis (Whitelam and Halliday, 2007).

Table 3 : Effect of LED lights on Chlorophyll content and Net Photosynthetic Rate at 15, 30 and 45 DAP.

Treatment	Chlorophyll content (mg g^{-1})			Net Photosynthetic Rate (µmol CO_2 m^2 sec^{-1})		
	15 d	30 d	45 d	15 d	30 d	45 d
Fluorescent	1.73	3.87	5.33	6.32	12.72	14.97
Red	4.98	6.24	8.52	10.26	19.43	22.47
Blue	3.73	5.93	7.49	7.21	13.42	27.15
80%R+20%B	5.23	8.36	10.94	14.82	29.52	32.12
White	1.63	2.74	3.82	6.42	11.34	13.84
CD 0.05	0.49	0.55	0.74	0.69	2.73	1.93
S.E(m) ±	0.16	0.18	0.25	0.23	0.92	0.65

Table 4 : Effect of LED lights on Stomatal Conductance and Net Respiration Rate at 15, 30 and 45 DAP.

Treatment	Stomatal conductance (mole H_2O m^2 sec^{-1})			Net respiration Rate (µmol CO_2 m^2 sec^{-1})		
	15 d	30 d	45 d	15 d	30 d	45 d
Fluorescent	0.027	0.043	0.085	0.343	2.572	4.109
Red	0.32	0.54	0.118	0.573	2.147	4.104
Blue	0.124	0.153	0.238	3.742	7.274	9.908
80%R+20%B	0.148	0.164	0.33	5.218	10.258	12.507
White	0.021	0.032	0.07	0.613	2.471	3.794
CD 0.05	0.03	0.024	0.038	0.39	0.98	0.87
S.E(m) ±	0.027	0.043	0.085	0.343	2.572	4.109

Net Respiration Rate (µmol CO_2 m^2 sec^{-1})

The net respiration rate in potted chrysanthemum plants was recorded at a 15 days interval at 15, 30 and 45 days, respectively. An overall increase in NRR was observed with the progression of days (Table 4). It was observed that the maximum NRR (12.50 µmol CO_2 m^2 sec^{-1}) was attained in the treatment with combination of 80% Red and 20% Blue LEDs at 45 days intervals. However, white LED treatment demonstrated a minimum increase in NRR (3.79 µmol CO_2 m^2 sec^{-1}) among all

other treatments. The table indicates that net respiration rate results are statistically significant at all day intervals. Similar results were obtained by Leonardos *et al*. (2019) where RB lights leads to increase in transpiration and reduced water use efficiency in chrysanthemum. Partly, stomatal regulation and circadian rhythm are responsible for such transpirational losses.

Net Assimilation Rate (g cm^{-1} day^{-1})

The net assimilation rate in chrysanthemum cv. Zembla plants was investigated at a 15 days interval at 15, 30 and 45 days, respectively. The obtained results were statistically significant at different day's interval. An overall increase among net assimilation rate was observed due to all the artificial light treatments (Table 5). It was observed that the maximum net assimilation rate (1.28 µmol CO_2 m^2 sec^{-1}) was attained in the treatment with R+B, LED combination at 45 days intervals. However, white LED treatment (control) demonstrated a minimum increase in net assimilation rate (0.12 µmol CO_2 m^2 sec^{-1}) among rest of the treatments. Similar results were achieved by Jeong *et al*. (2014) where RB, LEDs cause to increase in net assimilation rate in chrysanthemum under various supplemental lighting systems. RB light leads to increase in dry matter production by enhancing the net assimilation rate (Goins *et al*., 1997).

Relative Growth Rate (g gm^{-1} day^{-1})

The relative growth rate in potted chrysanthemum plants was recorded at a 15 day's interval at 15, 30 and 45 days respectively. An overall increase in RGR was observed with the progression of days (Table 5). It was observed that the maximum RGR (0.014 g^{-1} day^{-1}) was attained in the treatment with R+B LED combination at 45 days intervals. However, white LED treatment demonstrated a minimum increase in RGR (0.008 g^{-1} day^{-1}) among all other treatments. The table indicates that

Table 5 : Effect of LED lights on Net Assimilation Rate and Relative Growth Rate at 15, 30 and 45 DAP.

Treatment	Net assimilation Rate (gm cm^{-1} day^{-1})			Relative Growth Rate (gm gm^{-1} day^{-1})		
	15 d	30 d	45 d	15 d	30 d	45 d
Fluorescent	1.563	1.241	0.125	0.018	0.015	0.013
Red	3.271	2.427	1.164	0.025	0.014	0.014
Blue	2.364	1.167	1.176	0.023	0.012	0.011
80%R+20%B	3.364	2.635	1.283	0.029	0.014	0.014
White	2.281	1.104	0.121	0.012	0.011	0.008
CD 0.05	0.13	0.073	NA	0.007	NA	0.003
S.E(m) ±	0.04	0.025	0.034	0.002	0.001	0.001

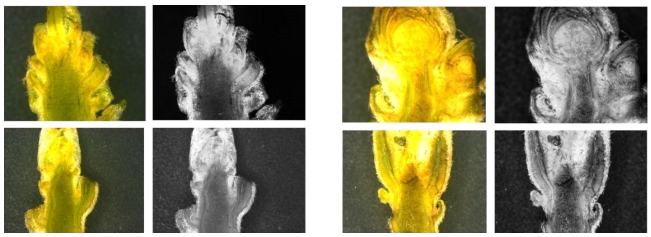

Fig. 3 : Bud Differentiation stages studied using a stereoscope.

relative growth rate results are statistically significant at 15 and 45 day's intervals. Bruggink and Heuvelink (1987) have postulated that relative growth rate (RGR) to be a function of mean daily light integrals. Further, modifications in red to far red ratio in LED light spectrum can alter plant growth patterns (Li and Kubota, 2009). This might be attributed towards light foraging capabilities and various cell wall modifying mechanisms. Contradictory findings were recorded where white and blue LED mixture promoted the growth patterns in green onions (Gao *et al*., 2020). Light spectra and their effects are species dependent and involve complex mechanisms.

Bud diameter (mm)

Bud diameter of plants under different light treatments was measured at fully developed stage. The results were statistically significant for all the treatments. The bud diameter was observed to increase with the influence of artificial lights (Table 6). It was observed that the maximum bud diameter (9.61 mm) was attained with treatment with R+B LED combination, followed by red LED treatments, respectively. However, blue LED treatment demonstrated a least progression in bud diameter (7.88 mm) among other treatments. Singh and Bala (2019) have also reported that maximum amount of photosynthetic assimilates were used for increase in flower size in chrysanthemum using LED lights. This resulted due to a higher partitioning coefficient over the experimental period.

Flower diameter (mm)

The flower diameter in chrysanthemum cv. Zembla plants was investigated at fully open stage. The obtained results were statistically significant for all the treatments. An overall increase in flower diameter was observed due to all the artificial light treatments (Table 6). It was observed that the maximum flower diameter (77.33 mm) was attained in the treatment with R+B, LED combination, followed by fluorescent LED treatment respectively. However, blue LED treatment demonstrated a minimum increase in flower diameter (60.31mm) among rest of the treatments.

Time taken for flower bud induction (days)

Time taken for flower bud induction of plants under

Table 6 : Effect of LED lights on Bud diameter (mm), Flower diameter (mm), Time taken for flower bud induction (days) and Flower weight (gm)

Treatment	Bud diameter (mm)	Flower diameter (mm)	Time taken for flower bud induction (days)	Flower weight (gm)
Fluorescent	9.168	73.048	82.2	6.21
Red	9.322	67.474	82.2	6.04
Blue	7.884	60.31	102.6	3.76
80%R+20%B	9.618	77.334	66.6	9.27
White	9.126	67.34	91.2	4.07
CD 0.05	0.62	2.89	4.23	1.19
S.Em±	0.21	0.98	1.43	0.4

Table 7 : Effect of different LED lights on gibberellic acid levels in chrysanthemum cv. Zembla.

Treatment	Fluorescent	Red	Blue	80%R+20%B	White	CD 0.05	S.E(m)±
GA_3 (ng/g)	28.26	53.28	14.92	66.07	45.54	14.24	4.52

different light treatments was measured at full development stage. The results were statistically significant for all the treatments. The time taken for flower bud induction was observed to increase with the influence of artificial lights (Table 6). It was observed that the maximum time for flower bud induction (102.6 days) was attained with blue LED treatment. However, 80% red and 20% blue LED treatment demonstrated a minimum time for bud induction (66.6 days) among other treatments. Nissim-Levi *et al*. (2019) have reported flower bud inhibition in three chrysanthemum cultivars under blue LED illumination. Inhibition rate of flowering using blue LED depends on the duration of light exposure. CmFTL3 gene levels are also modulated using LEDs which regulates flowering behaviour in chrysanthemum. However, Singh *et al*. (2019) have reported an earlier bud induction using blue LEDs.

Flower weight (g)

Flower weight of plants under different light treatments was measured at fully opened stage. The results were statistically significant for all the treatments. The flower weight was observed to increase with the influence of artificial lights (Table 6). It was observed that the maximum flower weight (9.27 g) was attained with R+B, LED combination followed by fluorescent LED treatment, respectively. However, blue LED treatment demonstrated a least progression in flower weight (3.76 g) among other treatments. RB light leads to increase in dry matter production by enhancing the net assimilation rate (Goins *et al*., 1997). Hence, assimilate partitioning is reflected in the subsequent flower weight in chrysanthemum. Whereas, low photon flux intensity due to lack of specific light spectra can cause reduced flower weight.

Effect of different LED lights on gibberellic acid levels in chrysanthemum cv. Zembla

As evident from the Table 7, describing relevant data on the way exposure to the different LED lights and their combinations impacts the levels of gibberellic acid hormone in the potted plants of chrysanthemum under the observations. All the results recorded were statistically significant for all the treatments (Table 7). The potted plants exposed to the combination of R+B LED (80%: 20%) recorded the maximum levels of GA_3 (66.07 ng/g). Whereas, the plants under exposure to blue LED lights demonstrated a minimum level of GA_3 levels (14.92 ng/g). The data clearly demonstrates that the gibberellic acid levels vary significantly with the exposure of the plants to different LED lights in chrysanthemum. The potted chrysanthemum plants exposed to the combined mixture of R+B LEDs (80: 20 recorded the maximum levels of GA_3 (66.07 ng/g), while under blue LED lights exposure demonstrated a minimum level of GA_3 levels (14.92 ng/g). The data clearly demonstrates that gibberellic acid levels vary significantly with the exposure to different LED lights in chrysanthemum. Jiang *et al*. (2010) have suggested that a stable ABA and GA_3 levels are necessary for crown bud formation in *C. morifolium*. Both the hormones play a key role in inflorescence differentiation and floral induction. Matsuo *et al*. (2019) have reported a higher level of bioactive GA and GA_4 under seedlings grown under red LED lights, while blue LEDs correlated negatively with the stem length in tomato. It was concluded that transcript levels of GA biosynthesis enzyme gene, *SIGA3ox3* were expressed more in red LED lights, while GA inactivation enzyme gene, *SIGA2ox7* increased with blue light intensity.

Conclusion

Hence, the application of supplemental dichromatic 80% red and 20% blue light proved advantageous for enhancing morphological and physiological parameters in chrysanthemum plants. Morphological parameters such as fresh and dry weight of leaves and stems, were notably augmented under this lighting conditions. Similarly, physiological parameters including chlorophyll content, net photosynthetic rate (NPR), stomatal conductance, net root respiration (NRR), net assimilation rate (NAR), and relative growth rate (RGR) were observed to increase significantly. Moreover, flowering parameters such as bud diameter, flower diameter, and flower weight were found to be highest under 80% red and 20% blue LED lighting, while registering the lowest values under blue LED treatment. Additionally, the earliest bud induction was observed in the plants under 80% red and 20% blue LED lights, whereas there was a delay in bud induction under blue LED lighting. Furthermore, gibberellic acid levels were highest under 80% red and 20% blue LED lighting and lowest under blue LED treatment. These findings collectively highlight the efficacy of dichromatic LED lighting, particularly 80% red and 20% blue, in optimizing the growth, development and biochemical responses of chrysanthemum plants, offering valuable insights for horticultural practices and greenhouse cultivation techniques.

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